Eleventh Keele Meeting on Aluminium

2015

The Natural History of Aluminium

Past, Present and Future

February 28th - March 4th

Book of abstracts

Lille, France

Hotel Couvent des Minimes

Keele University

Université de Lille 2 Droit et Santé

DigestScience

Fondation de recherche sur les maladies de l'appareil digestif et la nutrition

Eleventh Keele Meeting on Aluminium

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Keele University

Université de Lille 2 DROIT ET SANTÉ

DigestScience
Fondation de recherche sur les maladies de l'appareil digestif et la nutrition

The Natural History of Aluminium
Past, Present and Future

Scientific programme

Saturday 28th February 2015

17.00 Registration and Poster Assembly

19.30 Welcome to Meeting / Welcome Buffet

Sunday 1st March 2015

The Conference is Open!

Session 1
Environmental, Biological and Medicinal Chemistry

*Denotes presentation by a student.

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

08.55 Introduction by the Chair

9.00 Platform 1
Aluminium in the open ocean: simulation and observation.
Marco van Hulten (LSCE, France)

9.20 Discussion

9.30 Platform 2
Density-functional insights into the structure and formation of hydroxyaluminosilicates.
James Beardmore (Keele University, United Kingdom)

9.50 Discussion

10.00 Platform 3
Aluminium in biological environments: a computational approach.
Xabier Lopez (University of San Sebastian, Spain**)**

10.20 Discussion

10.30 **Coffee**

10.50 Platform 4
Identifying aluminium binding sites in amyloid β peptide by means of computational chemistry.
Jon Mujika (University of San Sebastian, Spain)

11.10 Discussion

11.20 Oral Poster 1
Effect of Al(III) on coenzyme NADH in solution.
Elena Formoso (University of San Sebastian, Spain)

11.25 Discussion

11.30 Platform 5
Irreversible denaturation of proteins through aluminium-induced formation of backbone ring structures.
Bo Song (Shanghai Institute of Applied Physics, China)

11.50 Discussion

12.00 Oral Poster 2*
A potentiometric, spectrophotometric and NMR study of protonation and formation equilibria of two new aluminium chelators.
Guadalupe Jaraquemada-Pelaez (University of Cagliari, Italy)

12.05 Discussion

12.10 Oral Poster 3*
A new series of kojic acid derivatives as ligands for aluminium.
Delara Mansoori (University of Cagliari, Italy)

12.15 Discussion

12.20 Platform 6*
The relationships between the physicochemical properties of aluminium-based adjuvants and their relative cytotoxicities in a THP-1 cell line.
Emma Shardlow (Keele University, United Kingdom)

12.40 Discussion

Chair's Summary

13.00 **Lunch**

Session 2
Bacteria, Fungi and Plants

*Denotes presentation by a student.

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

14.15 Introduction by the Chair

14.20 Platform 7
Fungal heterotrophic leaching of red mud: biogenic organic acid contribution to aluminium mobility in the environment.
Martin Urík (Comenius University in Bratislava, Slovakia)

14.40 Discussion

14.50 Platform 8*
Characterisation of aluminium toxicity in pollutant degrading bacteria.
Sean Booth (University of Pennsylvania, USA)

15.10 Discussion

15.20 Platform 9
Migration and transformation of inorganic and organic complexes of aluminium from soil to leaf through root, stem and twig of *Betula pendula*.
Marcin Frankowski (Adam Mickiewicz University in Posnań, Poland)

15.40 Discussion

15.50 Platform 10
Aluminium rhizotoxicity in solution culture: The past and the present.
Peter Kopittke (The University of Queensland, Australia)

16.10 Discussion

16.20 **Tea**

16.40 Platform 11*
Aluminium accumulation in three *Symplocos* species from Central Sulawesi.
Marco Schmitt (Ulm University, Germany)

17.00 Discussion

17.10 Oral Poster 4*
Comparative analysis of aluminium accumulation in leaves of several woody aluminium accumulators.
Eriko Maejima (Hokkaido University, Japan)

17.15 Discussion

17.20 Oral Poster 5
Differences and similarities in the characteristics of aluminium accumulation in various aluminium accumulators.
Toshihiro Watanabe (Hokkaido University, Japan)

17.25 Discussion

17.30 Platform 12
Signalling aluminium response in two contrasting rice cultivars.
Charlotte Poschenrieder (The Autonomous University of Barcelona, Spain)

17.50 Discussion

18.00 Platform 13
Sustainable crop growth and yields on acid soils.
Jiping Liu (Cornell University, USA)

18.20 Discussion

Chair's Summary

18.30 **End of the first day**

20.15 **Dinner**

21.15 **Poster session and BEER TASTING**

Monday 2nd March 2015

Session 3
Animal Models

*Denotes presentation by a student.

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

08.55 Introduction by the Chair

09.00 Platform 14
Aluminium toxicity in *Caenorhabditis elegans* experimental model of Alzheimer's disease.
Rosilene Kaiser (Federal Institute for Education, Science and Technology of Rio Grande do Sul, Brasil)

09.20 Discussion

09.30 Oral Poster 6*
Investigation of the toxicity of dairy industry effluents on the nervous system of *Caenorhabditis elegans*.
Vanessa Oliveira (Federal Institute for Education, Science and Technology of Rio Grande do Sul, Brasil)

09.35 Discussion

09.40 Oral Poster 7
Experimental intoxication with aluminium chloride induces behavioural and mitochondrial alterations in mice and decreased viability, proliferation and morphological changes in neurospheres.
Jessié Gutierres (Federal University of Santa Maria, Brasil)

09.45 Discussion

09.50 Platform 15*
Aluminium as an environmental risk factor in visceral hypersensitivity.
Nicolas Esquerre (University of Lille, France)

10.10 Discussion

10.20 **Coffee**

10.40 Platform 16
The RAS/P13K pathway is involved in damage to long-term potentiation induced by acute aluminium treatment.
Qiao Niu (Shanxi Medical University, China)

11.00 Discussion

11.10 Oral Poster 8*
Non-apoptotic role of caspase 3 on long-term depression in area CA1 of the rat hippocampus following aluminium exposure in vivo.
Huifang Zhang (Shanxi Medical University, China)

11.15 Discussion

11.20 Platform 17
Aluminium can cross and damage the intestinal epithelial barrier *in vitro.*
Mathilde Body-Malapel (University of Lille, France)

10.40 Discussion

11.50 Platform 18*
Effects of aluminium adjuvants on social behaviour in mice.
Sneha Sheth (University of British Columbia, Canada)

12.10 Discussion

12.20 Oral Poster 9
Gene-toxin synergy in the brain of an autistic mouse model.
Dan (Alice) Li (University of British Columbia, Canada)

12.25 Discussion

12.30 Platform 19
Assessment of the neurotoxic effects of aluminium vaccine adjuvant injected into mice.
Guillemette Crépeaux (Paris Est University, France)

12.50 Discussion

13.00 Oral Poster 10
Fluorescent nanodiamonds as a relevant tag for the assessment of alum adjuvant particle biodisposition.
Housam Eidi (Paris Est University, France)

13.05 Discussion

Chair's Summary

13.10 **Lunch**

Free afternoon,

3.00-5.00 **Exclusive guided walking tour of the old Lille**

20.00 **Dinner in the heart of Lille in the estaminet* Barbue d'Anvers**

*an estaminet is a typical northern French inn

Tuesday 3rd March 2015

Session 4
Human Exposure

*Denotes presentation by a student.

