Tenth Keele Meeting on Aluminium

Illuminating and Elucidating Aluminium’s Exposome: From Geochemistry to Neurochemistry, from Microbe to Man.

Book of Abstracts

Saturday 23rd - Wednesday 27th February 2013

Norton Park Hotel, Winchester, England
The Scientific Programme ................................................................. 1

Abstracts.............................................................................................. 19

Session 1: Aluminium Chemistry ................................................................. 19
Session 2: Aluminium, Plants and the Environment ........................................ 33
Session 3: Aluminium Biochemistry ............................................................ 51
Session 4: Human Exposure to Aluminium .................................................... 68
Session 5: Aluminium, Alzheimer's and Other Neurological Diseases ............... 87
The JD Birchall Memorial Lecture ............................................................. 95
Scientific Programme

Saturday 23rd February 2013

17.00 Registration and Poster Assembly
19.30 Welcome to Meeting / Welcome Buffet

Sunday 24th February 2013

8.25 – 13.00: Session 1 - Aluminium Chemistry

Chair: Tamas Kiss (Szeged University, Szeged, Hungary)

8.25 - Introduction by the Chair

8.30 - Platform 1: Natural organic matter adsorption on a lateral AlOOH surface elucidated with *ab initio* molecular dynamics.

*Dominique Costa* (ENSCP Chemie-Paristech, Paris, France)

8.50 - Discussion

9.00 - Oral Poster 1: Adsorption of inositol phosphate on gibbsite (γ-Al(OH)3).

*Oleg Antzutkin* (Luleå University of Technology, Luleå, Sweden)

9.05 - Discussion

9.10 - Platform 2: Interaction between Al3+ and 2, 3-dihydroxyterephthallic acid as a model compound for Inogashira fulvic acid.

*Takushi Yokoyama* (Kyushu University, Higachi-ku, Fukuoka, Japan)

9.30 - Discussion
9.40 - Oral Poster 2*: Interaction between $\text{Al}^{3+}$ and simple dicarboxylic acids at pH 3.

Mayumi Etou (Kyushu University, Higachi-ku, Fukuoka, Japan)

9.45 - Discussion

9.50 - Platform 3: Detecting toxicant interactions in mixtures of toxicants. Special consideration of ionic toxicants.

Thomas Kinraide (USDA, Beaver, West Virginia, USA)

10.10 - Discussion

10.20 - COFFEE

10.40 - Platform 4: Computer-aided speciation of small aluminium silicate clusters in aqueous environments.

Giorgio Lanzani (University of Oulu, Oulu, Finland)

11.00 - Discussion

11.10 - Oral Poster 3: Aluminium-silicon interactions in mild pH and ionic strength conditions

Jaakko Rämö (University of Oulu, Oulu, Finland)

11.15 - Discussion

11.20 - Oral Poster 4: Computer simulations of the structure, formation and growth of hydroxyaluminosilicates, at the level of individual and collections of molecules

James Beardmore (Keele University, United Kingdom)

11.25 - Discussion
11.30 - Platform 5: **Can Al\(^{3+}\) promote the Fenton reaction and oxidative stress?**

*Xabier Lopez* (Euskal Herriko University, Donostia, Basque Country)

11.50 – Discussion

12.00 - Oral Poster 5: **Unveiling the coordination of aluminium to amyloid-β peptide with computational chemistry.**

*Jon Mujika* (Euskal Herriko University, Donostia, Basque Country)

12.05 – Discussion

12.10 - Platform 6: **Hydroxypyrones, a fascinating family of chelating agents for Al(III).**

*Guido Crisponi* (Citadella University, Cagliari, Italy)

12.30 - Discussion

12.40 - Oral Poster 6: **Complex formation equilibria of substituted phenols with Al\(^{3+}\) and Fe\(^{3+}\).**

*Miriam Crespo-Alonso* (Citadella University, Cagliari, Italy)

12.45 - Discussion

12.50 - Oral Poster 7*: **Complexation of Al(III) with hydroxypyridine(di)carboxylic acids as new possible chelating agents in neurodegenerative diseases.**

*Eva Sija* (University of Szeged, Szeged, Hungary)

12.55 Discussion

13.00 **LUNCH**
14.15 – 18.15: Session 2 - Aluminium, Plants and the Environment

Chair: **Lubos Boruvka** (Czech University of Life Science, Prague, Czech Republic)

14.15 - Introduction by the Chair

14.20 - Platform 7: **Molecular mechanisms of crop adaptation to acid soils.**

_Leon Kochian_ (USDA-ARS, Cornell University, New York, USA)

14.40 - Discussion

14.50 - Platform 8: **Cellular distribution of aluminium and other elements in leaves of native plant species of Brazilian Cerrados.**

_Leide de Andrade_ (Embrapa Cerrados, Planaltina, Brazil)

15.10 – Discussion

15.20 - Oral Poster 8*: **Effect of aluminium ions on hydrangea sepals and viburnum drupes.**

_Blaine Groat_ (Virginia Military Institute, Lexington, Virginia, USA)

15.25 - Discussion

15.30 - Oral Poster 9: **Aluminium, iron and manganese, do they limit the determination of anionic substances by ion chromatography?**

_Ondřej Drabek_ (Czech University of Life Sciences, Prague, Czech Republic)

15.35 - Discussion
15.40 - Platform 9: **Amelioration of iron toxicity, a mechanism for aluminium-induced growth stimulation in tea plants.**

*Charlotte Poschenrieder* (Universidad Autónoma de Barcelona, Spain)

16.00 - Discussion

16.10 - **TEA**

16.30 - Oral Poster 10: **Long term changes in aluminium concentrations and fluxes in Czech streams recovering from acidification.**

*Pavel Kram* (Czech Geological Survey, Prague, Czech Republic)

16.35 - Discussion

16.40 - Platform 10: **Effect of salicylic acid on the attenuation of aluminium toxicity in cell suspensions of Coffea arabica L.**

*Teresa Hernandez-Sotomayor* (CICY, Mérida, México)

17.00 - Discussion

17.10 - Oral Poster 11: **The role of diacylglycerol in aluminium toxicity in plants.**

*Přemysl Pejchar* (Academy of Sciences of Czech Republic, Prague, Czech Republic)

17.15 - Discussion

17.20 - Platform 11: **Aluminium-induced cell death involves both mitochondria and the vacuole in plant cells.**

*Yoko Yamamoto* (Okayama University, Kurashiki, Japan)

17.40 - Discussion
17.50 - Oral Poster 12: **Influence of Al⁴⁺ on light-induced membrane potential changes in *Nitellopsis obtusa* cells.**

*Vilma Kisnierienė* (Vilnius University, Vilnius, Lithuania)

17.55 - Discussion

18.00 - Platform 12: **Physiological and oxidative responses of two *Avena sativa* genotypes and *Cucumis sativis* seedlings to aluminium in nutrient solution.**

*Maria Rosa Schetinger* (Federal University of Santa Maria, Santa Maria, Brazil)

18.20 - Discussion

18.30 - Oral Poster 13*: **Differential speed of activation in antioxidant systems in three oat genotypes.**

*Luciane Pereira* (Federal University of Santa Maria, Santa Maria, Brazil)

18.35 - Discussion

18.40 - Oral Poster 14*: **The bioleaching of aluminium from solid aluminium oxide by a filamentous fungus.**

*Ivana Pifkova* (Comenius University in Bratislava, Bratislava, Slovakia)

18.45 - Discussion

18.50 - **END OF FIRST DAY**

20.15 - **DINNER**

21.15 - **POSTER SESSION AND WINE TASTING**
Monday 25th February 2013

8.25-13.20: Session 3 - Aluminium Biochemistry

Chair: Paula Goncalves (University of Aveiro, Aveiro, Portugal)

8.25 - Introduction by the Chair

8.30 - Platform 13*: Comparative study on the binding of Al\(^{3+}\) and nano-Al\(_{13}\) with salmon sperm DNA and calf thymus DNA.

   Fei Ma (Nanjing Normal University, Nanjing, China)

8.50 - Discussion

9.00 - Oral Poster 15: Carbon nanomaterials as modified electrodes for the determination of trace aluminium (III) in biological fluids using 8-hydroxyquinone.

   Xiao Di Yang (Nanjing Normal University, Nanjing, China)

9.05 - Discussion

9.10 - Platform 14*: Cholesterol effect on (Na+K+)ATPase inhibition by submillimolar aluminium.

   Madina Artykbayeva (University of Aveiro, Aveiro, Portugal)

9.30 - Discussion

9.40 - Oral Poster 16: Acute and chronic neurotoxicity of aluminium oxide nanoparticles in mice.

   Qinli Zhang (Shanxi Medical University, Taiyuan, China)

9.45 - Discussion
9.50 - Oral Poster 17: Aluminium and calcium homeostasis; influence of insulin-like growth factor 1 (IGF-1) on intestinal absorption.

Daniel Orihuela (Universidad Nacional del Litoral, Sante Fe, Argentina)

9.55 - Discussion

10.00 - COFFEE

10.20 - Platform 15: Age dependence in the accumulation and elimination of aluminium in rats.

Denise Bohrer (Federal University of Santa Maria, Brazil)

10.40 - Discussion

10.50 - Platform 16: Effect of long term exposure to aluminium and a high fat diet on NTPDase and 5’-nucleotidase activities in lymphocytes and platelets of rats.

Rosalene Kaizer Perin (Instituto Federal de Educação Ciência e Tecnologia do Rio Grande do Sul, Brazil)

11.10 - Discussion

11.20 - Platform 17: No effect of long term low dosage of Al maltolate towards Th2 immune response in young rats.

Guoo-Shyng Wang Hsu (Fu-Jen Catholic University, Taipei, Taiwan)

11.40 - Discussion

11.50 - Platform 18: Aluminium adjuvant-induced mitochondrial alterations.

Håkan Eriksson (Malmö University, Malmö, Sweden)

12.10 - Discussion
12.20 - Platform 19: **Administration of aluminium in vaccine-relevant exposures in neonatal mice is associated with long term adverse neurological outcomes.**

*Christopher Shaw* (University of British Columbia, Vancouver, Canada)

12.40 – Discussion

12.50 - Platform 20: **Aluminium enhances inflammation and decreases mucosal healing in experimental colitis in mice.**

*Mathilde Body-Malapel* (University of Lille 2, Lille, France)

13.10 - Discussion

13.20 - **LUNCH**

**FREE AFTERNOON**

20.00 - **DINNER**

21.15 – **FILM**: **Dirty Little Secret**: *The Aluminium Files* (English Language Premiere):

A Film by Bert Ehgartner
8.25 – 13.00: Session 4 – Human Exposure to Aluminium

Chair: **David Chettle** (McMaster University, Hamilton, Canada)

8.25 - Introduction by the Chair

8.30 - Platform 21: **Excessive aluminium accumulation in the bones of patients on long term parenteral nutrition.**

*Patrick Parsons* (State University of New York, Albany, USA)

8.50 - Discussion

9.00 - Oral Poster 18: **Assessing inter-laboratory performance for serum Al in the New York State Proficiency Testing programme; implications for monitoring exposure to Al in parenteral nutrition patients.**

*Pamela Kruger* (New York State Department of Health, Albany, USA)

9.05 – Discussion

9.10 - Oral Poster 19*: **Hot watery infusion of *Hibiscus sabdariffa* petals, a potential source of aluminium in the human diet.**

*Adela Fraňkova* (Czech University of the Life Sciences, Prague, Czech Republic)

9.15 - Discussion

9.20 - Oral Poster 20: **A pilot study measuring aluminium in bone in Alzheimer’s and referent subjects; work in progress.**

*David Chettle* (McMaster University, Hamilton, Canada)

9.25 - Discussion

Shunsuke Meshitsuka (Totorri University School of Medicine, Yonago, Japan)

9.50 – Discussion

10.00 - COFFEE


Philippa Darbre (University of Reading, Reading, UK)

10.40 – Discussion

10.50 - Platform 24: Aluminium chloride transforms cultured mammary epithelial cells.

Stefano Mandriota (University Hospital of Geneva, Geneva, Switzerland)

11.10 – Discussion

11.20 - Platform 25: The relationship between aluminium, carbonyls and interleukins in the microenvironment of normal and cancerous breast tissue.

Ferdinando Mannello (University “Carlo Bo”, Urbino, Italy)

11.40 - Discussion

11.50 - Oral Poster 21*: Physico-chemical characterisation of clinically-approved and research aluminium-based adjuvants.

Emma Shardlow (Keele University, Staffordshire, UK)

11.55 Discussion
12.00 - Platform 26: Aluminium-hydroxide induced macrophagic myofasciitis (MMF); predictive scores and biomarkers.

*Francois-Jerome Authier* (Paris Est-Creteil University, France)

12.20 - Discussion

12.30 - Platform 27: Death following human papillomavirus (HPV) vaccination; an auto-immune adjuvant-mediated adverse reaction?

*Lucija Tomljenovic* (University of British Columbia, Vancouver, Canada)

12.50 - Discussion

13.00 – LUNCH
14.25 - 16.40: Session 5 - Aluminium, Alzheimer's and Other Neurological Diseases

Chair: Leon Kochian (USDA-ARS, Cornell University, New York, USA)

14.25 - Introduction by the Chair

14.30 - Platform 28: Caspase-3 short hairpin RNA interference targeted to an Alzheimer’s disease animal model induced by aluminium blocks neural cell death and defects of learning and memory.

_Qiao Niu_ (Shanxi Medical University, Taiyuan, China)

14.50 - Discussion

15.00 - Platform 29: Aluminium entry into the brain; studies in the cerebral vasculature and in human brain microvessel endothelial (hBMEC) cells.

_Walter Lukiv_ (Louisiana State University Health Sciences Centre, New Orleans, USA)

15.20 - Discussion

15.30 - Oral Poster 22: Relationship of aluminium intoxication with neurodegenerative disease.

_Alessandro Fulgenzi_ (University of the Studies of Milan, Milan, Italy)

15.35 - Discussion

15.40 - Platform 30: Colocalisation of aluminium and iron in cell nuclei in the brains of patients with Alzheimer’s disease.

_Sakae Yumoto_ (Yumoto Institute of Neurology, Tokyo, Japan)

16.00 - Discussion
16.10 - Platform 31: Aluminium, illicit drugs, neuropsychiatric impairment and disability; an evidence-based approach.

Paolo Prolo (Swiss Disability Insurance, Bellinzona, Switzerland)

16.30 - Discussion

16.40 – TEA

**17.10 – 20.00: Final Session - The JD Birchall Memorial Lecture**

Final Session Chair: Chris Exley (Keele University, UK)

17.10 - Introduction by the chair to the JD Birchall Memorial Lecture

17.20 – Lecture: From the coordination chemistry to the biological chemistry of aluminium

Professor Tamas Kiss (University of Szeged, Szeged, Hungary)

18.20 – Discussion

18.30 – CONCLUSION OF MEETING

20.00 – CONFERENCE DINNER

---

**END OF SCIENTIFIC PROGRAMME**

N.B: *Denotes a presentation given by a student.
List of Additional Posters

Session 2 - Aluminium, Plants and the Environment

Poster 1:  
Labile Al content or BC/Al ratio in soil: what controls tree seedlings growth?  

*Lubos Boruvka* (Czech University of Life Sciences, Prague, Czech Republic)

Poster 2:  
The influence of altitude on Al speciation in samples originating from beach and spruce forests.  

*Lubos Boruvka* (Czech University of Life Sciences, Prague, Czech Republic)

Poster 3:  
Salicylic acid is involved in the mechanism of response to aluminum toxicity in cell suspensions of *Capsicum chinense Jacq.*  

*Armando Munoz-Sanchez* (CICY, Merida, Mexico)

Poster 4*:  
The combined effect of Aluminium and tritium on the plant cell membrane bioelectrical parameters.  

*Olga Sevriukova* (Vilnius University, Vilnius, Lithuania)

Poster 5:  
Physiological and oxidative stress responses to aluminium in three oat genotypes grown hydroponically.  

*Vera Morsch* (Federal University of Santa Maria, Brazil).

Poster 6*:  
Localisation of callose in rice tissue using aniline blue staining and immunofluorescence.  

*Ian Stokes* (Keele University, United Kingdom)
Session 3 - Aluminium Biochemistry

Poster 1*: Aluminium effect on *Escherichia coli* growth and death

*Madine Artykbayeva* (University of Aveiro, Aveiro, Portugal)

Poster 2*: Effects of aluminium maltolate ingestion on the immune response of SD neonates.

*Hsin-Ya Lin* (Fu-Jen Catholic University, Taipei, Taiwan)

Poster 3*: Heme oxygenase-1 induction by ROS-JNK pathway plays a role in aluminium-induced anaemia.

*Chia-Yeh Lin* (Fu-Jen Catholic University, Taipei, Taiwan)

Poster 4*: Transcellular transport of alumina nanoparticles: A study on blood-brain barrier model in *vitro*.

*Hui-ting Peng* (Shanxi Medical University, Taiyuan, China)

Poster 5*: Effects on long-term potentiation and the expression of AMPA receptor subunits in rat exposed to aluminum *in vivo*.

*Jing Song* (Shanxi Medical University, Taiyuan, China)
Session 4 – Human Exposure to Aluminium

Poster 1*: In vivo neutron activation analysis of aluminium in bone: further refinements.

Hedi Mohseni (McMaster University, Hamilton, Canada)

Poster 2: Modelling absorption efficiency of elements via oral exposure in humans.

Yen Le (Radboud University, Nijmegen, The Netherlands)

Poster 3*: Antiperspirants, aluminium salts and relationship with breast cancer.

Caroline Linhart (Innsbruck Medical University, Austria)

Poster 4: Selective elevation of circulating CCL2/MCP1 levels in patients with longstanding post-vaccinal macrophagic myofasciitis and ASIA.

Romain Gherardi (University of Paris Est, Creteil, France)

Poster 5: Tyrosine as a depot adjuvant for use in allergy-specific immunotherapy.

Simon Hewings (Allergy Therapeutics, Worthing, UK)

Poster 6: Aluminium adjuvants potentiate the immune response via interaction with dendritic cells – but where does the aluminium go?

Matthew Mold (Keele University, United Kingdom)
Session 5 - Aluminium, Alzheimer's and Other Neurological Diseases

Poster 1*: Cognitive disorders and tau-protein expression among retired smelting workers exposed to aluminium.

Xiao-ting Lu (Shanxi Medical University, Taiyuan, China)

Poster 2*: Spectrometric methods to analyse and quantify the silicon content of mineral waters.

Krista Jones (Keele University, United Kingdom)

N.B: *Denotes a poster presented by a student.
Abstracts

Session 1 - Aluminium Chemistry

Platform 1

Natural Organic Matter Adsorption on a lateral AlOOH surface elucidated with Ab Initio Molecular Dynamics

Alessandro Motta, a Marie-Pierre. Gaigeot b and Dominique Costa c

a Dipartimento di Scienze Chimiche, Università di Catania and INSTM UdR Catania, Viale A. Doria, 6 - 95125 - Catania, Italy, Email: a.motta@dipchi.unict.it
b Laboratoire Analyse et Modélisation pour la Biologie et l’Environnement, LAMBE UMR CNRS 8587, Université Evry val d’Essonne, Blvd F. Mitterrand, Bat Maupertuis, 91025 Evry, France & Institut Universitaire de France IUF, 103 Blvd St Michel, 75005 Paris, France, Email: mgaigeot@univ-evry.fr
c Laboratoire de Physico-Chimie des Surfaces (UMR 7045), ENSCP Chimie-Paristech, 11 rue P. et M. Curie, 75005 Paris, France, Email: dominique-costa@chimie-paristech.fr

The investigation of metal oxides/water interfaces at the molecular level represents a fundamental issue for the understanding of geochemical, physical and biological processes. Recently Ab Initio Molecular Dynamics (AIMD) is being developed and is applied here to elucidate the (101) boehmite AlOOH/water interface and the adsorption of glycine at this interface. The interest of this system lies in the strong occurrence of Al polymorph in natural soils, of the importance of natural organic matter (NOM) adsorption at the interface with water and of the key role of lateral crystalline facets in NOM adsorption, all taken into account in our study. The (101) boehmite surface at the interface with water, glycine in “bulk” water, glycine in “outer-sphere” and “inner sphere” configurations were considered.

Boehmite (101) is a naturally stepped surface, covered with mono-coordinated (μ1) OH groups placed at the step edge and di-coordinated (μ2) OH groups placed along the terraces.

Interfacial water is somehow frozen in specific orientations, H-bond donor or H-bond receptor with respect to the surface. The effect of surface on the water organisation is lost at 6 Å from the surface, where liquid bulk is fully recovered. Proton transfers are observed at the interface between μ1 and μ2 species involving a Grotthus mechanism based proton transfers between distant μ1/μ2 groups. A bridge of interfacial water molecules assists this Grotthus proton transfer.
This preliminary analysis of the OH surface network appeared as essential to foresee the most stable configurations for adsorption of glycine, which was not straightforward.

Several configurations for glycine adsorbed in the inner sphere mode were built, in which glycine substitutes either one surface OH or one HOH group. We found that most stable conformer of glycine was glycinate (respectively zwitterion) when substituting an OH group (respectively HOH group), thus allowing the conservation of the surface charge.

Glycine inner-sphere adsorption was shown to be the most favorable adsorption mode, with either a glycinate located at the terrace (most favourable, \(<\Delta E^{K-S} > = -161.3 \text{ kJ/mol}\)) or a zwitterion located at the step (second most favourable, \(<\Delta E^{K-S} > = -113.6 \text{ kJ/mol}\)). An outer-sphere adsorption of a zwitterionic glycine at the boehmite interface is found less favourable in energy \(<\Delta E^{K-S} > = -20.5 \text{ kJ/mol}\). Glycine adsorption was also found to passivate the surface as it hinders the previously evidenced Grotthus based proton transfer at the step.

Adsorption of Inositol Phosphate on Gibbsite ($\gamma$-Al(OH)3)

Asma Mohammed\textsuperscript{a}, Anna-Carin Larsson\textsuperscript{a}, Maika Ruyter-Hooley\textsuperscript{b}, Bruce B. Johnson\textsuperscript{b}, Michael J. Angove\textsuperscript{b} & Oleg N. Antzutkin\textsuperscript{ac}

\textsuperscript{a}Chemistry of Interfaces, Luleå University of Technology, Luleå, SE-97187, Sweden
\textsuperscript{b}Colloid and Environmental Chemistry Laboratory, La Trobe University, Bendigo, Victoria, 3552 Australia
\textsuperscript{c}Department of Physics, Warwick University, CN4 7AL, U.K.

Phytic acid (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) and its ions, which are collectively denoted as IP6, has been recognized as the predominant form of organic phosphorous (P) form in soil and animal manure. In addition, more than 90\% of the P in seeds exists as inositol phosphate. IP6 is synthesized in terrestrial ecosystems by plants largely as the myo isomer with this isomer often comprising 100\% of the total inositol phosphates in nutritionally important legumes, cereals and oil seeds.

IP6 is readily complexed by soil minerals resulting in its accumulation over time. Since it is prevalent in soils, both its availability to plants and its potential for leaching into water bodies are of particular interest. IP6 is present in aquatic environments where it contributes to the growth of toxin-producing cyanobacteria, which currently pose the most pervasive threat to global water quality. Although inositol phosphates are a very important component of the global P cycle because of their relative abundance in soils and sediments, their origin, bioavailability and mobility in the environment are poorly understood. A fundamental factor determining the bioavailability and mobility is the mode of sorption of IP6 within soils and sediments. However, the effect of pH and IP6 concentration on sorption by soil clays, homogenous clays, aluminosilicates and ferric hydroxides has been the subject of only scarce investigations.

In this work we investigate the sorption of myo-IP6 onto gibbsite ($\gamma$-Al(OH)3). Surface speciation of IP6 on gibbsite was studied by solid state 31P MAS solid state NMR. For assignment of surface species, aluminium-IP6 complexes were synthesized and studied by FT-IR and multinuclear (31P, 13C, 27Al) MAS NMR spectroscopy. These spectroscopic data were correlated with adsorption macroscopic measurements at different pH and IP6 concentrations.
Interaction between Al\(^{3+}\) and 2, 3-dihydroxyterephthalic acid as a model compound for Inogashira fulvic acid

Takushi Yokoyama\(^a\), Mayumi Etou\(^a\) and Shuqin Bai\(^b\)

\(^a\) Department of Chemistry, Faculty of Science, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan. Email: yokoyamatakushi@chem.kyushu-univ.jp

\(^b\) College of Environment and resources, Inner Mongolia University

To evaluate the toxicity of Al\(^{3+}\) in hydrosphere, the interaction between Al\(^{3+}\) and fulvic and humic acids which are abundant natural organic compounds should be investigated. In this investigation, in order to elucidate the interaction between Al\(^{3+}\) and Inogashira fulvic acid (IFA), we synthesized a 2, 3-dihydroxyterephtharic acid (DHTPA) as a model compound of functional group of IFA. As shown in Fig. 1, the DHTPA has two COOH groups and two phenolic OH groups in the molecule. Using a calibration method for \(^{27}\)Al NMR spectra, the interactions between Al\(^{3+}\) and IFA were investigated at pH 3 to avoid the hydrolysis of Al\(^{3+}\). The DHTPA predominantly forms a 2:1 Al-DHTPA complex whose structure is similar to Al-salicylic chelate complex. In addition, the conditional binding constants of the Al-DHTPA complex (\(K_D\)) and the Al-IFA complex (\(K_I\)) were determined. The \(K_D\) value (2.5\(\times\)10\(^{-3}\) (dm\(^3\)/mol)) was quite similar to the \(K_I\) value ((1.14~1.62)\(\times\)10\(^{-3}\) (dm\(^3\)/mol)), suggesting that DHTPA is a suitable model compound for functional group of IFA.

![Figure 1: Structure of 2, 3-dihydroxyterephthalic acid (DHTPA).](image)
Interaction between aluminium ion (Al\(^{3+}\)) and simple dicarboxylic acid at pH 3

Mayumi Etou* and Takushi Yokoyama

Department of Chemistry, Faculty of Science, Kyushu University, 6-10-1, Hakuzaki, Higashi-ku, Fukuoka, 812-8581, Japan. Email: mayumie@mole.rc.kyushu-u.ac.jp

Due to the consideration of Al detoxification by simple carboxylic acid, the interaction between Al\(^{3+}\) and three dicarboxylic acids (oxalic acid (OX), malonic acid (MA) and succinic acid (SU)) at pH 3 was investigated by \(^{13}\)C and \(^{27}\)Al NMR techniques under various ligand/Al molar ratios. In this research, we focused on the chemical state of ligand in Al-complexes which is also important as well as that of Al atom. In addition, from the deconvolution of \(^{27}\)Al NMR spectrum and quantitative \(^{13}\)C NMR spectrum of each sample solution, the structure of each complex was defined. From these analyses, in OX system, it was suggested that the peak at 16 ppm in \(^{27}\)Al NMR spectrum is originated from two complex species (Al(OX)\(_3^{-}\) and Al(OX)\(_2^{-}\)) and the ratio of each complex depends on the OX/Al molar ratio. In MA system, it was also suggested that the three complexes (Al(MA)\(^2^{-}\), Al(MA)\(_3^{-}\) and Al(MA)\(^7+\)) are contained in the peak at 2 ppm in \(^{27}\)Al NMR spectrum.

Detecting Toxicant Interactions in Mixtures of Toxicants. Special Consideration of Ionic Toxicants

Thomas B. Kinraide, Yimin Wang, T. T. Yen Le, Peng Wang, Peter M. Kopittke

Toxicants, whether natural constituents (e.g., soil Al\(^{3+}\)), pollutants, or potentially intoxicating therapeutic drugs, often occur in mixtures. The total toxicity of toxicant mixtures can often be described closely by one or more assumed models of combined effects. Within each model, toxicants may or may not appear to interact so that one toxicant may appear to enhance the toxicity of another (synergism) or reduce the toxicity of another (antagonism). The occurrence of toxicant interaction often cannot be demonstrated unambiguously because the appearance of interaction depends upon the assumed model of combined effects. The problem is exacerbated in the case of ionic toxicants whose apparent interaction depends also upon the effect of each toxicant upon membrane surface electrical potential (\(\psi_0\)). Small systematic errors in the computation of toxicant-induced \(\psi_0\) can influence the appearance of interaction. Nevertheless, we present criteria by which interaction among ionic toxicants may be demonstrated in some cases.
Computer-aided speciation of Small Aluminium Silicate Clusters in Aqueous Environment

Giorgio Lanzani a, Tiina Leiviskä a,b, Satu Huhtakangas a, Carole A. Morrison c, Ari P. Seitsonen d, Marcella Iannuzzi d, Jürg Hutter d, Kari Laasonen e, Jaakko Rämö a and Simo O. Pehkonen f

a Thule Institute, University of Oulu, Oulu, Finland,
b Department of Process and Environmental Engineering, University of Oulu, Oulu, Finland,
c School of Chemistry, The University of Edinburgh, Joseph Black Building, Edinburgh, Scotland, United Kingdom,
d Institute of Physical Chemistry, University of Zurich, Zurich, Switzerland,
e Department of Chemistry, Aalto University, Espoo, Finland,
f Chem. Eng. Program, Masdar Institute of Science and Technology, Masdar City, Abu Dhabi, UAE.

The chemistry of aluminium silicates in water is quite unknown, although it is at the basis of many water treatment and environmental processes. There are still several uncharted factors concerning the structure and chemical composition of these species that need to be clarified, in order to develop novel and energy efficient chemicals for coagulation and adsorption purposes. The relative stability of small aluminium silicate clusters has been studied with computational techniques as constrained molecular dynamic and metadynamic simulations to obtain in-signs useful to the better speciation of the compounds originated from these species in aqueous environments. Zeta potential measurements, ICP-OES and FE-SEM analysis have been combined with the computational techniques above mentioned to confirm the finding obtained from the structural analysis. Overall, it was possible to conclude that additions of silicon to the system affect drastically the progress of hydrolysis and polymerisation of aluminium based complexes.
Aluminium-silicon interactions in mild pH and ionic strength conditions

Tiina Leiviskä a,b, Jaakko Rämö a, Giorgio Lanzani a, Satu Huhtakangas a, Kari Laasonen c, and Simo O. Pehkonen d

a Thule Institute, University of Oulu, Oulu, Finland,
b Department of Process and Environmental Engineering, University of Oulu, Oulu, Finland,
c Department of Chemistry, Aalto University, Espoo, Finland,
d Chem. Eng. Program, Masdar Institute of Science and Technology, Masdar City, Abu Dhabi, UAE.

The reaction of aluminium (1 mM) and silicon (0.1−2 mM) in water was studied at pH 6.2 and room temperature. A low ionic strength medium (conductivity ~0.4 mS/cm) was selected to be representative of natural water environment and domestic water conditions. For comparison, some experiments were carried also at higher ionic strength medium (0.1 M NaCl, 11-12 mS/cm). The solutions and precipitates were studied by ICP-OES, zeta potential, flow cytometry and FE-SEM. According to total Al and Si analyzes with ICP-OES Al precipitated totally when the silicon concentration was 0.5 mM or higher. The zeta potential decreased as the silicon concentration in solution increased. Particles formed with Al and Si could be detected with flow cytometry (0.2 µm detection limit) in both absence and presence of fluorescent Nile Red probe. The FE-SEM analyzes showed clearly the changes in Al:Si ratio of precipitates with different initial Al:Si ratio in solution.
Computer simulations of the structure, formation and growth of Hydroxyaluminosilicates, at the level of individual and collections of molecules

James Beardmore\textsuperscript{a}, Chris Exley\textsuperscript{a}, Xabier Lopez\textsuperscript{b}, Tiina Leivisk\textsuperscript{c}

\textsuperscript{a} Birchall Centre, Lennard Jones Laboratories, Keele University, Staffs England
\textsuperscript{b} Theoretical Chemistry, Chemical Faculty, University of the Basque Country, Donostia-San Sebastian, Spain
\textsuperscript{c} Chemical Process Engineering Laboratory, University of Oulu, Oulu, Finland

Hydroxyaluminosilicates (HAS) form by the condensation of silicic acid on an aluminium hydroxide template. Two discrete forms are observed, dependent upon the proportions of aluminium and silicic acid present in solution: HAS-A, with a Si:Al ratio of 0.5, and HAS-B, with a Si:Al ratio of 1.0. HAS-B requires a Si:Al ratio of 2.0 to form completely, yet HAS-A is understood to be a prerequisite particle for its formation [1]. Understanding the formation and growth of HAS species has implications on areas such as treatment of municipal water, and aluminium toxicity in natural waters such as lakes or forest soils. A new computational model is presented, which takes a Markov-Chain Monte Carlo approach [2] to simulate the interactions between possible aluminium hydroxide, HAS-precursor and HAS particles, in order to investigate the stages and species involved in the formation and growth of HAS. The model simulates chemical reactions at the level of collections of discrete particles [3], and is populated with particles and informed of permissible interactions by considering both quantum chemical calculations performed elsewhere, and information from the literature. The model is a modification of earlier work [4], and is described and some initial results presented.

[1] Exley C. (2012), Coordination Chemistry Reviews 256, 82-88
Can Al (III) promote Fenton Reaction and Oxidative Stress?

X. Lopez a, F. Ruipérez a, J. I. Mujika a, J. M. Ugalde a and C. Exley b

a Kimika Fakultatea, Euskal Herriku Unibertsitatea (UPV/EHU) and Dondostia International Physics Center (DIPC), P. K. 1072, 20080 Donostia, Euskadi (Spain).
b Birchall Centre for Inorganic Chemistry and Materials Science, Keele University, Staffordshire, UK

The possibility for an Al-superoxide complex to reduce Fe (III) to Fe (II), promoting oxidative damage through Fenton reaction, is investigated using highly accurate ab initio methods and Density Functional Theory in conjunction with solvation continuum methods to simulate bulk solvent effects. It is found that the redox reaction between Al-superoxide and Fe (III) to produce Fe (II) is exothermic. Moreover, the loss of an electron from the superoxide radical ion in the Al-superoxide complex leads to a spontaneous dissociation of molecular oxygen from aluminium, recovering therefore an Al$^{3+}$ hexahydrated complex. As demonstrated in previous studies, this complex is again prone to stabilize another superoxide molecule, suggesting a catalytic cycle that augments the concentration of Fe (II) in the presence of Al (III). Similar results are found for Al(OH)$^{2+}$ and Al(OH)$^{2+}$ hydrolytic species. Our work reinforces the idea that the presence of aluminium in biological systems could lead to an important pro-oxidant activity through a superoxide formation mechanism.
Unveiling the coordination of aluminium to amyloid-β peptide with computational chemistry

Jon I. Mujika, Xabier Lopez, Jesus M. Ugalde

Theoretical Chemistry, Chemical Faculty, University of the Basque Country, Donostia-San Sebastian, Spain

The implication of aluminium in Alzheimer’s disease (AD) has been investigated for more than twenty years, but there is not yet a conclusive answer to the question of how aluminium is directly involved in the disease. One of the hallmarks of AD is the aggregation of Amyloid-β (Aβ) peptides to form soluble oligomers first, and ultimately insoluble aggregates. Several in vivo studies found the presence of Al(III) in this aggregates, and in vitro experiments confirm that Al(III) accelerates the growth rate of them.

Most of the investigations centered on aluminium focus on the biophysics of the A aggregates formed with the metal, providing relevant information about their growing pattern. However, unlike with copper or zinc, little is known about the coordination mode of aluminium to the Aβ peptide. In this sense, quantum chemistry is a suitable tool to get access into the intrinsic coordination mode of Al(III) to Aβ peptides, as it allows characterizing the systems into an atomic level.