Chair: *Philippa Darbre* (University of Reading, United Kingdom)

8.25 Introduction by the Chair

8.30 Platform 20

Elucidating upon the cellular uptake of aluminium based adjuvants and the amyloidogenic $A\beta_{42}$ peptide 'antigen' in a monocytic THP-1 cell line.

Matthew Mold (Keele University, United Kingdom)

08.50 Discussion

09.00 Platform 21

Aluminium treatment of THP-1 monocytic cell line induces alterations in inflammatory and proteolytic pathways.

Ferdinando Mannello (University 'Carlo Bo' of Urbino, Italy)

09.20 Discussion

09.30 Oral Poster 11*

Effect of aluminium on migration of oestrogen unresponsive MDA-MB-231 human breast cancer cells in culture.

Ayse Bakir (University of Reading, United Kingdom)

09.35 Discussion

09.40 Platform 22*
Antiperspirants, aluminium salts and breast cancer: Preliminary data from a clinical trial.
Caroline Linhart (Medical University of Innsbruck, Austria)

10.00 Discussion

10.10 **Coffee**

10.30 Platform 23*
A pilot study measuring aluminium in bone in Alzheimer's and reference subjects: Work in progress.
Hedi Mohseni (McMaster University, Hamilton, Canada)

10.50 Discussion

11.00 Platform 24
Aluminium and bone: New clinical circumstances where aluminium deposition occurs in the calcified matrix of bones.
Daniel Chappard (University of Angers, France)

11.20 Discussion

11.30 Oral Poster 12
Study of aluminium in cancellous and compacted bone in humans.
Anetta Ziola-Frankowska (Adam Mickiewicz University in Posnań, Poland)

11.35 Discussion

11.40 Platform 25*
Impact of aluminium on human fertility.
Jean-Philippe Klein (University of Lyon, France)

12.00 Discussion

12.10 Oral Poster 13*
Aluminium and silicon in human sweat.
Krista Jones (Keele University, United Kingdom)

12.15 Discussion

12.20 Platform 26
Sea vegetables and aluminium.
Shunsuke Meshitsuka (Tottori University, Japan)

12.40 Discussion

12.50 Demonstration
DEVEXI Health Analytics Research Database: Applications in human exposure to aluminium.
Claire Dwoskin (Childrens Medical Safety Research Institute, USA)

13.05 Discussion

Chair's Summary

13.10 **Lunch**

Session 5
Human Health

All Platform Presentations in Session 5 are 15 minutes plus 5 minutes for Discussion
*Denotes presentation by a student.

Chair: *Cecile Vignal* (University of Lille, France)

14.25 Introduction by the Chair

14.30 Platform 27
Aluminium and health: A century of controversy, research and regulation.
Florence Hachez-Leroy (University of Lille, Nord de France, France)

14.45 Discussion

14.50 Platform 28
Aluminium-induced NF-κB -mediated pro-inflammatory signalling and amyloidogenesis in human brain microglial cells and tissues.
Walter Lukiw (Louisiana State University Health Sciences Center, USA)

15.05 Discussion

15.10 Platform 29
Colocalisation of aluminium and iron in neurodegenerative diseases.
Sakae Yumoto (Yumoto Institute of Neurology, Tokyo, Japan)

15.25 Discussion

15.30 Platform 30
Aluminium intoxication and treatment with chelation therapy.
Maria Elena Ferrero (University of Milan, Italy)

15.45 Discussion

15.50 Platform 31
Can 5-hydroxy-2-(hydroxymethyl)pyridine-4(1H)-one tautomer cure diseases of aluminium overload?
Joanna Lachowicz (University of Cagliari, Italy)

16.05 Discussion

16.10 Platform 32
Clinical features and functional brain imaging in patients with persisting aluminium hydroxide-induced macrophagic myofasciitis (MMF).
François-Jérôme Authier (Paris Est University, France)

16.25 Discussion

16.30 Platform 33
Aluminium-induced entropy in biological systems.
Robert Davidson (PhyNet Inc., USA)

16.45 Discussion

16.50 Platform 34
Predictors of costs of care in aluminium-related dementia: A stress simulation hypothesis.
Paolo Prolo (Swiss Disability Insurance, Bellinzona, Switzerland)

17.05 Discussion

Chair's Summary

17.10 **Tea**

Final Session

Chair: *Chris Exley* (Keele University, UK)

17.30 Introduction by the chair to the JD Birchall Memorial Lecture

17.35 The JD Birchall Memorial Lecture
Plants do some surprising things to deal with toxic aluminium in soil!
Leon Kochian (Cornell University, USA)

18.35 Discussion

18.45 **CONCLUSION OF MEETING**

20.00 Conference Dinner

List of Additional Posters

*Denotes presentation by a student.

Session 1

Poster 1*
Application of ion selective electrodes and optical sensors for the detection of aluminium(III) ions in clinical samples.
Lukasz Mendecki (Keele University, United Kingdom)

Session 2

Session 3

Poster 1*
Experimental reproduction of ovine ASIA syndrome: From symptoms to genomics.
Javier Asín (University of Zaragoza, Spain)

Poster 2*
Effects of aluminium on miR29 and beta amyloid (1-42) in rat brain.
Linping Wang (Shanxi Medical University, China)

Session 4

Poster 1*
Effect of aluminium on DNA damage and DNA repair in MCF10A immortalised non-transformed human breast epithelial cells.
Abdullah Farasani (University of Reading, United Kingdom)

Poster 2
Microcrystalline tyrosine (MCT): Its use a depot adjuvant in allergy immunotherapy, future perspectives and applications.
Matthew Heath (Allergy Therapeutics, United Kingdom)

Poster 3*
The fate of L-tyrosine in immunotherapy: What happens at the injection site?
Iulia Neagu (Keele University, United Kingdom)

Poster 4*
The use of antiperspirants containing aluminium salts and its relation to breast cancer: Methods and implementation of biospecimen sampling.
Dominik Panosch (Medical University of Innsbruck, Austria)

Poster 5*
Aluminium in human brain tissue: Measurement and imaging.
Ambreen Mirza (Keele University, United Kingdom)

Poster 6
Aluminium is deposited in the calcified matrix of bone exostoses.
Daniel Chappard (University of Angers, France)

Poster 7
Al exposure from food contact materials made of aluminium
Veronika Fekete (Scientific Institute of Public Health, Brussels, Belgium)

Poster 8
Accumulation of aluminium in human eye tissue: a role in age-related macular degeneration?
Alex Langford-Smith (Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester, Manchester, UK)

Session 5

Poster 1
Small sensory fiber neuropathy in patients with aluminium hydroxide-induced macrophagic myofasciitis (MMF)
François-Jérôme Authier (Paris Est University, France)

Poster 2
Do we need a holistic understanding about the effects and working mechanism of aluminium?
Esko Meloni (Enopop T:mi, Finland)

---------- Thanks to our sponsors ----------

Aluminium in the open ocean: simulation and observation

Marco van Hulten
Laboratoire des Sciences du Climat et l'Environnement (LSCE)

The objective of this study is to come to a better understanding of the behaviour of Al in the ocean. Recently, many accurate measurements in the open ocean have been performed as part of the Geotraces programme. According to these measurements, dissolved Al concentrations in the open ocean (away from coastal areas) are in the order of 1 nM in the Pacific and polar oceans, while they reach up to 50 nM in the surface waters of the Atlantic and Indian Oceans (Middag et al. in preparation).
We used a global biogeochemical general circulation model to simulate Al in the ocean. In the first version of this model the measured dissolved Al concentration in the upper part of the ocean has been simulated reasonably well with only a dust source and adsorptive scavenging as the removal process (Van Hulten et al. 2013). In a new study the simulation is significantly improved by the addition of a sediment resuspension source that depends on the amount of sedimented absorbed Al and the concentration of silicic acid in bottom water (Figure 1). This supports the idea that the most significant sources of Al to the ocean are dust deposition and sediment resuspension (Van Hulten et al.2014).