The presentation will be divided into three main parts. In the first one we will introduce the previous studies concerning Al(III) and the Aβ aggregates. Then, we will explain briefly the capabilities of quantum chemistry and how can contribute to a better understanding of the problem. Finally, we will present some preliminary results in which we have characterized multiple coordination modes of the metal to the Aβ peptides. With all these results, we will aim to shed some light on how Al(III) interacts with the Aβ peptides.
Hydroxypyrones, a fascinating family of chelating agents for Al$^{III}$.

Leonardo Toso$^a$, Valeria M. Nurchi$^a$, Guido Crisponi$^a$, Miriam Crespo-Alonso$^a$, Joanna I. Lachowicz$^a$, Sergio M. Marques$^b$, Maria Amelia Santos$^b$, Juan Niclós-Gutiérrez$^c$, Alicia Domínguez-Martín$^c$, Josefa M. González-Pérez$^c$, Zbigniew Szewczuk$^d$

$^a$ Dipartimento di Scienze Chimiche e Geologiche, Cittadella Universitaria, I-09042 Monserrato Cagliari (Italy)
$^b$ Centro Quimica Estrutural, Instituto Superior Tecnico-UTL, Av. Rovisco Pais, 1049-001 Lisboa (Portugal)
$^c$ Department of Inorganic Chemistry, Faculty of Pharmacy, University of Granada, E-18071 Granada (Spain)
$^d$ Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383, Wroclaw, Poland

The use of chelating agents for iron and aluminium has found increasing attention in the last thirty years [1-2]. In order to design new coordinating molecules we presented the equilibria of kojic acid and some of its derivatives [3-4]. Kojic acid, a natural product obtained from certain species of moulds, presents good chelating properties for hard metals such as Fe$^{III}$ and Al$^{III}$ as well as its dimer [3]. Evidence was given of the formation of ML, ML$_2$, and ML$_3$ complexes of both metal ions with kojic acid, confirmed by the X-ray structure of a FeL$_3$ complex. In addition, various protonated M$_2$L$_2$ and ML$_2$ complexes of the former dimer have been observed. On the basis of pFe value (23.1), and of its ability to scavenge iron from inside cells, we extended the investigation to related compounds 2 and 3. A huge advantage of these molecules is that they are easy and cheap to produce (the starting materials, kojic acid and vanillin, are not expensive).
In $\text{M}_2\text{L}_2$ complexes formed with ligands 1-3, each metal ion is coordinated by two CO-C(OH)-chelating moieties, one from each coordinating molecule. Actually, the length of the linker between the two kojic units prevents the coordination of both kojic units to the same metal. Therefore, aimed at obtaining more efficient kojic acid derivatives, we have designed and synthesized a new set of bis-kojic ligands: 4, 5, and 6, whose linkers are differentiated both in terms of type and length. The compound 7 was also synthesized, useful in determining the acid properties of the nitrogen atom in the linker. The complex formation equilibria with $\text{Al}^{\text{III}}$ studied by potentiometry, UV-Vis spectrophotometry, $^1\text{H}$ NMR spectroscopy and ESI-MS will be described and discussed.

Complex formation equilibria of substituted phenols with Al\textsuperscript{III} and Fe\textsuperscript{III}.

Leonardo Toso, Valeria M. Nurchi, Guido Crisponi, Miriam Crespo-Alonso, Joanna I. Lachowicz

Dipartimento di Scienze Chimiche e Geologiche, Cittadella Universitaria, I-09042 Monserrato Cagliari (Italy)

The stability of complexes formed between iron and aluminium with different chelating agents characterized by oxygen based coordinating groups depends on a variety of factors, among which the kind of coordinating groups and the effect of substituents [1-3]. These factors can play a fundamental role in determining the protonation and the complex formation constants, and therefore the strength of the chelators. In order to give evidence of these effects on simple molecules we present here the protonation, and the complex formation equilibria of Al\textsuperscript{III} and Fe\textsuperscript{III} with three easily available ligands, o-vanillin, 3-metoxysalycilic acid and 3-nitrosalycilic acid [3].

The complex formation equilibria with Al\textsuperscript{III} and Fe\textsuperscript{III}, studied by potentiometric techniques, UV-Vis spectrophotometry and \textsuperscript{1}H NMR spectroscopy, will be presented, and the differences in the chelating properties of the three ligands will be discussed on the basis of the pFe and pAl values.

Complexation of Al(III) with hydroxypyridine(di)carboxylic acids, as a new possible chelating agents in neurodegenerative disorders

Éva Sija\textsuperscript{a}, Valerio B. Di Marco\textsuperscript{b}, Tamás Jakusch\textsuperscript{c}, Annalisa Dean\textsuperscript{b}, Alfonso Venzo\textsuperscript{d}, Tamás Kiss\textsuperscript{a,c}

\textsuperscript{a}Bioinorganic Chemistry Research Group of the Hungarian Academy of Sciences, Dóm tér 7. H-6720 Szeged, Hungary
\textsuperscript{b}Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131 Padova, Italy, e-mail: valerio.dimarco@unipd.it
\textsuperscript{c}Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7. H-6720 Szeged, Hungary, e-mail: tkiss@chem.u-szeged.hu
\textsuperscript{d}CNR, Institute of Sciences and Molecular Technologies, via Marzolo 1, 35131 Padova, Italy

Although aluminium is the most abundant element in the earth’s crust, mostly found in the form of stable chemical compound therefore nearly unavailable for the living organism and has not become a biological by essential element. Nowadays through the human activity our body are exposed several soluable aluminium salt. The aluminium can be transported by transferrin to several places in the organism where can accumulate and cause biological dysfunctions. In many cases the neurodegenerative disorders like Alzheimer’s disease (AD) is associated with aluminium toxicity. Although its role is not clear several study reported aluminium deposits in the AD brain. The chelation therapy has been shown effective to mobilize the aluminium from the deposit. The two well established chelators (desferal and deferiprone) have several drawbacks. We proposed some hydroxypyridine(di)carboxylic acids as a potential chelating agents for Al. In the present poster we show the complexation properties of these ligands with Al(III).
Molecular Mechanisms of Crop Adaptation to Acid Soils

Leon Kochian\textsuperscript{a}, Jiping Liu\textsuperscript{a}, Jurandir Magalhaes\textsuperscript{b}, Miguel Piñeros\textsuperscript{a}, Lyza Maron\textsuperscript{a}, Jon Shaff\textsuperscript{a}, and Claudia Guimaraes\textsuperscript{b}

\textsuperscript{a}Robert W. Holley Center for Agriculture and Health, USDA-ARS, Cornell University, Ithaca, NY 14853. E-mail: lvk1@cornell.edu.
\textsuperscript{b}Embrapa Maize and Sorghum, Sete Lagoas, Brazil.

Plants have evolved different mechanisms for dealing with toxic metals in the environment, and these can involve avoidance (exclusion from a plant tissue/organ) or true tolerance (sequestration in an internal compartment). The best characterized metal resistance mechanisms involve crop adaptation to aluminium (Al) toxicity, which is a worldwide problem that arises in acid soils (pH <5); in these soils toxic forms of Al are solubilized into the soil solution, damaging roots and resulting in reduced water and nutrient uptake.

The genetic variability for Al resistance within many crop species has been utilized by plant breeders to enhance Al resistance. But beyond this, genetic variability in Al resistance has been an excellent experimental resource that is being mined by researchers to elucidate the molecular basis for this trait. Because of the agronomic importance of Al toxicity, research on the identification of crop Al resistance genes has attracted significant interest from a number of laboratories around the world.

This talk will focus on a well-documented Al resistance mechanism based on Al activation of specialized transporters localized to the root tip that release organic acids into the rhizosphere, where they chelate and prevent Al from entering the root. To date, many of the Al resistance genes in crop plant species that have been identified are from two different families of membrane transporters mediating this root organic acid efflux. For both types of transporters, Al-inducible regulation of transporter gene expression plays an important role in differential Al resistance. We also are finding that regulation of the functioning of these organic acid transporters also plays an important role in differential resistance. In this presentation I will focus on the regulation of expression of the major sorghum Al tolerance gene \textit{SbMATE}, as well as the role of protein-protein interactions in the regulation of citrate transport mediated by the SbMATE protein.

The identification of genes conferring Al resistance now provides the necessary molecular tools to more effectively address a worldwide agronomic problem that is only exceeded by drought stress with regards to abiotic limitations to crop production.
Cellular distribution of Aluminium and other elements in leaves of native plant species of Brazilian Cerrados

Leide Rovênia Miranda de Andrade\textsuperscript{a}, Françoise Watteau\textsuperscript{b}, Leila Maria Gomes Barros\textsuperscript{c}, Guillaume Echevarria\textsuperscript{b}

\textsuperscript{a}Embrapa Cerrados, BR 020, Km 18, PO Box. 08223, ZC 73301-970, Planaltina, DF, Brazil.
\textsuperscript{b}Laboratoire Sols et Environnement, INRA, Nancy-Université, BP172, 54505 Vandœuvre-lès-Nancy, France
\textsuperscript{c}Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica W5 Norte, Brasília, DF, Brasília, DF, ZC 70770-900, Brazil

The Brazilian native species \textit{Qualea grandiflora} (Qg) and \textit{Vochysia pyramidalis} (Vp) present levels above 0.1 kg% Al dry leaves, contrasting with \textit{Sclerolobium paniculatum} (Sp), a tolerant but Al excluder species. We used a TEM microscopy coupled to an EDX detector in order to locate and semi-quantify Al and other elements in cellular compartments of leaves of those species collected from mature trees and from seedlings grown in nutrient solution. The leaves had different patterns of Al distribution in cells. In Qg, Al was mainly located in the chloroplasts, while in Vp, it was in cuticle and epidermal cell walls. Aluminium was not detected in any tissue compartments of Sp. Concentrations of S and Cl in the chloroplasts of both Al accumulators suggests that they may be important counter ions for Al\textsuperscript{3+} in the chloroplasts. N in chloroplasts of Qg indicates that the mechanisms of N reduction were not impaired by the presence of Al.
Effect of Aluminum Ions on Hydrangea Sepals and Viburnum Drupes

Blaine B. R. Groat* and Henry D. Schreiber

Department of Chemistry, Virginia Military Institute, Lexington, VA 24450. Email: groatbb@mail.vmi.edu

Delphinidin-3-glucoside as its flavylinium cation is the source of the red colour in the sepals of many hydrangea inflorescences (blooms). Upon introduction of aluminum ions, this same anthocyanin pigment complexes with Al(III) in the sepals to generate a characteristic blue colour. Chemical studies of the interaction of Al(III) with delphinidin in model solvent systems, as well as of Al(III) with sepal extracts, have identified the core structure of this blue complex to be Al(III) anchoring a quinoidal base anionic form of delphinidin-3-glucoside with a flavylinium cationic form stacked on top. Further research has compared the interaction of Al(III) with sepal extracts as a function of bloom stage in order to understand the fading of many blue blooms of hydrangea back to red before their senescence. Furthermore, studies of Al(III) interactions with extracts from variously-coloured drupes (berries) of viburnum have determined the possibility of aluminum-induced colour changes in these natural systems.
Aluminium, Iron and Manganese; do they limit determination of anionic substances by means of IC?

Václav Tejnecký, Christopher Ash, Luboš Borůvka and Ondřej Drábek

Department of Soil Science and Soil Protection. Faculty of Agrobiology, Food and Natural Resources. Czech University of Life Sciences Prague, CZ – 165 21 Prague 6, Czech Republic. E-mail address: drabek@af.czu.cz.

Low Molecular Mass Organic Acids (LMMOA) are small in content but essential for chemical processes that affect the entire soil environment. Knowing their amount and speciation is required for realistic equilibrium modelling of soil chemical processes and transport mechanisms. There have been a number of proposed methods for the analysis of LMMOA. Ion exchange chromatography (IC) with hydroxide elution has proven to be a useful tool for the determination of LMMOA in many different water-based samples. However, the influence of multivalent cations (often present in environmental samples) on IC results has not yet been sufficiently studied. In order to assess the influence of Al, Fe and Mn on the amount of LMMOA determined by IC, an extensive set of model solutions were prepared and immediately analysed by means of IC. These experimental results were compared to expected values and also to results provided by the chemical equilibrium model - PHREEQC.
Amelioration of Iron Toxicity, a Mechanism for Aluminium-induced Growth Stimulation in Tea Plants

Roghieh Hajiboland b, Roser Tolrà a, Juan Barceló a and Charlotte Poschenrieder a

a Lab. Fisiología Vegetal, Facultad Biociencias Universidad Autónoma de Barcelona, E-08913 Bellaterra Spain
b Plant Science Department, University of Tabriz, 51666 Tabriz, Iran

Tea plants are well adapted to acid mineral soils. Not only they tolerate the hyper-accumulation of large shoot Al concentrations, but even show growth stimulation by Al. There is no experimental evidence for an essential role of Al in any organism and the mechanisms of this Al-induced growth stimulation are poorly investigated. Beneficial effects, due to amelioration of some latent stress, are main reason for growth stimulation by non-essential trace elements. Here we provide results from mineral content analysis and haematoxylin and morin staining that support the view that Al can stimulate growth of tea plants due to alleviation of latent iron toxicity.

Oral Poster 10

Long-term changes in aluminium concentrations and fluxes in Czech streams recovering from acidification

Pavel Kram, Oldrich Myska, Jakub Hruska, Vladimir Majer, Anna Lamacova & Filip Oulehle,

Czech Geological Survey, Klarov 3, 11821 Prague 1, Czech Republic

Hydrochemical changes in the Slavkov Forest (SF) were assessed on two spatial scales. Synoptic stream surveys based on 148 sampling points within 820 km² area were performed in 1991, 2001 and 2009. Aluminium (Al) fractions were determined in soil waters (10-90 cm) and stream waters in three small catchments covered by Norway spruce plantations in 1990-2012. The catchments represent contrasting geochemical environments (acidic granite catchment LYS, neutral amphibolite catchment NAZ, alkaline serpentinite catchment PLB). Inorganic monomeric Al (Ali) was the dominant fraction at LYS (mainly as Al-F complexes and Al³⁺), however particulate Al (Alp) was dominant at PLB and NAZ and in most SF sampling points. Long-term declines of Ali and total Al were attributed to the acidification recovery, with the most pronounced change observed in drainage waters at LYS. However, total Al and Alp have increased at some high pH sites, like PLB.
Effect of salicylic acid on the attenuation of aluminium toxicity in cell suspensions of *Coffea arabica* L.

S.M.Teresa Hernández-Sotomayor, Abril Chan-May, Yahaira Cab-Guillén and J. Armando Muñoz-Sanchez

Unidad de Bioquímica y Biología Molecular de Plantas. Centro de Investigación Científica de Yucatán, A.C. Calle 43 No. 130. Chuburná de Hidalgo. C.P. 97200. Mérida, Yucatán, México. E-mail: ths@cicy.mx.

Aluminium (Al) is the most abundant metal on Earth’s crust (7% of all elements). The toxicity of this metal is widely documented in tropical acid mineral soils. It is the most limiting factor in the productivity of crop species.

Coffee is one of the most important crops worldwide, economically speaking. This crop grows on acid soils where the availability of Al$^{3+}$ is greater; therefore coffee yield is limited by the toxic effects of this element. We have developed a biological model in which suspension cells of *Coffea arabica* have been used. Salicylic acid (SA) is an important endogenous signaling molecule in plant defense, which may occur in response to various types of biotic and abiotic stress. Studies have shown that SA is a plant regulator of several physiological processes and is essential in the expression of some defense genes. In this study, we will present the results of the effect of SA on aluminum toxicity in cell growth, as well as the research of the possible targets in the signal transduction pathway linked to phosphoinositides and protein phosphorylation.

Research founded by CONACYT grant (98352) for SMTHS and two research assistant fellowships (SNI 4422 and CICY 217-CD2) for YCG.
The role of diacylglycerol in aluminium toxicity in plants

Přemysl Pejchar a, Daniela Kocourková a, Martín Potocký a, Radovan Fišer b, Kateřina Schwarzerová c, Jan Martinec a

a Institute of Experimental Botany, v. v. i., Academy of Sciences of the Czech Republic, Prague, Czech Republic
b Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, Prague, Czech Republic
c Department of Experimental Plant Biology, Faculty of Science, Charles University in Prague, Prague, Czech Republic

The very rapid inhibitory effect of Al exposure on root elongation suggests involvement of signalling processes. Here we focused on the earliest changes in the plasma membrane and phospholipid signalling. We pre-labelled plant cells with fluorescent derivative of phosphatidylcholine and assayed for pattern of fluorescently labelled products. In cells treated with AlCl₃, there was a significant decrease of labelled diacylglycerol (DAG). Spectrofluorometric measurements of the isolated plant plasma membrane showed that Al induced a reduction in membrane fluidity. Application of the membrane fluidizer, benzyl alcohol, restored partially membrane fluidity, root growth and increased the amount of labelled DAG during first 30 min of Al treatment. Furthermore, Al-inhibited growth was partially restored by addition of exogenous DAG. Currently, we are testing effect of DAG on Al-reduced membrane fluidity. The potential role of DAG in aluminium toxicity will be discussed.

This work was supported by CSF grant No. P501/12/P950.
Aluminium-induced cell death process involves both mitochondria and vacuole in plant cells

Yoko Yamamoto\textsuperscript{a}, Koki Kariya\textsuperscript{a}, Yoshiyuki Tsuchiya\textsuperscript{a}, Tijen Demiral\textsuperscript{b} and Takayuki Sasaki\textsuperscript{a}

\textsuperscript{a} Institute of Plant Science and Resources, Okayama University, Chuo 2-20-1, Kurashiki, Japan.
\textsuperscript{b} Department of Biology, Faculty of Science and Art, Harran University, Sanliurfa, Turkey

In plant system, aluminium (Al) toxicity is observed in acidic soils where Al ions attack actively growing cells at root apical meristem, leading to cell elongation inhibition and cell death, accompanying the production of reactive oxygen species (ROS). We have used actively growing cultured tobacco cell lines as a model system of meristematic cells. Metabolomic analyses of a pair of isogenic cell lines, SL (wild-type) and an Al-tolerant line, suggest that a mechanism to prevent ROS production observed in the Al-tolerant line could be a shift of energy metabolism from the oxidative phosphorylation accompanying ROS production in mitochondria to lactate fermentation in cytosol. Using another wild-type cell line, BY-2, the collapse of vacuole was observed, together with an enhancement of the activity of vacuolar processing enzyme (VPE) under Al stress. Taken together, in plant cells, both mitochondria and vacuole play key roles to execute cell death under Al stress.
Influence of Al$^{3+}$ on light-induced membrane potential changes of *Nitellopsis obtusa* cells

Vilma Ksnierienë, Jonas Burneika, Indrė Lapeikaitė, Olga Sevriukova, Vidmantas Sakalauskas

Department of Neurobiology and Biophysics, Vilnius University M.K.Ciurlionio 21/27, LT-03101, Vilnius, Lithuania. E-mail: vilma.kisnieriene@gf.vu.lt.

Some responses of plant cells to aluminium are related to the alteration of plasma membrane properties. The electrophysiologic response pattern could be used to evaluate the effect of aluminium on the activity of ion transport systems in plant membrane. Membrane potential of *Nitellopsis obtusa* is very sensitive to light/dark and dark/light transition. Typical reaction to dark/light transition consists of two phases – a rapid transient depolarization of the membrane potential followed by a slow membrane hyperpolarization. We investigated kinetics of light-induced membrane potential changes when *Nitellopsis obtusa* cells were incubated in the standard solution at pH4.2 and in the solution with Al$^{3+}$ at pH4.2 for 20 h. in the darkness. We investigated the effects of blue light and found that photoelectric reaction is suppressed by application of 1 mM Al$^{3+}$. Aluminium but not pH caused this effect. Impact of Al$^{3+}$ could be related to the inhibition of the H+-ATPase activity.
Physiological and oxidative stress responses of two *Avena Sativa* genotypes and *Cucumis Sativus* Seedlings to Aluminium in nutrient solution


*Federal University of Santa Maria, Departament of Chemistry, Graduate Program in Toxicology Biochemistry, Rio Grande do Sul, Santa Maria, Brazil*

Oat and cucumber seedlings were grown in nutrient solution (pH 4.0) with 0 and 20 mg/L aluminum. At 12, 24, 36 h after aluminum addition, root length, biochemical parameters: catalase, ascorbate peroxidase, lipid peroxidation were determined. Regardless of exposure time, root length of tolerant genotype did not decrease with any aluminum treatments. Root length of sensitive genotype and cucumber seedlings were decreased when compared with controls. Aluminum supply caused lipid peroxidation only in sensitive genotype and cucumber root (at 12, 24, 36 h). In sensitive genotype and cucumber seedlings catalase was activated only at 36 h, however, catalase activity was compensated by increased activity of ascorbate peroxidase at 24, 36 h. In tolerant genotype the enzymes were activated at 12, 24, 36 h. Data for root growth suggested lipid peroxidation in sensitive genotype of oat and cucumber root may be effect of aluminum toxicity and increased production of reactive oxygen species.

Financial Support: CNPq, CAPES and FAPERGS.
Differential Speed of Activation in Antioxidant System in Three Oat Genotypes


Federal University of Santa Maria, Departament of Chemistry, Graduate Program in Toxicology Biochemistry, Rio Grande do Sul, Santa Maria, Brazil

Oat seedlings were grown in nutrient solution (pH 4.0) with 0 and 20 mg/L Al. At 12, 24, 36 h after Al addition, root length and biochemical parameters: catalase, ascorbate peroxidase and superoxide dismutase activities, lipid peroxidation, ascorbic acid, non-protein thiol group were determined. Regardless exposure time, root length of tolerant genotype did not decrease with any Al treatments. Al supply caused lipid peroxidation and root length decreased only in roots of sensitive genotype (at 12, 24, 36 h). In sensitive genotype, the enzymes were activated only at 24 or 36 h. In tolerant and intermediate genotypes, the enzymes were activated at 12, 24, 36 h. Tolerant and intermediate genotypes, Al presence at 12 h provoked an increase in AsA levels. This increase may have contributed to ROS detoxification and suggests their participation in Al detoxification, since root growth inhibition and lipid peroxidation were not observed in these genotypes.
The bioleaching of aluminium from the solid aluminium oxide by the filamentous fungus

I. Pifková*, M. Urík, M. Bujdoš, M. Kolenčík, P. Matúš

Comenius University in Bratislava, Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Mlynská dolina 1, 842 15 Bratislava 4, Slovakia, e-mail: pifikova@fns.uniba.sk

Some microscopic filamentous fungi produce acidic metabolites which can leach aluminium in soil from its immobile phase to the soil solution. In this article, we evaluated bioleaching of the solid aluminium oxide (Al₂O₃) by the common soil fungus Aspergillus niger. We have also observed the effect of soil organic matter (in form of humic acids) coverage of Al₂O₃ surface on bioleaching of aluminium. The growth medium with aluminium oxides (0.25 g/L), whose surface was in some groups treated with humic acids (from 1.2 to 6.4 mg/g Al₂O₃), was inoculated with a spore suspension of A. niger and cultivated at 25 °C. During cultivation the pH values of media decreased significantly (to 2.8) affecting the change of aluminium content in biomass and growth medium. No new mineral phases emerging from the interactions of oxides with fungal metabolites were observed during cultivation in solid substrate. Our results suggest that the (non-enzymatic) metabolites of filamentous fungus A. niger significantly promote the release of aluminium from the solid phase to the surrounding cultivation medium (32.6 mg/L at pH 3.4), while the activity of H⁺ ions solely in the solution affects the bioleaching efficiency insignificantly. Humic acids adsorbed to the surface of the solid Al₂O₃ reduced the amount of aluminium released into the solution (from 42.56±0.19 to 26.38±3.03 mg/L), while the content of aluminium in the biomass increased (from 0.84±0.01 to 4.79±2.89 mg/g). This suggests indirect influence of humic acids on membrane permeability of A. niger for aluminium ions.

Acknowledgements: The work was supported by Science and Technology Assistance Agency under the contracts No. APVT-20-010204 and LPP-0038-06 and by Scientific Grant Agency of Ministry of Education of Slovak Republic and the Slovak Academy of Sciences under the contract No. VEGA 1/0860/11 and No. 1/0778/11.
Labile Al content or BC/Al ratio in soil: what controls tree seedlings growth?

Luboš Borůvka, Marek Batysta, Václav Tejnecký, Ondřej Drábek, Antonín Nikodem, Josef Kratina

Department of Soil Science and Soil Protection. Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, CZ – 165 21 Prague 6, Czech Republic. E-mail address: boruvka@af.czu.cz

Though the fact that Al cations in soil solution manifest toxic effects on plants is generally accepted, there is no consensus whether the high Al concentration is always dangerous, or whether the balance between base cations (BC) and Al in soil solution described by molar BC/Al ratio is more important. The aim of this study was to analyze the relationship between tree (Picea abies L. and Fagus sylvatica L.) seedlings growth and the content of Al and base cations (calcium and magnesium) in soil solution. A two-years pot experiment with different intensity and chemical composition of irrigation waters was carried out.

Increased levels of Al addition caused limited growth of the seedlings. However, addition of base cations reduced the negative effect of Al. High concentrations of Al in tissues seem not to be toxic in case of sufficient base cations supply.
The influence of altitude on Al speciation in samples originating from beach and spruce forests

Luboš Borůvka\textsuperscript{a}, Vít Šrámek\textsuperscript{b}, Václav Tejnecký\textsuperscript{a}, Věra Fadrhonsová\textsuperscript{b}, Antonín Nikodem\textsuperscript{a}, Ondřej Šebek\textsuperscript{c}, Karel Němeček\textsuperscript{a}, Ondřej Drábek\textsuperscript{a}

\textsuperscript{a}Department of Soil Science and Soil Protection. Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, CZ-165 21 Prague 6, Czech Republic. E-mail address: boruvka@af.czu.cz (L. Borůvka)
\textsuperscript{b}Forestry and Game Management Research Institute, Strnady 136, CZ-156 04 Prague 5, Czech Republic.
\textsuperscript{c}Laboratories of the Geological Institutes, Faculty of Science, Charles University in Prague, Albertov 6, CZ-128 43 Prague 2, Czech Republic

Effects of potentially toxic Al forms in strongly acid forest soils are generally known. A number of studies described the difference in soil Al speciation under European beech (\textit{Fagus sylvatica} L.) and Norway spruce (\textit{Picea abies} L.) forests. In most cases, beech forest came out as more favourable vegetation with lower toxic Al forms proportion in soil compared to spruce forest. This contribution is focused on the effect of altitude on this difference. Two sample sets were used: samples collected in different localities of the whole Czech Republic, and an individual elevation transect in the Jizera Mts. It was found that the positive effect of beech with respect to soil Al is generally limited to the altitude range best suitable for this tree species. At higher altitudes (around 1000 m) the advantage of beech vegetation over the spruce one becomes less pronounced, as spruce trees are better suited to the higher altitudes.
Salicylic acid is involved in the mechanism of response to aluminium toxicity in cell suspensions of *Capsicum chinense* Jacq.

**Armando Muñoz-Sanchez, S.M. Teresa Hernández-Sotomayor, Yahaira Cab-Guillén**

*Unidad de Bioquímica y Biología Molecular de Plantas. Centro de Investigación Científica de Yucatán, A.C. Calle 43 No. 130. Chuburná de Hidalgo. C.P. 97200. Mérida, Yucatán, México. E-mail: arms@cicy.mx.*

Aluminium (Al) toxicity is widely documented in tropical acid mineral soils. It is the most limiting factor in the productivity of crop species. As well as the third most abundant element on Earth’s crust (7%). In Yucatecan Peninsula located in Mexico, chile is one of the most important crops, economically speaking. This crop grows in soils with a characteristic alkaline condition where the availability of Al^{3+} should be difficult; therefore chile crop can be considered a plant not susceptible to the toxic effects of this element. Taking advantage of this factor we use *Capsicum chinense* Jacq. in order to better understand the mechanism of tolerance and response to Al toxicity. We have developed a biological model in which suspension cells of *C. chinense* Jacq. have been used. Salicylic acid (SA) is recognized as an important endogenous signaling molecule in plant defense, which may occur in response to various types of biotic and abiotic stress. Many studies have shown SA as a plant regulator of several physiological processes. However, it is not very clear how the participation of SA acts as a response mechanism response for aluminum toxicity.

In this study, using cell suspension of *C. chinense* Jacq. a system not susceptible to Al, we will present the results of the effect of SA on aluminum response in cell growth, and the subcellular location of Al, as well as the research of the modifications in SA endogenous levels and the SA effects on cell structure under aluminum toxicity conditions.

Research founded by CONACYT grant (98352) for SMTHS and two researches assistant fellowships (SNI 4422 and CICY 217-CD2) for YCG.
The combined effect of Aluminium and tritium on the plant cell membrane bioelectrical parameters

Olga Sevriukova*, Vidmantas Sakalauskas, Indrė Lapeikaite, Jonas Burneika, Kristina Panaciova, Vilma Ksnieriene

Department of Neurobiology and Biophysics, Vilnius University M.K.Ciurlionio 21/27, LT-03101, Vilnius, Lithuania. E-mail: olga.sevriukova@gmail.com

Aluminium (Al) toxicity is now undeniable but its interaction with other contaminants and effect of their different combinations with Al that naturally occurs remains largely unexamined. While tritium (3H) is considered as a sensitizer of the effect of other pollutants especially through the antioxidant system, we supposed that 3H may influence the impact of Al on the plant cell. Bioelectrical characteristics of the plant cell membrane are one of the most sensitive indicators of rapid cell response due to environmental stress. Using unique Characaen cells we investigated the effect of single Al and Al combined with 3H on the mechanisms participating in generation of action potential. Obtained results showed that Al affects the bioelectrical properties during excitation of the membrane and 3H can modify the effect of Al in different ways: it can increase the effect of Al, inhibit it or even evoke the effect of an opposite sign.
Physiological and Oxidative Stress Responses to Aluminium of Three Oat Genotypes Grown Hydroponically


Federal University of Santa Maria, Departament of Chemistry, Graduate Program in Toxicology Biochemistry, Rio Grande do Sul, Santa Maria, Brazil

Aluminium toxicity is serious problem in Brazilian soils. Selecting oat genotypes is important strategy to produce this crop on these soil types. Aluminium-sensitive, intermediate and tolerant oat genotype seedlings were grown in nutrient solution (pH 4.0) with 0, 5, 10, 20, 30 mg/L Al. After 10 days, Al concentration in both root/shoot systems of all genotypes increased with increasing Al levels. Sensitive roots have highest values of aluminium accumulated- their growth was the most affected. This study showed that sensitive seedlings presented higher catalase, ascorbate peroxidase, and superoxide dismutase activities. However, their antioxidant system was unable to overcome toxicity resulting in negative effects such as lipid peroxidation, H$_2$O$_2$ content in this genotype. Intermediate and tolerant genotypes showed resistance to aluminium toxicity. PH measurements in boxes of tolerant genotypes showed important increase in pH values. Results suggest tolerant genotypes have mechanisms of external detoxification and intermediate genotypes have mechanisms of internal detoxification.

Financial support: CNPq, FAPERGS, CAPES
Localisation of callose in rice tissue using aniline blue and immunofluorescence staining.

Ian Stokes\textsuperscript{a}, Panagiotis Apostolakos\textsuperscript{b}, Pantelis Livanos\textsuperscript{b} & Christopher Exley\textsuperscript{a}.

\textsuperscript{a}The Birchall Centre, Lennard-Jones Laboratories, Keele University, United Kingdom.
\textsuperscript{b}Department of Botany, Faculty of Biology, University of Athens, Athens 15784, Greece

Following up a possible link between callose and silica deposition in plants (Law & Exley, 2010), a pair of staining methods were used to localise callose in \textit{Oryza sativa} for comparison with known sites of silica deposition. These methods were aniline blue staining and secondary immunofluorescence.

Callose fluoresces white or light blue when stained with aniline blue. In immunofluorescence, an antibody is raised specifically against callose. A secondary antibody carries a fluorophore and is raised against the primary antibody. When observed under fluorescent light of a specific frequency, the fluorophore will fluoresce green, showing areas of callose deposition.

These images can then be compared to images of silica structures obtained via acid digestion and PDMPO staining of \textit{Oryza sativa} samples.

Comparative study on the binding of Al\textsuperscript{3+} and nano-Al\textsubscript{13} with salmon sperm DNA and calf thymus DNA

Fei Ma\textsuperscript{a*}, Guo Xiang Wang \textsuperscript{a}, Ren Fang Shen \textsuperscript{b}, Xiao Di Yang \textsuperscript{a,b*}

\textsuperscript{a} Jiangsu Key Laboratory of Environmental Change and Ecological Construction, Jiangsu Key Laboratory of New Power Batteries, Nanjing Normal University, Nanjing 210097, China
\textsuperscript{b} State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Science, Nanjing 210008, China

The toxicity of aluminum (III) is caused by its bioavailability and adverse effects on health and persistence in the environment. Al\textsuperscript{3+} could disrupt biological membranes and cells and appears to play a toxicity role in pathological conditions \cite{1}. How about nano-Al\textsubscript{13}? In order to quantitatively elucidate the effects of Al\textsuperscript{3+} and nano-Al\textsubscript{13} on biomacromole, the interactions on salmon sperm DNA and calf thymus DNA are comprehensive researched as models. According to the spectral techniques: such as UV-vis spectroscopy, FTIR spectra, Raman spectra and circular dichroism approaches, the binding modes, binding sites and binding ability were comparative studied. The results indicated that nano-Al\textsubscript{13} displayed stronger effect on DNA than Al\textsuperscript{3+}, and salmon sperm DNA underwent more binding ability than calf thymus DNA. More information was showed in the following table.
### Binding Modes

<table>
<thead>
<tr>
<th>Samples</th>
<th>Binding Modes</th>
<th>Binding Sites</th>
<th>Binding Ability [L/mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon sperm</td>
<td>Hypochromic</td>
<td>Al$^{3+}$…N3-O2/cytosine</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Phosphate group binding</td>
<td>Al$^{3+}$…N7-C8/guanine</td>
<td>1.12×10$^{6}$</td>
</tr>
<tr>
<td></td>
<td>Non-classical/electrostatic binding</td>
<td>Al$^{3+}$…C4=O/thymine</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Hyperchromic</td>
<td>PO$<em>2$…Al$</em>{13}$…N7-C8/guanine</td>
<td>8.96×10$^{6}$</td>
</tr>
<tr>
<td>Calf thymus</td>
<td>Double-helix damage</td>
<td>Al$_{13}$…C4=O/thymine</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Groove/electrostatic binding</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### References:


Carbon nanomaterials modified electrodes for determination of trace aluminum (III) in biological fluids using 8-hydroquinone

Yong Zheng Tang a, Xue Chen b, Chong Zheng Xu b, Xiao Di Yang a,b

a Key Laboratory for Soft Chemistry and Functional Materials of Ministry of Education, School of Chemical Engineering, Nanjing University of Science and Technology, Nanjing, 210094, China
b Jiangsu Key Laboratory of New Power Batteries, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing, 210097, China

Electroanalytic direct determination of trace aluminum (III) is a difficult subject because of the highly negative reductive potential of Al(III) and interference of H+. However, the developing methods for the determination of trace Al (III) in biological samples is a hot topic owning to the recognition of the potential toxic effects of this element. In this paper, two novel carbon nanomaterials modified electrodes were developed by use of multi-walled carbon nanotubes (MWCNT) and chemical reduced graphene nanosheets (CRG), and those modified electrodes can detect Al (III) in biological fluids using 8-hydroquinone (8-HQ) as sequestering agent. The modified electrode was characterized by cyclic voltammetry (CV), transmission electron microscopy (TEM) and infrared spectra (FTIR). The electrochemical behaviors of 8-HQ with and without Al (III) at the modified electrodes were investigated by linear sweep voltammetry (LSV). The experimental results showed the electrochemical activities of the new carbon material modified electrode were improved greatly compared with the bare glassy carbon electrode. This novel method based on the linear decrease of LSV anodic peak current of 8-HQ with the concentration of Al (III) added. Under the optimum experimental conditions (pH 7.5, 0.1mol/L Tris-HCl buffer solution, 2x10⁻⁴mol/L 8-HQ), the linear range and detection limit were 7x10⁻⁷mol/L to 8x10⁻⁶mol/L, 3.1x10⁻⁷mol/L for MWCNT; 7x10⁻⁷mol/L to 8x10⁻⁶mol/L, 1.7x10⁻⁷mol/L for CRG. A modified electrode was used to detect for 8 times, and the relative standard deviation of the detection results for 1x10⁻⁶mol/L Al (III) were 3.2%(MWCNT) and 2.4%(CRG), respectively. The modified electrode exhibited good stability and had been used to detect aluminum concentration in biological fluids including human serum, cerebrospinal fluid and ascites, and the recoveries were 88.7% to 92.9% (MWCNT) and 92.3% to 96.1% (CRG) in the application of the method.