References

Hulten, M.M.P. van, A. Sterl, J.-C. Dutay, A. Tagliabue, M. Gehlen, Baar, H.J.W. de, and R. Middag (2013). "Aluminium in an ocean general circulation model compared with the West Atlantic Geotraces cruises". In: J. Mar. Syst. 126, pp. 3–23. doi: 10. 1016/j.jmarsys.2012.05.005. arXiv: ao-ph/1202.4679.
Hulten, M.M.P. van, A. Sterl, R. Middag, H. de Baar, M. Gehlen, J.-C. Dutay, and A. Tagliabue (2014). "On the effects of circulation, sediment resuspension and biological incorporation by diatoms in an ocean model of aluminium". In: Biogeosciences 11 (14), pp. 3757–3779. doi: 10.5194/bg-11-3757-2014. arXiv: ao-ph/1405.5752.
Middag, R., Hulten, M.M.P. van, Aken, H.M. van, M. Rijkenberg, L. Gerringa, P. Laan, and Baar, H.J.W. de (in preparation). "Dissolved Aluminium in the Ocean Conveyor of the West Atlantic Ocean: Mirror Image of the Biological Cycle?"

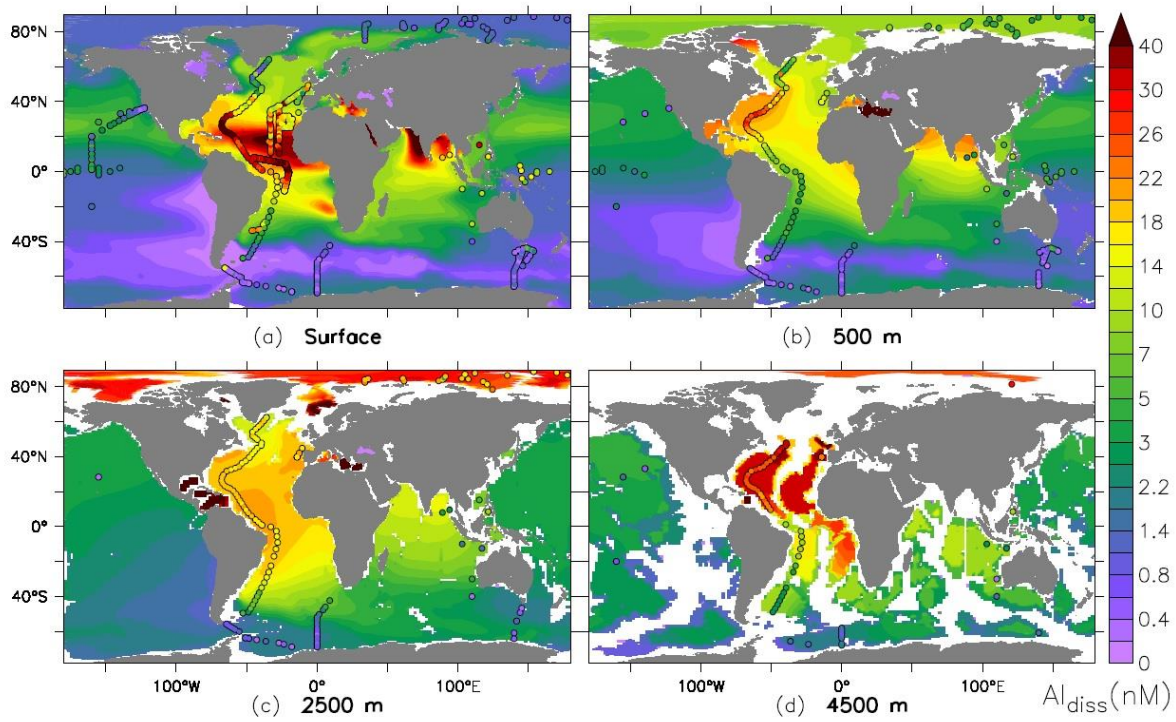

Figure 1: The simulated dissolved Al concentration (nM) of a simulation with sediment resuspension at four depths (500 yr after forking from a steady-state simulation without a sediment source). The respective observations (same depths) are presented as coloured dots.

Density-functional insights into the structure and formation of hydroxyaluminosilicates

James Beardmore[a], Xabier Lopez[b], Christopher Exley[a]
[a] Birchall Centre, Keele University, Keele, Staffs ST5 5BG, England
[b] Kimika Fakultatea, Euskal Herriko Unibersitatea, Av de Tolosa, 54, 20018 Donostia-San Sebastian, Gipuzkoa, Spain

Aluminium is the third most abundant element in the lithosphere after oxygen and silicon. Despite this, it has no known biological function, due to its historical lack of biological availability. The toxic effects of biologically-available aluminium include fish death in acidified lakes, low crop yields, and neurological damage in humans. Silicon, in the form of silicic acid, $Si(OH)_4$, acts as a natural antagonist to aluminium, and in many circumstances ameliorates its toxicity. The underlying reaction is possibly the condensation of $Si(OH)_4$ on to an aluminium hydroxide ($[Al(OH)_3]_n$) template to form hydroxyaluminosilicates (HAS), and is investigated here using density functional theory. Calculations performed at the B3LYP/6-311++G(3df,2p) level of theory have been used to show how aluminium hydroxide favours a structure where aluminium atoms are linked via double hydroxy bridges, but condensation of silicic acid onto these structures may open these bridges to form single-bridged HAS particles. The energetics of formation are investigated, comparing the competition between growth of $[Al(OH)_3]_n$ units, condensation of $Si(OH)_4$ on $[Al(OH)_3]_n$ units (HAS formation), and aggregation of HAS units themselves. Although the reaction energetics imply a theoretical possibility of early, transient HAS species with an Al:Si ratio inconsistent with both HAS_A and HAS_B, such species are not observed in the lab. The quantum chemistry results are simplified and used as basic "rules" in a many-body systems approach built on a Monte Carlo lattice-gas automaton, which reproduces some of the experimentally-observed behaviour.