References:
Cholesterol effect on \((Na^+/K^+ )\) ATPase inhibition by submillimolar aluminium

V.S. Silva, L. Oliveira, P. Moraes, M. Artykbayeva* and P.P. Gonçalves

CESAM, Department of Biology, University of Aveiro, Aveiro, Portugal

The question whether different cholesterol contents in synaptosomal membranes modify the inhibitory effect of aluminium on the \((Na^+/K^+ )\)ATPase was addressed. The aluminium effect on the enzyme was also measured in spheroplasts isolated from *Escherichia coli*, since its inner membrane does not contain cholesterol.

Synaptosomal \((Na^+/K^+ )\)ATPase activity was significantly reduced by micromolar AlCl\(_3\) added in vitro and when aluminium was orally administered to rats. The oral administration of colestipol reduces the cholesterol content and concomitantly inhibits the \((Na^+/K^+ )\)ATPase. In vitro cholesterol depletion reduced the aluminium inhibitory effect on synaptosomal \((Na^+/K^+ )\)ATPase. Conversely, the \((Na^+/K^+ )\)ATPase activity of spheroplast prepared from *E. coli* cells previously exposed to submillimolar AlCl\(_3\) concentrations remained identical to the measured in control conditions. Moreover, the enzyme activity remained unchanged when AlCl\(_3\) (micromolar range) was added directly to the reaction medium.

In conclusion, the results strongly support our hypothesis that aluminium effect on \((Na^+/K^+ )\)ATPase activity implicates the reduction of interacting protomers within the oligomeric ensemble of the membrane-bound \((Na^+/K^+ )\)ATPase.

This work was supported by the grant SFRH/BPD/14677/2003 from FCT, the Portuguese Ministry of Sciences, Technology and High Education.
Acute and Chronic Neurotoxicity of Aluminum Oxide Nanoparticles on Mice

Qin-li Zhang, Lina Jia, Junwei Ji, Cuicui Ge, Xiujun Qin, Qiao Niu

Department of Occupational Health, School of Public Health, Education Ministry Key Laboratory of Cell Physiology, Shanxi Medical University, Taiyuan 030001, China

Given the widespread use of aluminium oxide nanoparticles (Al$_2$O$_3$ NPs), systematic tests for understanding the uncertain health aspects of Al$_2$O$_3$ NPs is necessary. In this study, ICR mice were used as in vivo model to assess the neurotoxicity of Al$_2$O$_3$ NPs, which were given to the mice through gavage (5g/kg weight) for once only and transnasal drip (50 mg/kg weight) for 30 days, mice treated with 0.9% normal saline and alumina micro-particles were used as black and bulk controls. Our study suggested that the Al$_2$O$_3$ NPs were excreted by kidney at a short-term period, and would do mild acute harm to neurobehavioral performance and functions of liver, spleen and kidney. While, ICP-MS examination showed that Al$_2$O$_3$ NPs accumulated and distributed in mice, liver, kidney as well as brain in the 30th days. The mice sub-chronically exposed to nano-alumina showed cognitive impairment symptoms, i.e., agitation, vesania, prostration and cognitive performance decrement. There were significant increment of BBB permeability and reduction of brain tight junction proteins’ expression in nano-alumina treated mice compared to those in controls. The mechanism was mediated by induction of lipid peroxidation and reactive oxygen species (ROS). Furthermore, abundant of mitochondria disruption and autophagy were observed in the brain slices, indicating that mitophagy, as a new found cell death pathway, might be involved in the procedure.

The research work was supported by National Natural Science Foundation of China, 30972456, 81241098)
Aluminium (Al) disturbs several aspects of calcium (Ca) homeostasis in mammals, including the intestinal absorption. We have shown these effects are influenced by some factors involved in body growth, as thyroid hormones. Pituitary growth hormone (GH) and its physiologic mediator Insulin-like growth factor I (IGF-1) have a major role in linear bone growth during childhood and adolescence, and also can promote intestinal Ca absorption by a mechanism independent of vitamin D signalling.

In the present study, we analysed the in vivo and in vitro effects of Al on different components of intestinal Ca transport by using radioisotopic techniques ($^{45}\text{Ca}$), in young and old rats treated with IGF-1. The results showed that the effect of Al upon both Ca-uptake kinetic parameters and transcellular Ca flux across small intestine epithelium was increased by IGF-1 in young but not in old rats. These findings could imply that the actions of Al on Ca homeostasis would be more deleterious in early stages of growing organisms, since have been demonstrated that IGF-1 declines throughout lifetime.

This work is supported by grant C.A.I+D 2009-12/B354-PROG 069, U.N.L, Argentina
Age dependence in the accumulation and elimination of aluminum in rats

Marlei Veiga, Sandra Ribeiro, Patrícia Mattiazzi, Cristina Bandero, Queli Lenz, Mauro Silveira, Carlos Mello, Denise Bohrer

Department of Chemistry, Departamento de Physiology and Pharmacology, Federal University of Santa Maria, Brazil.

It has been shown that the aluminum delivered to preterm infants via parenteral nutrition exceeds the limit of 5 µm/kg/day set by the US Food and Drug Administration. In an earlier work we demonstrated that the Al intake by preterm infants in a NICU was about 15 µg/kg. From this amount only 44% was eliminated in the urine. In this work we evaluated the effect of the administration of such amount of aluminum in newborn rats. Al organ deposition and possible developmental and cognitive damages were evaluated. The study also investigated the administration of higher amount of Al not only to newborn rats but also to rats considered adults (2-month old) and elderly (4-month old). Based on the corporeal surface equivalency, a dose of 0.12 mg Al/kg/day was administered to 20 newborn male Wistar rats for a period of 10 days. Every day after the second day of life the animals were evaluated regarding developmental conditions. On day 7 the animals started being evaluated for auditory stimulus and on day 19 for other behavioral tests such as object recognition and open field tests. Twenty days after the last administration, 10 animals were killed by decapitation and the organs were removed. The remainders were killed at the day 40. To a second group of newborn rats and to the other two groups of rats (2- and 4-month old) a dose of 24.8 mg/kg/day was administered, and the animals were killed following the same protocol, after 20 and 40 days. Only the newborns in this group had developmental and cognitive functions evaluated. The results showed no significant differences on the Al tissue accumulation between the group that received 0.12 mg/kg/day and the control group. On the other hand, in the higher dose groups, accumulation occurred in all tissues for all ages but in higher extent among newborns (ca 50% higher). However, after 40 days, while the Al content of all tissues decreased more than 50% in this group, among the elderly the Al content increased in the liver, mussel, bones and cortex and remained constant in the kidneys and heart. The cortex presented the most impressive result: while a reduction of 78% (0.42 to 0.33 mg/kg) was observed among the newborns, an increase of 400% (0.10 to 0.40 mg/kg) among the 2-month old and 800% (0.05 to 0.40 mg/kg) among the 4-month old rats occurred. Although the results for development and behavior were not conclusive and no significant differences were observed between the low Al dose and control groups, the higher dose group results are impressive. The older the animal the lower the elimination rate was. An interval of twenty days reduced the Al level in some tissues of the newborns to practically the same of the controls, whereas among the elderly the Al level remained the same or even increased within the same time interval.
Effect of long-term exposure to aluminium and high fat diet on NTPDase and 5'-nucleotidase activities in lymphocytes and platelets of rats

Rosilene Rodrigues Kaizer\textsuperscript{a}, Cínthia Melazzo Mazzanti\textsuperscript{a}, Roberta Schmatz\textsuperscript{b}, Jessié Martins Gutierres\textsuperscript{b}, Daniele Brolo Martins\textsuperscript{a}, Vera Maria Morsch\textsuperscript{b} and Maria Rosa Chitolina Schetinger\textsuperscript{b}

\textsuperscript{a}Instituto Federal de Educação Ciência e Tecnologia do Rio Grande do Sul – IFRS Campus Sertão, Rodovia RS 135, Km 25, Distrito Eng. Luiz Englert, Sertão, RS Brazil
\textsuperscript{b}Programa de Pós-Graduação em Ciências Biomédicas: Bioquímica Toxicológica, CCNE, Universidade Federal de Santa Maria, Av. Roraima, 97105-900, Santa Maria, RS, Brazil.

Aluminium (Al) is recognized as a neurotoxic agent and has been related to Alzheimer’s disease (AD). High fat diets increased the fibrollogenesis, characteristic of AD. The present study evaluates the effect of long-term exposure to Al plus high fat diets, on NTPDase and 5'-nucleotidase activities in lymphocytes and platelets of rats. The rats were treated by gavage with AlCl\textsubscript{3} 50 mg/kg day, for three months and were divided into twelve groups (n=5): (1) control; (2) Citrate; (3) Al/citrate; (4) Al; (5) high fat saturated diet (HFSD); (6) HFSD/citrate; (7) HFSD plus Al/citrate; (8) HFSD/Al; (9) high fat monounsatured diet (HFMD); (10) HFMD/citrate; (11) HFMD plus Al/citrate; (12) HFMD/Al. ATP, ADP and AMP hydrolysis in lymphocytes and platelets was enhanced in all groups treated only with Al or Al plus high fat diets (p<0.05). These results indicate the ability of Al plus high fat diets to elicit the immune function, increasing the adenosine levels, product of ATP hydrolysis, a potent anti-inflammatory molecule.
No effect of long-term low dosage of Al maltolate toward Th2 immune response in young rats

Guoo-Shyng W. Hsu, Wen-Mein Wu, Tzu-Jung Kou

Department of Nutritional Science, Fu-Jen Catholic University, Taipei, Taiwan

Allergies affect nearly three-hundred million people over the world. The occurrence of allergy is associated with the stronger response of type 2 T helper cell (Th2). Aluminum (Al), commonly used as an adjuvant to induce allergy-type responses in animal asthma model, was found high in certain infant formulas. Previous studies in our laboratory confirmed that Al-overload (13 µg Al/g b.wt/day as AlCl3) could increase serum and tissue Al contents as well as enhanced Th2 response in neonatal rats. The present study aimed to investigate if lower Al loading (similar to the high Al content in the commercial infant formula) with longer feeding period would cause stronger Th2 response also. The 3-day-old breast-fed pups were divided into three groups with gavage of 0 (Control, C), 0 (Maltol, M) and 1.3 (Al maltolate, ALM) µg Al/g b.wt /day respectively till weaning. Animals were continuously fed with semi-purified diet (AIN93G) and drinking water with (Al maltolate, 25 mg Al/L) or without Al till12- week of age. Results indicated that ALM animals had significantly lower IgG and IgA, compared to the other groups. However, there was no sign of immune response toward Th 2 under the blood and tissue Al levels in this study.
Aluminium adjuvant induced mitochondrial alterations

Lars Ohlsson\textsuperscript{a}, Anna Darabi\textsuperscript{b}, Peter Siesjö\textsuperscript{b} and Håkan Eriksson\textsuperscript{a}

\textsuperscript{1}Department of Biomedical Laboratory Science, Faculty of Health and Society, Malmö University, SE-205 06 Malmö, Sweden
\textsuperscript{2}Glioma Immunotherapy group, Division of Neurosurgery, Department of Clinical Sciences, BMC D14, Lund University, SE-221 84 Lund, Sweden

The mitochondria are organelles of vital importance for the cell and supply cells with chemical energy in the form of ATP. In addition to being the main suppliers of cellular energy, mitochondria are also involved in several other cellular processes as signalling, cell differentiation, apoptosis and innate immune activation.

Phagocytosing cells have shown alterations in mitochondrial conformation and functions after \textit{in vitro} incubation with aluminium based adjuvant. In peripheral monocytes and in cell lines of monocytic origin, the mitochondrial content rapidly increases showing a stabilized membrane potential and increased production of reactive oxygen species (ROS). The concept that mitochondria are crucial initiators for innate immune signalling has recently been proposed and the functional coupling between aluminium adjuvant and mitochondria may constitute a direct link between aluminium salts and immune activation.
Administration of aluminium in vaccine-relevant exposures in neonatal mice is associated with long-term adverse neurological outcomes

Lucija Tomljenovic a, Yongling Li a, Christopher A. Shaw b

a Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, University of British Columbia, 828 W. 10th Ave, Vancouver, BC, V5Z 1L8, Canada
b Department of Ophthalmology and Visual Sciences, Program in Experimental Medicine and the Graduate Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, 828 W. 10th Ave, Vancouver, BC, V5Z 1L8, Canada

We previously found that a highly significant correlation exists between the amounts of aluminium (Al) administered to preschool children through routine paediatric vaccinations and the current prevalence of autism spectrum disorders (ASD) in Western countries. These findings prompted our current research in animal models which aims to determine whether Al vaccine adjuvants represent a neurodevelopmental hazard at amounts equivalent to those found in vaccines recommended for American and Scandinavian children (designated as “high Al” and “low Al” schedules respectively). For this purpose three groups of C57BL/6 mice each consisting of 14 animals (males and females) were injected with 6 doses of either “high Al”, “low Al” or saline placebo, mimicking as closely as possible the timing of the paediatric vaccination schedule. At approximately 6 months of age, the mice were subjected to a battery of behavioural tests. Mice of both sexes injected under the “high Al” schedule showed a highly significant increase in anxiety (p=0.0005 males; p=0.0001 females) and a marked reduction in exploratory behaviour (p=0.013 males; p=0.0001 females) compared to controls. Females however were more severely affected, showing significant increase in anxiety even at “low Al” (p=0.034). In addition, males but not females receiving “high Al” were significantly more lethargic and less active than control males or those on the “low Al” schedule (p<0.0001). Finally, both males and females in the “high Al” group showed a highly significant and sustained increase in body weight (p=0.0005 males; p=0.001 females). In summary, our current results show that administration of Al in vaccine-relevant exposures in neonatal mice is associated with long-term adverse neurological and metabolic outcomes.
Aluminum Enhances Inflammation and Decreases Mucosal Healing in Experimental Colitis in Mice

Mathilde Body-Malapel, Guillaume Pineton de Chambrun, Florence Deknuydt, Madjid Djouina, Nicolas Esquerre, Frédéric Altare, Christel Neut, Marie Claire Arrieta, Thirumala-Devi Kanneganti, Jean-Frédéric Colombel, Antoine Cortot, Pierre Desreumaux, and Cécile Vignal.

Background & objectives: The increase of inflammatory bowel diseases (IBD) in developing countries has brought to attention the potential role of environmental pollutants as causative factors in their pathophysiology. Despite the known toxicity of aluminum and its gut interaction, aluminum effects on intestinal homeostasis have not been investigated so far. The objectives of our study were to evaluate the effect of aluminum, at a dosage relevant to common exposure, on intestinal inflammation in murine models of colitis and to explore underlying mechanisms in vitro.

Results: Aluminum worsened intestinal inflammation in mice with TNBS and DSS induced colitis and decreased epithelial cell renewal compared with control animals. In vitro, aluminum induced granuloma formation and synergized with lipopolysaccharide to stimulate the expression of inflammatory cytokines by epithelial cells.

Conclusions: We demonstrated that exposure to environmentally relevant doses of aluminum increased the intensity and duration of intestinal inflammation, providing strong evidences that aluminum might be an environmental factor involved in IBD pathophysiology.
Aluminium effect on *Escherichia coli* growth and death

V.S. Silva, P. Moraes, M. Artykbayeva* and P.P. Gonçalves

*CESAM, Department of Biology, University of Aveiro, Aveiro, Portugal*

Aluminium toxicity has been recognized in many situations where exposure is heavy or prolonged. Aluminium is ubiquitous in our environment and its bioavailability is increased under acidic conditions. The mechanism of toxic action of aluminium continues to remain poorly understood.

The toxicity of aluminium to *E. coli* has been studied during the exponential and stationary phase in Boillon-Miller growth liquid medium. Addition of AlCl$_3$, in the millimolar range, drastically reduced the survival ability of *E. coli* either during exponential or stationary phase. The application of logistic growth models to analysis of the effect of aluminium on bacterial growth allowed determining the values of growth and death rates and their apparent values in the presence of different aluminium concentrations.

Preliminary data suggest that AlCl$_3$ could induce the generation of two *E. coli* subpopulations with different sensitivities for aluminium. Whether *E. coli* develops a persistence or resistance phenotype in response to stress induced by aluminium is under evaluation.

This work was supported by the grant SFRH/BPD/14677/2003 from FCT, the Portuguese Ministry of Sciences, Technology and High Education.
Effects of aluminum maltolate ingestion on the immune response of SD neonates

Hsin-Ya Lin*, Wen-Mein Wu, Guoo-Shyng W. Hsu

Department of Nutritional Science, Fu-Jen Catholic University, Taipei, Taiwan. Email: elsa07110308@yahoo.com.tw

This study was conducted to understand the effects of Al maltolate on immune system of neonates, and whether those animals with higher plasma and/or tissues Al levels are more susceptible to specific antigen. Six-day-old breast-fed pups were divided into four groups with gavage twice a day of 0 (Control, C), 0 (Maltolate, M), 0.43 (low Al maltolate, LALM) and 1.3 (high Al maltolate, HALM) μg Al/g b.wt/day respectively for 15 days till animals were sacrificed. Cell proliferation and cytokine concentration of thymocyte, splenocyte and MLN lymphocyte were measured. Results showed that Al content in serum, spleen; liver and kidney were elevated with increased dietary Al maltolate which Al level is equivalent to infant formula. Meanwhile, cell proliferation or cytokine levels of splenocytes, thymocytes and MLN lymphocytes were also changed. In conclusion, the higher Al content in the body affected immune response.
Heme oxygenase-1 induction by ROS-JNK pathway plays a role in aluminium-induced anaemia

Chia-Yeh Lin\textsuperscript{a}, Wei-Chun Hsiao\textsuperscript{b}, Chang-Jen Huang\textsuperscript{c}, Cheng-Fu Kao\textsuperscript{b} & Guoo-Shyng W. Hsu\textsuperscript{a}

\textsuperscript{a}Ph.D. Program of Nutrition and Food Sciences, Fu-Jen Catholic University, Taipei, Taiwan.
\textsuperscript{b}ICOB, Academia Sinica, Taipei, Taiwan.
\textsuperscript{c}IBC, Academia Sinica, Taipei, Taiwan.

Aluminium (Al) overload is correlated to microcytic, hypochromic anaemia. One possible mechanism is that Al may impede heme biosynthesis through the activity changes of its biosynthesis enzymes. However, how aluminium affects these enzymes and the molecular mechanisms are not clear. Here, we showed that hemoglobin concentration and hematocrit were decreased in Sprague-Dawley rats after long-term exposure of Al. Meanwhile, the activity of aminolaevulinic acid dehydratase in rat liver was reduced, but aminolaevulinic acid synthetase and heme oxygenase (HO) activities were enhanced. The increase of HO activity was due to the up-regulation of HO-1 mRNA and protein, an inducible HO isozyme. Furthermore, reactive oxygen species (ROS) mediated c-Jun N-terminal kinase (JNK) activation was found to be critical for HO-1 induction by Al, because HO-1 level in rat hepatocytes was diminished when N-acetyl-L-cysteine (ROS scavenger) or JNK inhibitor was used in combination with Al. Thus, Al elevates HO-1 expression through ROS-JNK pathway, which may enhance HO activity to degrade heme and lead to microcytic anaemia.
Transcellular transport of alumina nanoparticles: A study on blood-brain barrier model in vitro

Hui-ting Peng*, Fu-pin Gao, Qin-li Zhang, Qiao Niu

School of Public Health, Shanxi Medical University, Taiyuan 030001, China. Email: niuqiao55@163.com.

Alumina nanoparticles (nano-alumina) have been widely used in the environment. However, little is known concerning the potential adverse effects on the brain exposed to alumina nanoparticles. The present study focuses on the hypothesis that nano-alumina can increase the permeability of blood-brain barrier (BBB) and enter cells by endocytosis. In vitro studies were carried out in BBB models, which were co-cultured of endothelial cells and astrocytes from newborn rat brains using transwells. The integrity and functionality of BBB were checked, followed by exposure of alumina nanoparticles at various concentrations and time points. Rutin and cy5.5 labeled nanoalumina were used to sign the permeability of BBB, which were increased by nano-alumina in a dose- and time-dependent manner. Cellular uptake of nano-alumina particles were monitored by time-lapse photography using confocal microscope, and endocytosis was considered as one of the main routes of transport. These results indicate that BBB can be affected by nano-alumina. The relationship between BBB damage and neurotoxicity induced by nano-alumina still needs further research.

The research work was supported by National Natural Science Foundation of China, 30972456, 8124109
Effects on Long-term Potentiation and the Expressions of AMPA Receptor Subunits in Rat Exposed to Aluminium in vivo

Jing Song*, Ying Liu, Hui Fang Zhang, Qiao Niu

Department of Occupational Health, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi 030001, China. E-mail: niuqiao55@163.com

AMPA receptor trafficking appears to be involved in the mechanism of long-term potentiation (LTP) in hippocampus. It has also been proposed that the aluminium may have suppressive effects on LTP in hippocampus. Here we explored the changes in LTP of CA1 region and protein expressions of AMPAR subunits (GluR1 and GluR2) both in total and in membrane-enriched extracts from rat hippocampus after acute and subchronic aluminium exposure. Acute aluminium treatment, by intracerebroventricular injection (i.c.v.) with different dose of aluminium-maltolate complex (Al(mal)3), produced a dose-dependent suppressive effect on LTP in hippocampus and a dose-dependent decrease of GluR1 and GluR2 in membrane extract, but similar changes were not been found in total extract, suggesting a trafficking of the AMPA receptor subunits from synaptic sites to intracellular pools in the hippocampus. In rats receiving different dose of Al(mal)3 by intraperitoneal injection (i.p.) for 8 weeks, the dose-dependent suppressive effects on LTP and the expressions of AMPA receptor subunits both in membrane and in total extracts were all been found in subchronic aluminium treatment, suggesting not only a trafficking from synaptic sites to intracellular pools but also low protein expression of AMPA receptors subunits. It was concluded from the results that Al(mal)3 obviously suppressed the LTP in rat hippocampal CA1 region in a dose-dependent manner in vivo, it may be related to the trafficking and low protein expression of AMPA receptor subunits. However, the mechanisms underlying this observation need further investigation.

Supported by NSFC 30972512
Excessive aluminium accumulation in the bones of patients on long-term parenteral nutrition

Pamela C. Kruger\textsuperscript{a}, Aubrey L. Galusha\textsuperscript{b}, Lyn Howard\textsuperscript{c}, Patrick J. Parsons\textsuperscript{a,b}

\textsuperscript{a}Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health, Albany, NY, USA
\textsuperscript{b}Department of Environmental Health Sciences, School of Public Health, The University at Albany, State University of New York, Albany, NY, USA.
\textsuperscript{c}Department of Medicine, Division of Gastroenterology and Nutrition, Albany Medical College, Albany, NY, USA

Aluminium (Al) contamination of parenteral nutrition (PN) solutions remains a concern for long-term PN patients. In 2004, the FDA required a reduction in the Al content of large volume parenteral ingredients used in PN solutions, but small volume ingredients remain unregulated. Thus, long-term PN patients remain at risk for Al accumulation. Using a well-validated analytical method based on electrothermal atomic absorption spectrometry, we measured Al accumulation in autopsy bones from seven patients who had received PN for short bowel syndrome over a period of 2–21 years, and compared bone Al levels with those in living control patients undergoing hip or knee replacement. Markedly elevated bone Al levels (P <0.0001) were found in all but one patient who received PN for only two years before death. Even greater Al accumulation (up to 56 µg/g dry weight) was found for PN patients who developed late renal impairment. We conclude that long-term adult PN patients continue to be at risk for Al toxicity.
Assessing inter-laboratory performance for serum Al in the New York State Proficiency Testing program: implications for monitoring exposure to Al in PN patients

Pamela C. Kruger\textsuperscript{a}, Mary Frances Verostek\textsuperscript{a}, Lyn Howard\textsuperscript{b}, Patrick J. Parsons\textsuperscript{a,c}

\textsuperscript{a}Laboratory of Inorganic and Nuclear Chemistry, Wadsworth Center, New York State Department of Health, Albany, NY, USA
\textsuperscript{b}Department of Medicine, Division of Gastroenterology and Nutrition, Albany Medical College, Albany, NY, USA
\textsuperscript{c}Department of Environmental Health Sciences, School of Public Health, The University at Albany, State University of New York, Albany, NY, USA

Bone Al measurements are the best indicators of Al body burden, since this element accumulates to the greatest extent in bone. However, obtaining uncontaminated bone biopsies is not only difficult; it is highly invasive and impractical for routine monitoring purposes. Measuring serum (or plasma) Al may be a suitable alternative for monitoring exposure and accumulation in patients with elevated Al body burdens. However, obtaining reliable serum Al data is not straightforward, and requires considerable skill. Generally, analytical methods fall into two categories: those based on electrothermal atomic absorption spectrometry (ETAAS), and those based on inductively coupled plasma – mass spectrometry (ICP-MS). However, laboratories may implement these methods quite differently. Here we examine interlaboratory performance for serum Al in the New York State Proficiency Testing (NYS PT) program. We also review the performance of our lab’s well-validated ETAAS method, and explore its potential for monitoring excess Al accumulation in parenteral nutrition patients.
Hot-watery infusion of *Hibiscus sabdariffa* petals, a potential source of Al in human diet

Jan Malík\textsuperscript{a}, Adéla Fraňková\textsuperscript{a}, Jiřina Száková\textsuperscript{a}, Ilona Šperlingová\textsuperscript{b}, Aleš Vaněk\textsuperscript{a}, Ladislav Kokoška\textsuperscript{c} and Ondřej Drábek\textsuperscript{a}

\textsuperscript{a}Faculty of Agrobiology, Food and Natural Resources, CULS Prague, Prague, Czech Republic
\textsuperscript{b}National Institute of Public Health, Prague, Czech Republic
\textsuperscript{c}Institute of Tropics and Subtropics, CULS Prague, Prague, Czech Republic

The dark red petals of *Hibiscus sabdariffa* L. (known also as Roselle) are one of the most common tea-like plant materials consumed worldwide, often in amounts which substitute fresh water intake. According to our previous study, the amount of Al in hot-watery infusion can reach up to 1 mg/L.\textsuperscript{1}

Therefore, we tried to describe Al transport from the raw material into infusion. The raw material was decomposed using HNO\textsubscript{3} in a laboratory microwave unit.\textsuperscript{2} For infusion preparation, the amount of 1g was leached for 2, 5, 10 and 15 minutes in 100 mL of 90°C deionised water. The Al content was analysed by ICP-OES.\textsuperscript{3} Aluminium bioavailability screening \textit{in vitro} was then carried out using a Physiologically Based Bioavailability Test (simulating the consequent digesting processes in the mouth, stomach, duodenum and small intestine)\textsuperscript{4} and Simple Bioavailability Extraction Test (detailed simulation of digestion in the stomach).\textsuperscript{5} The \textit{in vivo} test of Al absorption was also carried out. The comparison of \textit{per os} consumed and urinary rejected Al was observed for 11 healthy volunteers.\textsuperscript{6} At first, a urine sample was collected for one week before treatment as a control. One litre of hibiscus tea (containing at least 0.5 mg of Al) was then served for 20 days to 10 individuals (one left as a negative control). After the treatment, urine was collected for another 10 days. The ICP-OES results of urine were converted according to creatinine content, analysed using HPLC.\textsuperscript{7}

We made the following observations: at least 70\% of Al that leached in 15 minutes can also be found in solution after 2 minutes. We assumed that the main part of the Al digestion took place in in the stomach. More than 20\% of Al appears to pass the human body to the intestine. A part may be absorbed here together with water absorption or leave the body with faeces. The daily consumption of hibiscus tea seems to be strongly affecting the common amount of bioavailable Al. In contrast to the pre-treatment phase, the human body appears to excerpt Al irregularly even during the hibiscus tea drinking or in the post-treatment period.
According to our results, we do not recommend the excessive daily consumption of hibiscus hot-watery infusion to humans highly sensitive to Al (those suffering from renal failures, infants).

1) Malik et al. (2009). 8th Keele Meeting on Aluminium, p. 58
2) Vanek et al. (2009). J Geochem Explor, 102: 7-12
5) Intawongse & Dean (2008). Environ Pollut, 152: 60
A pilot study measuring aluminium in bone in Alzheimer’s and referent subjects: work in progress


Medical Physics & Applied Radiation Sciences, McMaster University, Hamilton, Canada
Department of Medicine, McMaster University, Hamilton, Canada
Department of Physics, Ryerson University, Toronto, Canada
Atomic Energy of Canada Limited, Chalk River Laboratories, Ontario, Canada
University of Florida Proton Therapy Institute, Jacksonville, FL, USA

Aluminium is measured non-invasively in human subjects using in vivo neutron activation analysis. A person’s hand is exposed to a low radiation dose of neutrons. Aluminium present in the bones can undergo the $^{27}$Al(n,γ)$^{28}$Al reaction. Another key reaction is $^{48}$Ca(n,γ)$^{49}$Ca. After the 45 s irradiation, the subject moves to a set of NaI(Tl) detectors configured in a 4π array. The $^{28}$Al decays with a half life of 2.25 m, emitting a γ-ray of energy 1.78 MeV, and $^{49}$Ca decays with a half life of 8.72 m, emitting a γ-ray of energy 3.08 MeV. A series of ten 1 minute counts of γ-ray energy spectra are recorded from the detector array and these are analysed particularly for the 1.78 MeV and 3.08 MeV peaks, from which the amounts of aluminium and calcium respectively are inferred. In fact, the ratio of aluminium to calcium is used for quantification, because this is independent of details of exact irradiation and counting configurations and can readily be corrected for any variations in irradiation, transfer or counting time.

Subjects diagnosed with Alzheimer’s Disease, using the Diagnostic and Statistical Manual for Mental Disorders criteria, have been enrolled in the pilot study, as have referent subjects of similar age. All subjects are 60 years or over. Each subject has given informed consent or, in the case of some of the Alzheimer’s sufferers, consent has been given by a delegated decision maker. Measurements began in spring of 2012 and the pilot study is intended to encompass measurements of 20 Alzheimer’s subjects and 20 referents. This paper will present the results available up to February 2013.
Absorption and metabolism of aluminium, and its influence on gene expressions to cause diseases

Shunsuke Meshitsuka

Tottori University School of Medicine, Yonago, 683-8503 Japan

It is shown that the absorption rate of aluminium is depended upon foods and drinks. Although aluminium is excreted rapidly into urine, aluminium stays in longer periods in bodies after taking excess amount of aluminium. Accumulation of aluminium in the body has been linked to disease conditions. The toxic effects of aluminium to neuronal cells were examined to show apoptotic cell death via endoplasmic reticulum stress, implicating an influence of aluminium on the gene expression. Also, the astrocyte-neuron interaction was altered in the presence of aluminium and was important in the process of toxic effects in the central nervous system. In addition, it was shown that chronic exposure of aluminium might be a cause of essential hypertension due to the up-regulation of renin, confirmed by RT-PCR and Western blotting experiments in the dose dependent treatments and the time course observation in kidneys.

Effect of aluminium on migratory and invasive properties of human breast cancer cells in culture.

P.D. Darbre and E Iskakova

School of Biological Sciences, Biomedical Sciences Section, University of Reading, Reading RG6 6UB, UK. E-mail p.d.darbre@reading.ac.uk

Exposure to aluminium may be a contributory factor in breast cancer development and aluminium has been measured in human breast tissue. However, since mortality results from breast tumour growth at metastatic sites rather than in the breast itself, we have investigated whether long-term exposure of human breast cancer cells to aluminium could alter their migratory and invasive properties and so contribute to the metastatic processes of breast cancer. MCF-7 human breast cancer cells exposed to aluminium long-term (>30 weeks) showed increased migration in wound healing assays and increased invasion through matrigel using xCELLigence technology. Alterations to E-cadherin and β-catenin were observed on Western immunoblotting and immunocytochemistry suggesting that aluminium might influence the processes of epithelial-mesenchymal transition.
Aluminium Chloride Transforms Cultured Mammary Epithelial Cells

Stefano J. Mandriot\textsuperscript{a,b}, Raphaëlle Buser\textsuperscript{a,b}, Laurence Lesne\textsuperscript{a,b}, Marc Ansari\textsuperscript{a,b}, Fabienne Gumy-Pause\textsuperscript{a,b}, Dominique Belin\textsuperscript{c} and André-Pascal Sappino\textsuperscript{d}.

\textsuperscript{a}Faculty of Medicine, Department of Pediatrics, Geneva, Switzerland.
\textsuperscript{b}University Hospitals of Geneva, Division of Pediatric Oncology, Onco-Hematology Unit, Geneva, Switzerland.
\textsuperscript{c}Faculty of Medicine, Dept of Pathology and Immunology, Geneva, Switzerland.
\textsuperscript{d}Clinique des Grangettes, Geneva, Switzerland.

Aluminium salts used as antiperspirants have been incriminated as contributing to breast cancer incidence in Western societies. At the time we started our work, very little epidemiological or experimental data confirmed or infirmed this hypothesis. We recently reported that in normal human mammary epithelial cell models, physiologically relevant concentrations of aluminium lead to an early induction of DNA double strand breaks, transient cell proliferation arrest, and cellular senescence. Upon continuous exposure to aluminium, this was followed by loss of contact inhibition and anchorage-independent growth. Aluminium had no similar effects on human keratinocytes or fibroblasts, and was not detectably mutagenic in bacteria. More recently, we extended these observations to cultured mouse mammary epithelial cells. Our observations challenge the safety ascribed to aluminium’s widespread use in underarm cosmetics. Based on our findings, we are currently developing an animal model to further explore the transforming effects of aluminium on mammary epithelial cells.
Relationship among Aluminium, Carbonyls and Interleukins in normal and cancerous breast microenvironment

Ferdinando Mannello, Daniela Ligi, Matteo Canale

Dept of Biomolecular Sciences, Section of Clinical Biochemistry, Unit of Cell Biology, University “Carlo Bo”, 61029 Urbino, Italy

Human breast cancer (BC) is the most common malignancy but the current screening tools miss up to 40% of BC, suggesting the urgent need for non-invasive, intraductal screening for early detection (Mannello, Expert Opin Med Diagn 2008;2:1221). It is well known that nipple aspirate fluid (NAF) represents the mirror of the metabolic pathways occurring in the mammary gland during pathological conditions (Mannello et al, Expert Rev Proteomics 2009,6:43). The bio-molecular analysis of NAF allows the identification of both early biomarkers of BC risk and possible targets for therapeutic approaches.