Aluminum in Biological Enviroments. A computational Approach

Xabier Lopez, Jon I Mujika, Elena Formoso, Noelia Luque and Jesus M. Ugalde
Kimika Fakultatea, Euskal Herriko Unibertsitatea UPV/EHU, and Donostia International Physics Center (DIPC), P.K. 1072, 20080 Donostia, Euskadi, Spain.
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The increased availability of aluminum in biological environments, due to human intervention in the last century, raises concerns on the effects that this so far "excluded from biology" metal might have on living organisms. Consequently, the bioinorganic chemistry of aluminum has emerged as a very active field of research. However, the experimental determination of structure and affinities of Aluminum-Bioligand complexes is not without difficulties and theoretical methods have emerged as a fundamental tool to unveil aluminum biochemistry. In the present talk I will review some of the recent advances made by our group on this field. Various theoretical methods (CASSCF, DFT, QM/MM and molecular dynamic simulations) were used to characterize a variety of Aluminum–-Bioligand complexes. We will focus on the effect that Aluminum could have in the tautomerization equilibria of α-ketoglutarate, the importance of Aluminum-phosphate interactions and the possibility of superoxide stabilization by Aluminum.

[1] *J.I. Mujika, E. Rezabal, J. M. Mercero, F. Ruipérez, D. Costa, J. M. Ugalde, X. Lopez.* Computational And Structural Biotechnology Journal, **2014**, 9, e201403002
[2] *Jon I Mujika, Jesus M. Ugalde and X. Lopez,*
J. Phys. Chem B, **2014**, 118, 6680–6686 (2014). DOI: 10.1021/jp502724w
[3] *N. B. Luque, Jon I. Mujika, E. Rezabal, J. M. Ugalde, X. Lopez*,
“Mapping the affinity of aluminum(III) for biophosphates: interaction mode and binding affinity in 1:1 complexes” Physical Chemistry Chemical Physics, **2014**, 16, 20107-20119 DOI: 10.1039/C4CP02770A

Identifying aluminum binding sites in amyloid Aβ peptide by means of computational chemistry

Jon I. Mujika, Xabier Lopez, Jesus M. Ugalde
Kimika Fakultatea, Euskal Herriko Unibertsitatea (UPV/EHU) and Donostia International Physics Center (DIPC), Donostia, Basque Country (Spain)

Aluminum has been identified as a risk factor in Alzheimer's disease (AD)[1]. One of the main hallmarks in AD is the formation of extracellular deposits, known as senile plaques. These plaques consist of insoluble amyloid deposits composed primarily of aggregates of amyloid-β (Aβ) peptides in their fibril form. Several studies indicate that metal ions seem to influence Aβ folding process, aggregation and deposition. Besides, Al(III) was identified in the core of senile plaques[2].

However, most of the investigations centered on aluminum focus on the biophysics of the Aβ aggregates formed in presence of the metal, providing relevant information about their growing pattern. However, unlike with copper or zinc, little is known about the coordination mode of aluminum 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 Aβpeptides, as it allows characterizing the systems into an atomic level.

Due to the lack of unambiguous data on the coordination mode of aluminum to Aβ peptides, different strategies were followed to tackle the problem. The investigation presented herein includes: i) survey of several databases in order to infer the preferential coordination mode of Al(III) in other systems, ii) calculations of small cluster models to determine which ligands constitute the most favorable binding site for Al(III), iii) based on these data, diverse structures of Al(III)-bound Aβ peptides were characterized. All the results, in overall, show a clear preference of Al(III) towards carboxylic groups, concluding that three of the aspartate and glutamate residues present in the Aβ peptide might be involved in the binding of Al(III).

[1] Tomljenovic, L. *J. Alzheimers Dis.* (**2011**), 23, 567
[2] Yumoto, S., Kakimi, S., Ohsaki, A., Ishikawa, A. *J. Inorg. Biochem.* (**2009**), 103, 1579

Effect of Al(III) on coenzyme NADH in solution

Elena Formoso, Jon I. Mujika and Xabier Lopez
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β-Nicotinamide adenine dinucleotide, NAD^+, and its reduced form β-dihydronicotinamide adenine dinucleotide, NADH, act as coenzyme in enzymes that are ubiquitously involved in biological redox processes and play an important role in the conversion of chemical energy to useful metabolic energy.[1] The NADH is composed of two 5'-nucleotides, AMP and dihydronicotinamide ribose 5'-phosphate, which are linked together by pyrophosphate bridge, see Figure 1.

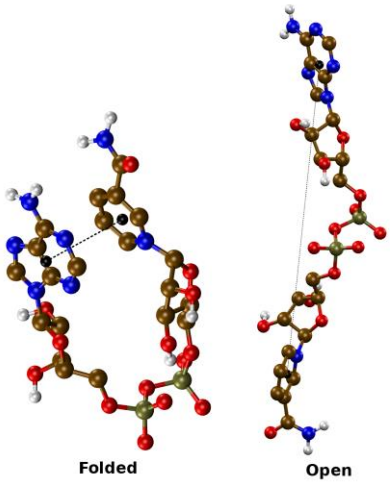

Figure 1: Folded and open conformation forms of NADH.

It is known that NADH two main limiting conformational forms existed in solution: extended/open form in which adenine and nicotinamide group lie apart and folded/stacked form in which the two base are stacked parallel to each other, see Figure 1. Normally, in solution these conformational states are in rapid equilibrium with each other, and the amount of each depends on the conditions of pH, solvent and temperature. The available literature data on metal ions induced the conformational changes of NADH are rather scarce. Actually, there are only a few conformational studies of Mn^{2+}, Li^+, Al^{3+}, La^{3+} and Eu^{3+} with NAD^+/NADH,[2-6] even though some metal ions have very important roles in the NADH participating enzymes reactions.[7]

In particular, we are interested on creating a systematic methodology to study the effect of Al(III) on bioligands. Nowdays, we are studing the conformational change of NADH in the presence of Al(III) by means of molecular dynamics and quantum calculations using state of the art techniques such as well-tempered metadynamics.[8] We would like to compare our

results with the study made by Yang et al.[6,9] some years ago. They analyzed by means of NMR and fluorescence spectra the effect of Al(III) on NADH and suggested that Al(III) favors the folded conformation of NADH.

References

1) R. Hull, P. Conger III, and R Hoobler, Biophys. Chem. **90**, 9 (2001)
2) R. Sarma and R. Mynott, J. Am. Chem. Soc. **95**, 7470 (1973)
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4) W. Saenger, B. Reddy, K. Mühlegger and G. Weimann, Nature **267**, 225 (1977)
5) P. Bayley and P. Debenham, Eur. J. Biochem. **43**, 561 (1974)
6) X. Yang, S. Bi, L. Yang, Y. Zhu, and X. Wang, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy **59**, 2561 (2003)
7) A. Louie and T. Meade, Chem. Rev. **99**, 2711 (1999)
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9) X. Yang, Q. Zhang, L. Li, and R. Shen, J. Inorg. Biochem. **101**, 1242 (2007)

Irreversible denaturation of proteins through aluminum-induced formation of backbone ring structures

Bo Song
Shanghai Institute of Applied Physics, Chinese Academy of Sciences, China

Aluminum is widely used, but extensive reports indicate that aluminum may be a critical factor in many neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease. Unfortunately, the underlying molecular mechanism is still poorly understood, which limits efforts to prevent and treat these diseases. Using a combined approach with both *ab initio* calculations and circular dichroism (CD), X-ray photoelectron spectra (XPS), nuclear magnetic resonance (NMR), we show that aluminum ions can induce a surprising formation of backbone ring structures in a wide range of peptides including the neurodegenerative disease related motifs, which largely destabilizes the protein and results in irreversible denaturation. This behavior benefits from the special bonding property of aluminum that the aluminum ion forms chemical bonds simultaneously with both the amide nitrogen and carbonyl oxygen atoms on the peptide backbone. Particularly, the XPS and NMR experiments strongly support the theoretical prediction that the aluminum ions bound in protein are largely reduced from trivalent Al ions, while simultaneously bonding with the nitrogen and oxygen atoms. These findings provide a molecular-level understanding of the potential mechanism underlying aluminum-induced neurotoxicity, and may be helpful in novel-drug design for aluminum-related diseases, even in clues for treatment of aluminum-polluted water.