In the BC microenvironment, inflammatory processes may lead to the overexpression of cancer-associated growth factors, proteases, and specific “acute-phase” proteins, resulting mainly from oxidative stress (Cichon et al, J Mammary Gland Biol Neoplasia 2010;15:389). Several previous studies have demonstrated that personal care products are potential contributors to the body burden of Aluminium (Al) and recent evidence has linked Al accumulation in breast tissue and breast cancer with Al-based antiperspirants (Exley et al., J Inorg Biochem. 2007;101:1344 – Darbre et al., J Inorg Biochem. 2011;105:1484). We have previously reported that an aluminium-rich microenvironment may be responsible for damaging breast tissue through protein oxidation and iron-driven inflammation (Mannello et al, J Appl Toxicol 2009;29:1 - and 2011,31:262).

With the present study, we have studied if NAF samples with Al-overload may be related to a breast microenvironment with an altered profile of Interleukins (ILs) and oxidized proteins (Carbonyls), predisposing breast tissue to cancer initiation/development. We have analyzed nipple aspirate fluids (collected from women affected by malignant diseases and benign lesions with different risk factors, and from control women not scheduled for biopsy) assaying the concentrations of ILs (by 17-BioPlex multiELISA kit), and Carbonyls (by ELISA kit), and Al with ICP-mass spectrometry.

We have discovered that the mean level of aluminium (a non-physiological component of the human breast) was significantly higher in Cancer NAF (Ca) (268.4±28.1 μg/L; n=19) than in...
NoCancer (NoCa) NAF (131.3±9.6 μg/L; n=16; P< 0.0001).

Significantly higher protein carbonyl concentration was found in NAF from invasive BC patients with Al-rich microenvironment (2.07±2.1 nmoles/mg prot; n=19) compared to control subjects with low Al content (0.37±0.05 nmoles/mg protein=16) (*** P<0.0001). Noteworthy, NAF levels of protein carbonyls are also significantly higher in women with premalignant conditions (like hyperplasia with atypia, papilloma with atipia) with Al-rich breast microenvironment respect to healthy control subjects (* P<0.001). The mean levels of inflammatory cytokines in Cancer NAF with high levels of Al was significantly higher respect to those found in NoCancer NAF containing low amounts of Al in breast microenvironment. In particular, we found significantly increased levels of pro-inflammatory ILs (IL-1, -6, -12, and TNFalpha) as well as chemoattractant cytokines (IL-8, MIP-1 and O No Ca NAF was found for the cytokine profile of IL-2, -4, -5, -10, -13, RANTES, and INF. Noteworthy, a highly significant correlation between carbonyls and IL-6 content (Y= 23.52x + 5.174, r2 =0.816, P<0.0001) was found in Cancer NAF contained increased levels of Al.

Actually, the use AL-based antiperspirants may explain the accumulation of aluminium in breast tissues, even if the reasons for the high levels of AL in cancer NAF remain unknown. According to the effects of Al on oxidative status in aluminium exposed humans (Celik et al, Clin Biochem, 2012, in press), for the first time, we described that in the breast cancer microenvironment high levels of Al might exert their toxic effects, modulating both the protein oxidation pathways and pro-inflammatory cytokines.
Physico-chemical characterisation of clinically approved and research aluminium-based adjuvants: An Infrared Spectroscopy and Particle Size study.

Emma Shardlow* and Christopher Exley

The Birchall Centre, Lennard-Jones Laboratories, Keele University, STAFFS, ST5 5BG, United Kingdom

The individual physico-chemical properties of both clinically approved and research aluminium-based adjuvants may influence their mechanisms of action in biological systems. Information upon such is disparate and generally unequivocal. Functionality analysis of two clinically approved preparations (aluminium oxyhydroxide and aluminium hydroxyphosphate) and one research preparation (50:50 aluminium hydroxide and magnesium hydroxide) using ATR-FTIR demonstrated that all samples exhibited varying degrees of hydroxyl functionality (3700-3000cm\(^{-1}\)). Phosphate group vibrations were also detected in aluminium hydroxyphosphate (1500-1000cm\(^{-1}\)) and the presence of carbonate group vibrations were detected in the research sample (1500-1450cm\(^{-1}\)). The particle size distributions of these adjuvant preparations when introduced into a biological fluid mimicking that found at the site of vaccine injection were analysed using a Malvern Zetasizer ZS (0.3nm-10µm). Aluminium oxyhydroxide maintained a stable particle size over a period of 24 hours (1-5µm) whereas all other preparations demonstrated a decrease in particle size within an hour of introduction.

Research funded by Keele Acorn and Dwoskin Foundation
Aluminium hydroxide-induced macrophagic myofasciitis (MMF): predictive scores and biomarkers

Nilusha Ragunathan-Thanagarajah a, Christine Le Beller b, Anne Fustier c, Jean-Louis Brasseur c, Romain K. Gherard a,d, Pierre Boutouyrie b, Stéphane Laurent b, François-Jérôme Authier a,d

aTeam 10, INSERM U955, Paris Est-Creteil University, and dReference Centre for Neuromuscular Diseases, Henri Mondor Hospital, Creteil, France; 
bPharmacovigilance Centre, Georges Pompidou European Hospital, Paris, France 
cRadiology Department, Pitié-Salpêtrière Hospital, Paris, France.

MMF is characterized by: (1) a clinical syndrome with arthromyalgias, chronic fatigue, and neurocognitive dysfunction; and (2) a specific myopathological lesion witnessing long-term persistence of vaccine-derived aluminium hydroxide within body. Diagnosing MMF requires performing muscle biopsy, a procedure not suitable for the routine investigation of all myalgic patients, and incompatible with large-scale epidemiological surveys. To overcome this difficulty, we set up non-histological tools for diagnosing MMF. In 130 consecutive vaccinees with chronic myalgias, 42/130 (32.3%) had MMF. From the analysis of 173 clinical and laboratory parameters, we developed bioclinical scores based on multivariate logistic regression model, with sensitivity and specificity ranging from 50% to 88% and from 36.4% to 78.1%, respectively. To improve the detection of MMF, we evaluated the predictive value of musculoskeletal ultrasonography in a cohort of 141 myalgic patients who underwent deltoid muscle biopsy. In this series, MMF was detected in 45/141 patients (31.9%). Ultrasonography was abnormal in 37/45 patients with MMF (82.2%) and in 61/96 negative patients (63.5%) (p<0.05), its sensitivity and negative predictive value being 82.2% and 81.4%, respectively.
Death following human papillomavirus virus (HPV) vaccination: an autoimmune adjuvant-mediated adverse reaction?

Lucija Tomljenovic\textsuperscript{a} and Christopher A. Shaw\textsuperscript{b}

\textsuperscript{a}Neural Dynamics Research Group, Department of Ophthalmology and Visual Sciences, University of British Columbia, 828 W. 10\textsuperscript{th} Ave, Vancouver, BC, V5Z 1L8, Canada

\textsuperscript{b}Department of Ophthalmology and Visual Sciences, Program in Experimental Medicine and the Graduate Program in Neuroscience, University of British Columbia, Vancouver, British Columbia, 828 W. 10th Ave, Vancouver, BC, V5Z 1L8, Canada

Herein reported is the case of a 19-year-old female who had no relevant medical history and was not taking drugs and who expired in her sleep, approximately 6 months after her third and final aluminium-adjuvanted human papillomavirus virus (HPV) vaccine booster. Her symptoms started after the first HPV vaccine injection and consisted of unexplained fatigue, muscle weakness, tachycardia, chest pain, tingling in the extremities, irritability, mental confusion and periods of amnesia (memory lapses). The coroner’s autopsy was unremarkable and failed to determine the exact cause of death. In contrast, our histological examinations of brain specimens showed accumulations of aluminium, marked activation of the complement membrane attack complex (MAC), deposition of immunoglobulin (Ig)-complexes and marked immunoreactivity for several pro-inflammatory markers in cerebellar Purkinje cells and hippocampal neurons. This pattern of immune system activation in the absence of brain infection indicates an abnormal triggering of the immune response in which the immune attack appears to be directed towards self-tissue. Notably, activation of MAC and deposition of Ig-complexes typically occurs in neurodegenerative diseases which have an underlying immune trigger and is not a typical feature of a normal brain in adolescence. From the medical history of the patient it appears that the most relevant immune trigger of the observed autoimmune CNS pathologies was the aluminium-adjuvanted HPV vaccine.
In vivo neutron activation analysis of aluminium in bone: further refinements

W. Matysiak\textsuperscript{1,2}, J.Z. Atanackovic\textsuperscript{3}, H. K. Mohseni\textsuperscript{1}, S.H. Byun\textsuperscript{1}, W.V. Prestwich\textsuperscript{1}, N.D. Priest\textsuperscript{3}, D. Cowan\textsuperscript{4}, M.J. Inskip\textsuperscript{1}, D.R. Chettle\textsuperscript{1}

\textsuperscript{a}Medical Physics & Applied Radiation Sciences, McMaster University, Hamilton, Canada
\textsuperscript{b}University of Florida Proton Therapy Institute, Jacksonville, FL, USA
\textsuperscript{c}Atomic Energy of Canada Limited, Chalk River Laboratories, Ontario, Canada
\textsuperscript{d}Department of Medicine, McMaster University, Hamilton, Canada

In vivo neutron activation analysis of aluminium in bone is based on the $^{27}\text{Al} (n,\gamma)^{28}\text{Al}$ reaction. The first such systems were developed in the 1970s, in response to the occurrence of dialysis encephalopathy. At McMaster University a system has been developed using a Tandetron accelerator as a neutron source, by means of the $^7\text{Li} (p,n)^7\text{Be}$ reaction. Work on this system has been reported previously, including estimates of bone aluminium made in vivo as a spin-off of a study to measure the accumulation of manganese in bone. Successive improvements to this system have also been reported at previous Keele Meetings on Aluminium.

A measurement comprises irradiation with a low dose of neutrons to both the $^{28}\text{Al}$ and $^{49}\text{Ca}$ from the $^{48}\text{Ca} (n,\gamma)^{49}\text{Ca}$ reaction. Other elements are also activated, particularly chlorine and sodium from the $^{37}\text{Cl} (n,\gamma)^{38}\text{Cl}$ and $^{23}\text{Na} (n,\gamma)^{24}\text{Na}$ reactions. The subject or sample is transferred from the irradiation position to the set of nine NaI (TI) detectors configured in a 4π array. A series of short, typically one minute, counts is then collected usually for total of ten or fifteen minutes. In the γ-ray spectra a peak at 1.78 MeV is characteristic of $^{28}\text{Al}$ and a peak at 3.08 MeV is characteristic of $^{49}\text{Ca}$. These are quantified and their ratio is proportional to the aluminium to calcium ratio in bone.

Three areas of improvement are reported here. One is the use of anti-coincidence counting. The aluminium γ-ray at 1.78 MeV is close to and usually dominated by the larger peak at 1.64 MeV from $^{38}\text{Cl}$. However, the 1.64 MeV is in partial coincidence with a peak at 2.17 MeV also from $^{38}\text{Cl}$. Counting in anticoincidence discriminates against the $^{38}\text{Cl}$ peaks and therefore in favour of the $^{28}\text{Al}$ peak.

The $^{28}\text{Al}$ has a half-life of 2.25 m; $^{49}\text{Ca}$ has a half-life of 8.72 m. Both are characterised not only by their respective energies, but also by their respective half-lives. The overall data set
has been analysed in two dimensions, that is, energy and time, in order to characterise the amount of aluminium present with maximal precision. (The $\gamma$-ray peak from $^{49}$Ca was already sufficiently well marked not to require further improvement in precision.)

The original set of calibration standards, by comparison with which the ratio of 1.78:3.08 MeV peaks is converted to a ratio of aluminium to calcium ratio in bone, had higher aluminium concentrations than observed in members of the general public. So new calibration standards have been made and the calibration of the whole system re-examined with background levels of aluminium as the target measurement.
Modelling absorption efficiency of elements via oral exposure in humans

T.T. Yen Le and A. Jan Hendriks

Department of Environmental Science, Institute for Water and Wetland Research
Faculty of Science, Radboud University, Nijmegen, The Netherlands.
Email: yenle@science.ru.nl

For a variety of elements, uptake from oral exposure is the main source of accumulation in humans and should be considered in human risk assessment. Information about the absorption efficiency via this pathway is required to quantify the uptake via oral exposure as well as the total uptake. This information additionally provides insight into the trophic transfer of elements. Moreover, the absorption efficiency must be related to chemical properties of elements to increase the potential for extrapolation to a number of elements. Data on the absorption efficiency were collected from experimental studies on humans and animals and subsequently related to chemical properties of elements. The trend of variations in the absorption efficiency among elements, both metals and non-metals, could be explained well in relation to different element-specific properties. Moreover, food characteristics, such as the chemical form of the intake and diet composition, should be considered in combination with the chemical properties of elements to obtain more reliable estimations of the absorption efficiency and increase the extrapolation potential to different exposure conditions.
Antiperspirants with aluminium salts and the relation to breast cancer.

Caroline Linhart\textsuperscript{a}\textsuperscript{*}, Nicole Concin\textsuperscript{b}, Susanne Taucher\textsuperscript{b}, Herbert Lindner\textsuperscript{c}, Hanno Ulmer\textsuperscript{a}

\textsuperscript{a}Department of Medical Statistics, Informatics and Health Economics, Innsbruck Medical University, Schöpfstraße 41/1, 6020 Innsbruck, Austria

\textsuperscript{b}Department of Gynecology and Obstetrics, Innsbruck Medical University, Anichstrasse 35, 6020 Innsbruck, Austria

\textsuperscript{c}Division of Clinical Biochemistry and Protein Micro-Analysis Facility, Innsbruck Medical University, Innrain 80, 6020 Innsbruck, Austria

Studies of antiperspirants containing aluminium salts and their effect on breast cancer have shown conflicting results. We designed a study consisting of two parts. Case-control study: History of antiperspirant use will be compared between a group of 262 female breast cancer patients aged 20–85 years (n=131 cases and) and age-matched controls (n=131) without breast cancer. A personal interview regarding individual hygiene, life-style, and aluminium exposure will be performed. The study questionnaire is partly based on the MARIE study of the German Cancer Research Centre.

Cohort study approach: A total of 100 consecutive patients requiring breast biopsy will be recruited and different tissue parts of the breast region (axilla, lateral, middle, medial, fat, and connective tissue) will be collected for determination of aluminium with atomic absorption spectroscopy (AAS). Also, these patients will be interviewed. Results will be compared between patients with subsequent breast cancer diagnosis and patients with benign outcome.
Selective elevation of circulating CCL2/MCP1 levels in patients with longstanding post-vaccinal macrophagic myofasciitis and ASIA

Josette Cadusseau\textsuperscript{a,b,c}, Nilusha Ragunathan-Thangarajah\textsuperscript{a,b,d}, Mathieu Surenaud\textsuperscript{a}, Sophie Hue\textsuperscript{a}, François-Jérôme Authier\textsuperscript{a,b,d} and Romain K. Gherardi\textsuperscript{a,b,d}

\textsuperscript{a}Inserm, U955, Créteil, 94000, France
\textsuperscript{b}Université Paris Est, Faculté de Sciences et Technologie, Créteil, 94000, France
\textsuperscript{c}Université Paris Est, Faculté de Médecine, Créteil, 94000, France
\textsuperscript{d}AP-HP, Hôpital H. Mondor - A. Chenevier, Service d’Histologie, Centre de Référence Neuromusculaire GNMH, Créteil, 94000, France

Several medical conditions sharing similar signs and symptoms may be related to immune adjuvants. These conditions described as ASIA (Autoimmune/inflammatory Syndrome Induced by Adjuvants), include a condition characterized by macrophagic myofasciitis (MMF) assessing long-term persistence of vaccine derived-alum adjuvants into macrophages at sites of previous immunisation. Despite increasing data describing clinical manifestations of ASIA have been reported, biological markers are particularly lacking for their characterization and follow up. We report an extensive cytokine screening performed in serum from 44 MMF patients compared both to sex and age-matched healthy controls and to patients with various types of inflammatory neuromuscular diseases. Thirty cytokines were quantified using combination of LuminexR technology and ELISA. There was significant mean increase of serum levels of the monocyte-chemoattractant protein 1 (CCL2/MCP-1) in MMF patients compared to healthy subjects. MMF patients showed no elevation of other cytokines. This contrasted with inflammatory patients in whom CCL2/MCP-1 serum levels were unchanged, whereas several other inflammatory cytokines were elevated (IL1beta, IL5 and CCL3/MIP1alpha). These results suggest that CCL2 may represent a biological marker relevant to the pathophysiology of MMF rather than a non-specific inflammatory marker and that it should be checked in the other syndromes constitutive of ASIA.
Tyrosine as a Depot Adjuvant for use in Allergy Specific Immunotherapy

Simon J Hewings and Murray A Skinner

Allergy Therapeutics, Dominion Way, Worthing, West Sussex, BN14 8SA
Email: murray.skinner@allergytherapeutics.co.uk

Candidate allergy therapies have been evaluated to consider the benefits of using the natural product tyrosine as a depot adjuvant in subcutaneous immunotherapy (SCIT) rather than conventional adjuvants such as calcium phosphate and aluminium.

Tyrosine is naturally metabolised and we have investigated the pharmacokinetics of tyrosine showing it has a half-life at the injection site of 48 hours, thereby controlling active release and positive immune exposure. This is a particular benefit for allergy SCIT which is traditionally long course treatment, thereby minimising the accumulation of non-biodegradable adjuvant.

We have demonstrated that tyrosine can act as an adjuvant to induce increased IgG1 and IgG2 antibody production when the tyrosine is adsorbed to allergen preparations.

These findings form the basis for using tyrosine as a safe and efficacious SCIT platform applicable for treatment of any allergy.
Aluminium adjuvants potentiate the immune response via interaction with dendritic cells – but where does the aluminium go?

Matthew Mold\textsuperscript{a}, Håkan Eriksson\textsuperscript{c}, Peter Siesjö\textsuperscript{b}, Emma Shardlow\textsuperscript{a}, Christopher Exley\textsuperscript{a}

\textsuperscript{a}The Birchall Centre, Lennard-Jones Laboratories, Keele University, Staffordshire, ST5 5BG, UK.
\textsuperscript{b}Department of Clinical Sciences, Glioma Immunotherapy Group, The Rausing Laboratory, Division of Neurosurgery, BMC D14, Lund University, SE-221 84 Lund, Sweden.
\textsuperscript{c}Department of Biomedical Laboratory Science, Health and Society, Malmö University, SE-20506 Malmö, Sweden.

Since their inception in the 1920s, aluminium adjuvants (Al\textsubscript{ADJ}) remain to be the only approved adjuvants for use in human vaccinations. Al\textsubscript{ADJ} potentiate the immune response, however their mechanism of action remains unclear. Such lack of knowledge poses a significant barrier to the development of new and improved adjuvants for use in human vaccine preparations and raises questions over their safety. Whilst a consensus is yet to be reached upon the aetiology of the biological activities of Al\textsubscript{ADJ}, an intense research effort has purported to the role played by dendritic cells (DCs) in potentiating the immune response upon exposure to Al\textsubscript{ADJ}.

DCs (but not eosinophils, mast cells or macrophages) are thought to be critical for the adjuvanticity of Al\textsubscript{ADJ}. In response to Al\textsubscript{ADJ} the internalisation of the target antigen has been shown to be increased as has their intracellular duration. The subsequent binding of major histocompatibility complex class II (MHCII) to phagocytosed antigen, leads to an increased antigen presenting capacity on the surface of DCs. In addition Al\textsubscript{ADJ} are thought to sustain those MHCII complexes formed on DC surfaces. In combination with other co-stimulatory molecules including CD80/86, Al\textsubscript{ADJ} enhance the binding and activation of antigen specific CD4\textsuperscript{+} T cells subsequently triggering the adaptive immune response [1, 3].

Whether aluminium can or even does enter dendritic or T helper cells remains to be elusive. A recent viewpoint is that aluminium does not enter dendritic cells, rather its avid binding to the lipid plasma cell membrane of DCs induces the observed immune potentiation effects. Herein a model of Al\textsubscript{ADJ} associated immune potentiation aims to elucidate upon the cellular location of aluminium.

Caspase-3 Short Hairpin RNA Interference Targeted to Alzheimer’s Disease Animal Model Induced by Aluminium Blocks Neural Cells Death, and Defect of Learning and Memory

Qin-li Zhang, Na Li, Xia Jiao, Li Xu, Weili Guo, Qiao Niu

Ministry of Education Key Laboratory, Department of Occupational Health, Shanxi Medical University, Taiyuan 030001 PR China. Email: niuqiao55@163.com

Apoptotic pathway is believed to represent an important mechanism for the physiological or pathological neural cell death. It has been reported that aluminum (Al) can induce apoptosis in neural cells of rodent models and is associated with Alzheimer’s Disease (AD). Al treated primary cultured neural cells were transfected with PGCsil-GFP caspase-3 shRNA vectors. The results indicated that caspase-3 silencing significantly inhibited the apoptosis induced by Al and helped the survival of neural cells. We also determined the effects of caspase-3 silencing on AD animal models induced by Al. The results demonstrated that the learning and memory performance were significantly improved and the expressions of AD specific proteins (Aβ and Tau) significantly decreased in hippocampus of caspase-3 shRNAs intra-ventricular injected AD models than those of controls. The data suggest that caspase-3 shRNAs, as inhibitors of the apoptosis pathway, perform a neuro-protective effect, and knocking-down caspase-3 gene expression may provide a valuable method for AD therapy.

The research work was supported by National Natural Science Foundation of China, 30972456, 81241098, and Shanxi Research Fund for the Returned Scholar from Abroad, 2011-key project-3
Aluminium entry into the brain: studies in the cerebral vasculature and in human brain microvessel endothelial (hBMEC) cells

Walter J. Lukiw\textsuperscript{a}, Brandon M. Jones\textsuperscript{a}, Jian-Guo Cui\textsuperscript{a}, Yuan Yuan Li\textsuperscript{a}, S. Bhattacharjee\textsuperscript{a}, James M. Hill\textsuperscript{a}, Yuhai Zhao\textsuperscript{a}, Theodore P. A. Kruck\textsuperscript{b}, Maire E. Percy\textsuperscript{b}, JR Walton\textsuperscript{c}, Aileen I. Pogue\textsuperscript{a}

\textsuperscript{a}Neuroscience Center and Department of Neurology and Ophthalmology, Louisiana State University Health Sciences Center, New Orleans, LA 70112 USA; \textsuperscript{b}Neurogenetics Laboratory, Surrey Place Centre & Department of Physiology, University of Toronto, Toronto, ON M5S 1A8, Canada

\textsuperscript{c}Faculty of Medicine, University of New South Wales, Sydney, NSW 2204, Australia

Once biologically available aluminium bypasses gastrointestinal and blood-brain barriers, this environmentally-abundant neurotoxin has exceedingly high affinity for the large pyramidal neurons of the human hippocampus. The same anatomical regions of the brain are also targeted by the earliest signs of Alzheimer’s disease (AD) type neuropathology. The mechanism for the selective targeting of aluminium into the hippocampus of the brain is not well understood. In an effort to improve our understanding of this targeting and pathological aluminium entry system, in these studies we examined the aluminium content of the arteries that supply blood to the hippocampus, including the aorta, the common carotid artery, the internal cerebral artery and the middle and posterior cerebral arteries. In aged AD patients we found a gradient of increasing aluminium concentration with the highest aluminium levels in the posterior cerebral artery that immediately supplies blood to the hippocampus. Primary cultures of human brain microvessel endothelial (hBMECs) cells that line the microvasculature of the brain, were found to have an extremely high affinity for aluminium when compared to other endothelial and human brain cell types. Interestingly, hBMEC cells treated with nanomolar aluminium sulfate displayed NF-\textsuperscript{k}B-DNA activation and up-regulation of pro-inflammatory micro RNAs (miRNAs) that are characteristic of AD brain. Together, these results suggest that endothelial cells that line the cerebral vasculature and microvasculature and direct blood supply blood to the hippocampus, may have biochemical attributes conducive to directing and targeting aluminium to selective anatomical regions of the brain, such as the hippocampus, with downstream pro-inflammatory and pathogenic consequences.
Acknowledgements: Human brain tissues were provided in part by the Oregon State University Health Science Centre, the Louisiana State University Health Sciences Center Brain Bank, the Harvard Brain Tissue Bank, and by the Memory Impairments and Neurological Disorders (MIND) Institute at the University of California, Irvine Alzheimer’s Disease Research Center (UCI-ADRC; funded in part though NIA P50 AG16573). Thanks are also extended to the physicians, neuropathologists and families who have kindly consented to provide human brains for study. Research on the structure and function of aluminum, cerebral vascularization, NF-κB and miRNA expression, speciation and complexity in AD brain in the Lukiw laboratory were supported through Grant Number P20RR016456 from the National Center for Research Resources (NCRR), Translational Research Initiative (TRI) Grants from LSU Health Sciences Center New Orleans (WJL), an Alzheimer Association Investigator-Initiated Research Grant IIRG-09-131729 (WJL), and NIH NIA Grants AG18031 and AG038834 (WJL). The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Aging, National Center for Research Resources, or the National Institutes of Health.
Relationship of aluminium intoxication with neurodegenerative diseases

Alessandro Fulgenzi and Maria Elena Ferrero

Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano

The aetiology of neurodegenerative diseases (ND) seems to involve susceptibility genes and environmental factors. Heavy metals (HM), pesticides and phenols are considered major environmental pollutants (1). Following our study of a case of multiple sclerosis (MS) improvement due to removal of HM intoxication (2), we have examined the possible relationship between such intoxication and ND. We here report the data related to aluminium intoxication. It was present in 40% cases comprehensive of ND and healthy patients (total patients=426); in particular, the patients affected by MS represented the 70% of total ND. The mean value of aluminium present in the urine samples after intravenous treatment with the chelating agent EDTA was 127.12 μg/g creatinine (normal values ≤25). In our experience, treatment of patients with EDTA chelation therapy was able to improve patient symptoms.

References


Colocalization of aluminium and iron in cell nuclei in the brains of patients with Alzheimer’s disease

S.Yumoto\textsuperscript{a}, S. Kakimi\textsuperscript{b}, H. Souma\textsuperscript{b} and A. Ishikawa\textsuperscript{b}

\textsuperscript{a}Yumoto Institute of Neurology, Tokyo, Japan
\textsuperscript{b}Nihon University, Tokyo, Japan

There is increasing evidence that metal-induced oxidative stress plays a pivotal role in the pathogenesis of Alzheimer’s disease (AD). 8-Hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, was demonstrated in nuclear DNA in the AD brain. Iron is a transition metal capable of generating hydroxyl radicals that can oxidize DNA. Aluminium has been reported to facilitate iron-mediated oxidative reaction. In this study, elements contained in the cell nuclei in AD brains were examined by scanning electron microscopy equipped with energy-dispersive X-ray spectroscopy (SEM-EDX). Elevated levels of Al and Fe were demonstrated in the nuclei of nerve and glial cells. The highest concentrations of both Al and Fe were measured in the nucleoli of nerve cells. We hypothesized that Al and Fe colocalized in the nuclei of brain cells might be involved in the oxidation of nuclear DNA, eventually causing neurodegeneration and the development of AD.
Aluminium, illicit drugs, neuropsychiatric impairment and disability: an evidence-based approach.

Paolo Prolo

Swiss Disability Insurance, Bellinzona, Switzerland

Societal impact of substance abuse is substantial. Substance-related disorders have an impact of more than a trillion dollars worldwide. Claims because of either cognitive impairment or personality disorders have been increasing rapidly by subjects known for illicit drug use in our disability insurance setting. This is mostly true with subjects snorting heroin, smoking marijuana and/or so called “designer” drugs. Evidence-based guidelines are needed that use systematic literature searches and establish assessment specific criteria. We selected studies on cognitive impairment, dementia, all major psychiatric disorders linked to both traditional and “designer” illicit drugs, aluminium (Al), silica, manganese published from January 1966 to September 2012. As expected, none of those is a randomized study. However, there is enough evidence that identify dose-response relationships between Al, neuropsychiatric impairments and illicit drug use, which may provide not only new routes to disease treatment or lifestyle modification, but also raise stakeholders’ attention to the issue.
Cognitive disorders and tau-protein expression among retired smelting workers exposed to aluminium

Xiao-ting Lu, Rui-feng Liang, Zhi-jian Jia, Hao Wang, Bao-long Pan, Qin-li Zhang, Wei-li Guo, Xiu-liang Ji, Qiao Niu

Department of Occupational Health, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi 030001, China. E-mail: niuqiao55@163.com

Purpose: To evaluate the relationship among cognitive functions, tau-protein expression and exposure to aluminium.

Methods: 66 Al-exposed workers came from one aluminium electrolyzing plant, and 70 unexposed controls were selected. Cognitive functions were assessed by the Mini Mental State Examination (MMSE). The serum Al level was measured with inductively coupled plasma mass spectrometry (ICP-MAS). The tau-protein expression in human peripheral blood lymphocytes were analyzed by western blot, which is including total tau protein (tau5), p-tau396, p-tau262, p-tau231, p-tau181.

Results: Significantly higher internal doses of serum Al were found in the Al-exposed workers compared to the control group. Cognitive functions test showed significant difference in the MMSE scores, the scores of time and place orientation, short-term memory, calculation ability and language skills between the exposed and the control population. 12 mild cognitive impairment (MCI) cases in the exposed group and 4 MCI cases in the unexposed group were diagnosed respectively according to the total score of MMSE, and there was a significant difference between the two groups. Serum aluminium concentrations, year of education, age and gender were the main factors which affected the total score of MMSE. There was a significant correlation between MCI detection rate and serum aluminium concentration with logistic regression analysis. The protein expression of tau5, P-tau181, P-tau231, P-tau396 in MCI patients increased significantly higher than those of non-MCI groups. Significantly higher P-tau181, P-tau231, P-tau396 protein levels were found in the Al-exposed workers compared to the control group.

Conclusions: The cross-sectional study suggested that long-term exposure of aluminium can cause cognitive disorders, and may be a risk factor for MCI. Old, male, low cultural level and high serum aluminium level may be risk factors for cognitive impairment. P-tau181, P-tau231, P-tau396 may be useful in the monitoring of cognitive decline of Al-exposed workers.

Support in part by NFSC30910103003 and NFSC81001241, International Collaborative Project of Shanxi Province 2010081059.
Spectrometric methods to analyse and quantify silicon content in mineral water samples.

Krista Jones* and Christopher Exley

The Birchall Centre, Lennard-Jones Laboratories. Keele University, STAFFS, ST5 5BG, United Kingdom

Silicon is the second most abundant element in the Earth’s crust. Silicon containing compounds have been used to strengthen bones, hair as well as to enhance the immune system (Jurkic et al, 2013). Recently, scientists have been drawn to the therapeutic effects of orthosilicic acid, Si(OH)₄, the bioavailable form of silicon. These effects have been seen in patients suffering with neurodegenerative diseases, such as Alzheimer's Disease (Davenward et al, 2013). Although a mechanism has yet to be established, it is believed that silicon increases aluminium excretion and therefore alleviating the body burden of aluminium. Many varieties of mineral water advise a certain silicon content in mg/L, although this does not represent the content of silicic acid in the waters. In this investigation I have used the molybdenum blue method (R.K. Iler 1979) to colourimetrically quantify the concentration of silicic acid in these waters. Total silicon content was quantified by graphite furnace atomic absorption spectroscopy (GFAAS) (Exley et al, 2006). This compares the amount of orthosilicic acid with the total silicon content in the mineral water. Suppliers of these waters state total silicon values as opposed to the therapeutic orthosilicic acid content, of which the concentration can be considerably lower than suggested from the results shown in this investigation.


Aluminium, Copper, Zinc, Iron, Lead and diabetes risk in a colorectal screening

S. Polizzi, M. Ferrara, M. Bugiani, A. Schiavone, P. Panarisi.

Dept. of Oncological Prevention – Occupational and Environmental Health
NHS- ASLTO5 Carignano (TURIN) – ITALY. E-mail: mdl8to@cometacom.it

OBJECTIVE— Starting from the assumption that there are conflicting results from in vitro study, that have suggest the protective role of copper to prevent amyloidogenesis in pancreatic β cells, and clinical findings in humans that suggest a diabetic risk in subjects with elevated haematic levels of copper, we examined the association between serum trace metals (aluminum, copper, iron, lead, manganese, zinc) concentration and the risk of diabetes.

RESEARCH DESIGN AND METHODS— We examined the correlation among levels of some metals (Cu, Zn, Al, Fe, Pb) with glycated haemoglobin (HbA1c) in a cross-section study on 811 subjects, both male (479 cases) and female (332 cases), 59-69 years old, invited at national coloncancer screening programme by faecal occult blood test (FOBT) in Turin (ITALY). For every subject we obtained anthropometric measurements: weight, height and Body Mass Index – BMI.

We analyzed the Odd Ratio of diabetes risk (HbA1c ≥ 6.5%) according to the interquartile range of the metals’ levels.

RESULTS— We found an odd ratio (OR) of being at risk of diabetes, according to interquartile range of metals, as reported: Aluminium was correlated with a OR of 3.12 (CI 1.21-8.19) only for group 4; lead showed an OR of 0.32 (CI 0.16-0.61) and 0.54 (CI 0.30-0.97) respectively for group 3 and 4. We failed to detect any significant difference for the other metals examined, even if Cu and Zn seemed to have a trend, with protective effects from low to high quartile.

CONCLUSIONS— Our result show a significant, and opposite, effects of Aluminium and Lead on diabetes tendency, with the first increasing the risk and the latter reducing. Copper and zinc showed a trend in reducing the risk but these data were not statistically significant.
From the coordination chemistry to the biological chemistry of Aluminium

Tamás Kiss

University of Szeged, Department of Inorganic and Analytical Chemistry, Bioinorganic Chemistry Research Group of the Hungarian Academy of Sciences, University of Szeged, Dóm tér 7, H-6720 Szeged, Hungary. Email: tkiss@chem.u-szeged.hu

My first meetings with Al started with studying the coordination chemistry of Al with aromatic and aliphatic hydroxycarboxilyc acid derivatives, some of them having strong biological importance, e.g. salicylic acid, catechol derivatives, lactic acid, malic acid, citric acid. We studied the role of the position of the different substituents on the benzene ring on the Al binding ability, and found that carboxylate can behave as a strong enough anchoring donor to promote ionization and coordination of the very weakly acidic alcoholic-OH to the hard Al(III) in the acidic pH range to prevent hydrolysis of the metal ion. This was the start 30 years ago and the present is preparation of Al(III) (and other metal ion) chelators to control metal ion homeostasis in order to cure neurological disorders. And between? My adventures in Al chemistry. I will talk about that.

N.B: *Denotes a presentation given by a student. Presenting authors are underlined