A potentiometric, spectrophotometric and NMR study of protonation and formation equilibria of two new aluminium chelators.

M. Guadalupe Jaraquemada-Pelaez,[a)] Joanna I. Lachowicz,[a)] Guido Crisponi,[a)] Valeria. M. Nurchi,[a)] Maria Antonietta Zoroddu,[b)] Massimiliano Peana [b)]

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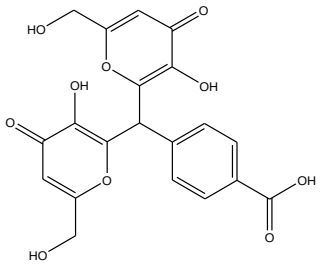

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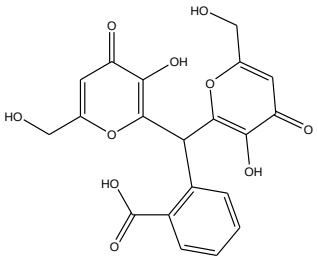

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In the frame of our research on Al^{III} and Fe^{III} chelators, a number of ligands containing two kojic acid units joined by different linkers were designed, synthesized, characterized by solid state X-ray diffraction and quantum chemical calculation, and their protonation and Al^{III} complex formation equilibria exhaustively studied with a variety of techniques (potentiometry, spectrophotometry, ESI-MS, NMR) [1-5]. The ligands bearing in the linker a vanillin/ortho-vanillin residue presented promising properties in Al^{III} chelation. Here we will present two analogous of the molecules of communication by Mansoori et al. in which the phenolic group was substituted by a carboxylic group. Being a phenolic group characterized by a pK ~ 9 and a carboxylic group by a pK ~ 4, this substitution was intended to evaluate how the value of protonation constant affects the pAl value. The topic of this presentation will be the study of protonation and Al complex formation equilibria by potentiometric, spectrophotometric and NMR methods.

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[3] L. Toso, G. Crisponi, V.M. Nurchi et al., *J. Inorg. Biochem*. **2013,** 127, 220-231
[4] L. Toso, G. Crisponi, V.M. Nurchi et al., *J. Inorg. Biochem*. **2014,** 130, 112-121
[5] V.M. Nurchi, G. Crisponi, M. Arca et al., *J. Inorg. Biochem*. **2014,** 141, 132-143

A new series of kojic acid derivatives as ligands for aluminium.

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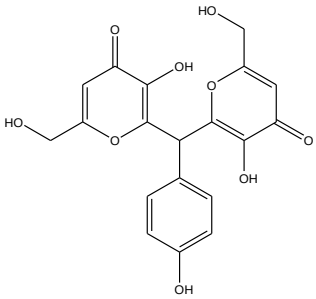
The use of chelating agents for iron and aluminium has found ever increasing attention [1-2]. The chelating agents nowadays in use, based on hydroxamic groups, hydroxyl-substituted pyridinones or aromatic ring systems, all present different drawbacks. We performed a systematic study on complex formation equilibria of various kojic acid derivatives with Fe^{III} and Al^{III} [3-7]. In particular the ligands bearing in the linker the vanillin and ortho-vanillin residues showed interesting chelating properties toward Al^{III} (pAl value 13.9 and 11.9, respectively). In this communication we will present three analogous ligands in which the methoxy group of vanillin was substituted by proton (**1**) and that of ortho-vanillin by a proton (**2**) or a nitro group (**3**), designed and synthesized to evaluate the influence of substituents on chelating properties.



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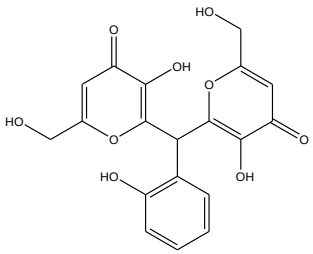

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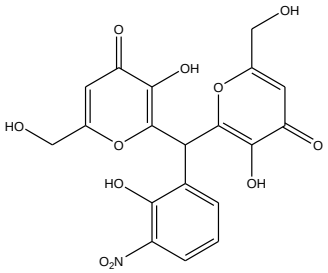

3

Protonation and Al complex formation examined by potentiometry, spectrophotometry and NMR will be discussed.

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[5] L. Toso, G. Crisponi, V.M. Nurchi et al., *J. Inorg. Biochem*. **2013,** *127*, 220-231
[6] L. Toso, G. Crisponi, V.M. Nurchi et al., *J. Inorg. Biochem*. **2014,** *130*, 112-121
[7] V.M. Nurchi, G. Crisponi, M. Arca et al., *J. Inorg. Biochem*. **2014,** *141*, 132-143

The relationship between the physicochemical properties of ABAs and their relative cytotoxicity in a THP-1 cell line

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Aluminium – based adjuvants (ABA) improve the efficacy of vaccinations by potentiating and tailoring the immune response to a co-administered pathogenic antigen. However, the aetiology and mechanisms surrounding ABA immunopotentiation *in vivo* remain generally equivocal and have yet to be fully elucidated. The cytotoxicity of both clinical and research preparations was investigated using a model monocytic leukaemia cell line (THP-1) via live/dead staining. Variance in individual compound cytotoxicity was concomitant with the differing physicochemical properties exhibited between ABAs, including solid state, particle size and zeta potential.
Research funded by Keele Acorn, Medical Research Council (MRC) & Dwoskin Foundation

Fungal heterotrophic leaching of red mud – biogenic organic acid contribution on aluminium mobility in the environment

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Bioleaching by 17 isolates of genus *Penicillium*, *Aspergillus* and *Eurotium* was compared to chemical leaching by citric, glutamic, oxalic and hydrochloric acids to evaluate fungal metabolite contribution on aluminium mobilization from hazardous red mud, toxic waste of aluminium refining, collected from an alumina plant near Kolontár in western Hungary. Fungus *A. niger* was the most efficient, with leached aluminium up to approximately 140.6 $mg.L^{-1}$ media concentration over 7-day cultivation. From genus *Penicillium*, the most efficient were species *Penicillium crustosum* and *P. palitans* with final 127 and 108 $mg.L^{-1}$ aluminium media concentrations, respectively. We discuss the significance of pH and organic acid production in this process, with comparison of biological and chemical leaching using model system with known organic acids concentrations. Furthermore, the effect of humic acid presence on microbial leaching was evaluated. The results suggest that fungal metabolites and bioaccumulation efficiency significantly promote the release of aluminium, and presence of biogenic organic acids have crucial role in red mud leaching. This work was supported by the Ministry of Education of Slovak Republic and the Slovak Academy of Sciences Scientific Grant Agency under VEGA contract Nos. 1/0836/15 and 1/0203/14.

Keywords aluminium, fungi, bioleaching, bioaccumulation

Characterization of Aluminium Toxicity in Pollutant Degrading Bacteria

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Human industrial activities release toxic pollutants into the environment. Bioremediation of organic pollutants is a cost-effective solution for preventing damaging effects in all exposed organisms. Bacteria are able to degrade organic pollutants but co-contamination with metals interferes with this process. Aluminium is a frequent co-contaminant of polychlorinated biphenyls, so bacterial cultures were grown on biphenyl in the presence of aluminium and analyzed using metabolomics to elucidate how metal toxicity specifically affects organic pollutant degradation. By identifying and quantifying the small molecules used and produced by cells a robust characterization of global cellular physiology was obtained. Al exerted its toxicity by causing DNA damage and interfering with energy producing pathways as biphenyl degradation and Krebs' cycle intermediates were accumulated, likely due to the destruction of Fe-S clusters present in many enzymes in these pathways. Knowledge of these mechanisms of toxicity can now be used to improve bioremediation strategies for co-contaminated sites.

Migration and transformation of inorganic and organic complexes of aluminium from soil to leaf through root, stem and twig of *Betula Pendula*

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The research presents the possible way of migration and transformation of inorganic and organic complexes of aluminium based on the results obtained for water extract of: soil, lateral root, tap root, stem, twig and leaf. The samples were collected in two different environments: (1) Chemical Plant in Luboń (contaminated area) and (2) Wielkopolski National Park (protected area).

The main aims of this research has been divided into two dependent parts: (I) methods development for simultaneous determination of inorganic (F^-, SO_4^{2-}, PO_4^{3-}) and organic (CH_3COO^-, $HCOO^-$, $CH_2(COO)_2^{2-}$, $C_2O_4^{2-}$, $C_3H_5O(COO)_3^{3-}$) anions which can create complexes of aluminium (HPIC method) and for speciation analysis of inorganic and organic complexes of aluminium by HPLC with DAD and fluorescence detection (II) determining how and in what way aluminium can be transport from soil to leaf and which anions plays an important role in this system.

Preliminary research indicates that both inorganic and organic aluminium ligands play an important role in migration of aluminium from soil to leaves. Determined concentration of Al-ligands was completely different for particular morphological parts of *betula pendula*, which was strongly connected with concentration of aluminium. It was also found that uptake of aluminium is limited by root system and with the concentration of e.g. Ca and Mg occurring in soil. Nevertheless a combination of the results obtained for aluminium, inorganic and organic ions and complexes of aluminium allows to confirm the results of the research.

The research was financed by the National Science Centre, Poland through research project DEC-2012/07/D/NZ8/01030

Aluminium rhizotoxicity in solution culture: The past and the present

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The rhizotoxicity of Al in acid soils has been known for > 100 years. Knowledge of soil solution composition has enabled Al toxic effects in soils to be examined in solution culture systems which mimic those in soils. However, use unrealistic solutions with 10-times ionic strength has produced erroneous results. Here, these problems are discussed based on kinetic and thermodynamic principles and data are presented. We have used a multidisciplinary approach, including high resolution kinematic analyses, molecular biology, rheology, and advanced imaging techniques, to determine the sequence of Al toxic effects. This along with appropriate nutrient solutions has now resulted in the identification of the primary lesion of Al toxicity in plant roots.

Aluminium accumulation in three *Symplocos* species from Central Sulawesi

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The aluminium-hyperaccumulating (i.e. > 1,000 mg/kg dry mass) genus *Symplocos* (Symplocaceae) is used as a mordant by Indonesian weavers for their traditional dying process. Because of destructive collecting methods, *Symplocos* trees become more and more endangered.
We collected samples from roots, leaves, bark and wood at three different sites along an altitudinal gradient (between 1,600 and 2,400 m.a.s.l.). MPAES analyses were conducted to measure the concentration of Al, Fe, Mg, K and Ca. Furthermore, the soil pH was measured, Al localisation in various tissues was examined, and roots were investigated for mycorrhizae. These results contribute not only to a better understanding of the Al transport and storage capacity within *Symplocos* trees, but also suggest that Indonesian weavers could achieve sustainable use by collecting old leaves from the forest floor. In fact, our findings show that Al-concentrations in leaves are age dependent, with the highest Al levels occurring in old leaves.

Comparative analysis of aluminium accumulation in leaves of several woody aluminium accumulators

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Some plants growing naturally in acidic soil can accumulate over 1000 mg kg^{-1} of aluminium (Al) in their leaves, whereas many other plants have developed the ability to exclude Al as an Al tolerance mechanism. The latter are called 'Al accumulators'. Al accumulators are widely distributed throughout the plant kingdom. However, the differences in the physiological and chemical characteristics of Al accumulation among various plant species are not well understood. We investigated the differences in Al accumulation concentration, chemical form and localisation in several woody Al accumulators. The characteristics of Al accumulation under hydroponic conditions in 2 Melastomataceae species (*Melastoma malabathricum* and *Tibouchina urvilleana*) were different, but *M. malabathricum* showed similarities to *Symplocos chinensis* (Symplocaceae). Inorganic monomeric Al and Al-oxalate complexes constituted the main soluble Al component in the leaves of the plant species investigated. However, their ratios differed among plant species, irrespective of their Al accumulation potential.

Differences and similarities in the characteristics of aluminium accumulation in various aluminium accumulators

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It is well known that high aluminium (Al) ion concentration in soil solutions is the most important factor in restricting plant growth in acid soils. Most plant species have developed the ability to exclude Al from roots (e.g. organic acid exudation) as a method of adapting to acid soils. In contrast, some species accumulate high concentrations of Al in their shoots. These species, called 'Al accumulators', are widely distributed throughout the plant kingdom. Here we discuss physiological characteristics of Al accumulation among different Al accumulator species within various vascular plant taxa. In angiosperms, Al accumulators are mostly found in woody eudicots, especially Ericales, Gentianales and Myrtales. Al accumulator species are also found in pteridophytes. Our study indicated that oxalate is a common ligand for a part of Al in the leaves of various Al accumulator species (including pteridophytes), whereas a high concentration of non-chelated monomeric Al was also detected in the leaves of woody eudicots. Moreover, microscopic analysis revealed that Al localisation in pteridophyte leaves was similar to that of angiosperms, although the latter show a higher accumulation of Al in epidermal cells than ferns.

Signaling aluminium response in two contrasting rice cultivars

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Rice (*Oryza sativa*) is the most Al tolerant crop among the small-grain cereals. Among other mechanisms, a higher expression of STAR1/STAR2 genes can at least in part be responsible for the inducible Al resistance in this species. Here we analyzed Al resistance in two contrasting rice varieties. All analyzed markers (root elongation, Evans blue and haematoxylin staining), indicated high Al-resistance in var. Nipponbare, while var. Modan was considerably less tolerant. Nipponbare accumulated much less root Al than Modan. In Nipponbare, but not in Modan, a strong , Al-induced expression of STAR1 and STAR2 was observed in roots after 24 to 48 h exposure. In Nipponbare, but not in Modan, a strong increase of jasmonic acid concentrations was found both in roots and shoots. The possible role of jasmonic acid and ethylene in Al-signaling and the activation of Al resistance will be discussed.

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Sustainable Crop Growth and Yields on Acid Soils

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Aluminum (Al) toxicity is a major limitation to crop yields on acid soils worldwide. Many crop species have evolved an Al resistance mechanism based on root efflux of Al-chelating organic acids. However, the use of organic acids for Al resistance can impose a significant carbon cost on crop plants and thus, negatively affect crop growth and yield on acid soils. Therefore, a finely-controlled Al-activated root organic acid exudation is essential not only for Al resistance but also for maintaining sustainable growth and yields under Al stress. Due to the extremely heterogeneous nature of acid soils, plants need to have a nimble and rapidly reacting Al response system in the root apex to achieve these goals. We previously isolated *SbMATE* that encodes an Al-activated root citrate efflux transporter underlying sorghum Al resistance. We recently have also identified a novel *Sorghum bicolor* metal-binding protein (SbMBP) that exhibits high affinity binding to SbMATE, and appears to regulate Al-activated citrate transport mediated by SbMATE. In this report, we will: ***1)*** demonstrate that SbMBP is an Al sensor that detects and responds to Al^{3+} toxicity in acid soils; ***2)*** elucidate the mechanisms underlying the well-controlled Al^{3+}-activated citrate exudation facilitated by SbMATE-SbMBP interactions in sorghum; and ***3)*** discuss the transferability of an optimized sorghum SbMATE-SbMBP system into other cereal crops. Our research should have immediate and practical applications for improving the yields of sorghum and other cereals on acid soils both in the US and in the tropics and subtropics where many developing countries reside.

Aluminium toxicity in *Caenorhabditis elegans* experimental model of Alzheimer's disease

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Aluminium is recognized as a neurotoxic agent and has been associated with Alzheimer disease etiology. Actually, the nematode Caenorhabditis elegans are highlighted as a predictive model to determine the neurological toxicity in mammalian organisms. The present study evaluates the aluminium toxicity and this role in promotion of AD, through of investigation of toxicological paradigms using the C. elegans behavior. This study was performed from of the determination of LD_{50} values of aluminium sulfate, to determine doses to long-term exposure to aluminium to worms. C. elegans were divided into five groups: control, only ultrapure water, and aluminium in doses of 5 mM, 2.5 mM, 1.0 mM and 0.5 mM. The long-term exposure to aluminium was performed for three days, compromises the period of the exposure since L1 to L4 larvae phases. To investigate the effects of aluminium exposure was determined the changes of endpoints of pharynx pumping rate and defecation frequency in C. elegans. The doses 5 mM and 2.5 mM promoting a decrease in feed and defecation frequency in worms. These results can be transferred and explored in human system due to the high homology between C. elegans and mammalian systems.

Investigation of toxicological paradigms of dairy industry effluents treated on the nervous system of Caenorhabditis elegans

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Aluminium is used as a flocculant agent in effluent of the industries, such as dairy effluent. The present study evaluates the effect of long-term exposure to aluminum and dairy industry effluent treated on endpoints of C. elegans behavior and biochemistry parameters. The nematode C. elegans is an important model organism can be used as a bioindicator organism of the environmental toxicity. Initially, was determined the concentration of aluminium present in the effluent treated (0.6mg/L), then the worms were treated through of the long-term exposed to dairy industry effluent and the aluminium residual concentration lasting in effluent post treatment. In fact, this study evaluate the hypothesis that dairy effluent and Al exposure can be related to neurodegenerative diseases promoting disruption between the enzyme activity reflecting on the behaviors parameters. All endpoints observed presented alterations after the long-term exposure to toxicants, showing an inhibition in the frequency of both behavioral parameters and AChE activity.

Experimental intoxication with aluminium chloride induced behavioural and mitochondrial alterations in mice and decrease viability, proliferation and changes in morphology of neurospheres.

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Neural stem cells cannot be studied *in vivo*, neurospheres provide a method to investigate neural precursor cells *in vitro*. Thus, it becomes relevant to investigate the role of Aluminum (chloride) during the cell cycle and differentiation of neural progenitor cells, and this study aims to define if: i) $AlCl_3$ in different concentrations [0.1 ηM, 10 ηM, 1µM and 100 µM] has the ability to change viability and proliferation of neurospheres; ii) $AlCl_3$ in the same concentrations [0.1ηM, 10 ηM, 1µM and 100 µM] has the ability to alters morphology of neurospheres *in vitro*. Our results showed that $AlCl_3$ decreased ($P<0.05$) the viability of neurospheres at 1 µM and 100 µM concentration. $AlCl_3$ alters the morphology of neurospheres reducing the diameter and number of this cells per well in 10 ηM, 1µM and 100 µM concentration. Furthermore, when $AlCl_3$ was added to the culture medium of the neurospheres it was observed a significantly decrease in the percentage of proliferating cells in the concentration of 10 ηM and 1µM of $AlCl_3$ ($P<0.05$). This study demonstrated that $AlCl_3$ can cause a disruption of neurogenesis and perturbation of neural progenitor cell homeostasis since that present reduction in proliferation, viability and the number and diameter of these cells which is directly related to the formation of neurospheres which has similar characteristics to the central nervous system (CNS). In this context, this model can be used in the future to investigate the mechanisms of aluminum neurotoxicity in the CNS.

Key words: $AlCl_3$; neurospheres; cell cycle; viability; progenitor cells.

Aluminum as an environmental risk factor for visceral hypersensitivity

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Introduction: Irritable bowel syndrome is a common disease characterized by chronic abdominal pain. Involvement of environmental factors is suspected in the development of this syndrome, especially because of the high prevalence in Western countries. The aim of our study was to analyze the effect of aluminium on visceral hypersensitivity in rodent models.

Results: Oral aluminum administration to rats induced a persistent dose and time dependent visceral nociception. This effect apparently arises from the ability of aluminum to increase mast cells degranulation and to modify the expression of genes with hyperalgesic and analgesic effects. Protease-Activated Receptor 2 implication was also discussed.

Conclusion: We demonstrated that exposure to environmentally relevant doses of aluminum promotes the development of visceral hypersensitivity with persistent effect over time.

The RAS/PI3K pathway involved in the damage on long-term potentiation of acute aluminum treatment

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The RAS/PI3K signal transduction pathway appears to be involved in the mechanism of AMPA receptor trafficking and Long-term potentiation (LTP). Our previous study showed that acute aluminum treatment obviously suppressed the hippocampal LTP of rats in vivo, and it may be relate to the decreased trafficking of AMPA receptor subunits. Here we explored the RAS activity of rat hippocampus after acute aluminum exposure and the antagonism of RAS activator EGF on hippocampal LTP suppressed by aluminum and on the PKB activity and the phosphorylation of GluR1 S831和S845. First, acute aluminum treatment, by intracerebroventricular injection (i.c.v.) with different dose of aluminum-maltolate complex ($Al(mal)_3$), produced a dose-dependent decrease of RAS activity in rat hippocampus. Second, the early suppression of hippocampal LTP by aluminum could be antagonized by the RAS activator EGF. Finally, the same changes with LTP were showed in the PKB activity and the phosphorylation of GluR1 S831和S845 after EGF treatment. It was concluded from the results that RAS→PI3K/PKB→GluR1 S831和S845 signal transduction pathway may be involved in the damage on hippocampal LTP by aluminum in rats. However, the mechanisms underlying this observation need further investigation.

Key words: aluminum; long-term potentiation; RAS; PKB; in vivo

Non-apoptotic role of caspase-3 of aluminium exposure on long-term depression in area CA1 of the rat hippocampus in vivo

*Huifang Zhang, Xiaojuan Yang , Qinli Zhang, Qiao Niu**

Exposure to aluminum (A1) causes learning and memory deficits in animals and humans. Long-term potentiation (LTP) and long-term depression (LTD), two forms of synaptic plasticity, are believed to underlie the mechanisms of learning and memory. Experimental evidence for a direct interference of A1 with mechanisms underlying neuronal excitability and plasticity has been provided by means of the model of LTP. In the present study, we investigate the effects of subchronic aluminium exposure on LTD. 40 SD rats were randomly distributed into four groups . Over two months , the rats in the saline group received daily intraperitoneal (i.p.) injection of 0.9% saline ,while the rats of Al-exposed groups received i.p. 1 0μM/kg, 20μM/kg, 40μM/kg $Al(mal)_3$ administrations, respectively. LTD in hippocampus was recorded. Our studies indicate that subchronic Aluminium exposure significantly and dose-dependently suppressed LTD in the rat hippocampal CA1 region in vivo. Emerging evidence suggests that in addition to their classical role in cell death, caspase-3 has a key role in modulating LTD. To investigate the mechanism of LTD impairment due to Al exposure, 48 SD rats were randomly distributed into 6 groups：control group， z-DEVD-fmk (caspase-3 inhibitor) group ， $Al(mal)_3$ group (2mM、10mM、50mM)and $Al(mal)_3$(50mM)+ z-DEVD-fmk group. Over 7 days , the rats in the control group received daily intracerebroventricular (i.c.v.) injection of 0.9% saline . $Al(mal)_3$ or z-DEVD-fmk are i.c.v. injected and drug pre-treatment was followed for one week. The hippocampal LTD were recorded by field potentiation technique *in vivo*. After LTD recording， the rate of apoptosis of hippocampus was detected by Annexin-Ⅴ and PI double staining method . Our studies indicate that with the increase of aluminum exposure dose , the apoptosis rate of hippocampus were increased , but LTD were gradually suppressed . In z-DEVD-fmk group , LTD was blocked . Consistent with previous report , caspase-3 are critical for LTD. In $Al(mal)_3$+ z-DEVD-fmk group, application of caspse-3 inhibitor reduced the apoptosis rate of hippocampus, but LTD were also suppressed. We conclude that aluminum may impaire hippocampal LTD by affecting the balance of caspase-3's role in cell death and synaptic plasticity .

Keywords Aluminium; LTD; caspase -3;

Aluminum can cross and damage intestinal epithelial barrier *in vitro*

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Background. Earlier studies have indicated that Aluminum exerts deleterious effect on intestinal inflammation and mucosal repair, suggesting that aluminum might be an environmental risk factor for Inflammatory Bowel Disease. However, little is known about the effects and association of Aluminum with the intestinal epithelial barrier.

Aim. Our objectives were to study the effects of aluminum on polarized intestinal epithelial cells monolayers in terms of permeability, crossing and cellular localization.

Results. Incubation of Aluminum Citrate (AluCi) and Aluminum Hydroxyde Rhodamine (AluRho) particles with non-differentiated Caco2 cells impaired barrier formation. Once polarized, incubation of Caco2 cells monolayers with AluCi and AluRho particles decreased intestinal permeability, assessed by transepithelial electrical resistance measurement, associated with a significant inhibition of Occludin and Claudin-7 mRNA levels. After 6 hours incubation with Caco2 cells monolayers, a significant increase of AluRho in the basal compartment was observed, as compared to cells treated with Rhodamine alone. Confocal microscopy revealed AluRho particles both inside and between intestinal epithelial cells.

Conclusion
As a whole, these data argue in favor of the ability of Aluminum to cross and to damage intestinal epithelial barrier *in vitro*.

Effects of aluminum adjuvants on social behavior in mice

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Background: Our group has demonstrated that significant correlations exist between rates of autism spectrum disorder (ASD) and total aluminum adjuvant given to children in several western countries. These correlations satisfied eight out of nine Hill criteria for causality. Experimental studies using early postnatal mice, given equivalent to human aluminum adjuvant injections, demonstrated a range of behavioural abnormalities. In order to broaden the behavioural outcomes, we have now repeated the previous studies and included tests of social behaviors.

Methods: A total of 51 neonatal male and female CD-1 mice pups were injected with 550 µg of Aluminum hydroxide gel (for experimental group) or saline (for controls) during the first 2 weeks of postnatal life. The mice were tested at weeks 8, 17 and 29 on behavioral tests for social interest and social novelty/memory. P-values were calculated using the Mann-Whitney test.

Results: Aluminum injected mice showed diminished social interest compared to controls at week 8 ($p=0.016$) and 17 ($p=0.012$). They also demonstrated abnormal social novelty/memory from controls at week 8 ($p=0.002$) and at week 29 ($p= 0.042$).

Conclusion: This is the first experimental study to demonstrate that aluminum adjuvants can impair social behavior if applied early in life. The relationship between the observed changes and those of ASD require further study.

Gene-toxin synergy in the brain of autistic mouse model

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In young children, a significant correlation exists between aluminum-adjuvanted vaccines and autism spectrum disorder (ASD), suggesting aluminum's involvement in the pathogenesis of ASD. To investigate how aluminum interacts with immune genes, we tested the expression level of 17 genes which have implicated function in both ASD and innate immune response in the brains of aluminum-injected mice. A spectrum of expression level change was observed in aluminum-injected mice. Some of the activators and effectors of macrophage were significantly up-regulated, while the NF-κB inhibitor and the excitatory neurotransmitter were remarkably down-regulated in aluminum-injected male mice. The decreased NF-κB inhibitor and increased inflammatory signal led to the activation NF-κB signaling pathway resulting in releasing more inflammatory proteins. Unlike male, female mice were less responsive to environmental insult as fewer genes with altered expression level were discovered. Moreover, the regional patterns of gene expression alterations exhibited gender difference. Altogether, these results strongly suggest that aluminum could impair brain function by interacting with key players of immune and neural system especially in males, while females may be protected from the aluminum toxicity by their more robust neurobiological system.

Keywords: vaccine safety, aluminum toxicity, ASD, innate immunity, gene-toxin interaction, NF-κB signaling pathway.

Assessment of the neurotoxic effects of aluminic vaccine adjuvant injected in mice.

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Aluminum hydroxide ($Al(OH)_3$) is a nanocrystalline compound used as a vaccinal adjuvant to increase the immune response to a antigen. Long-term biopersistence of alum associated with cognitive dysfunction and autoimmunity has been reported in adult patients but only few animal studies of $Al(OH)_3$ neurotoxicity are available.

Mice were intramuscularly injected with alhydrogel® or Al-containing vaccine (HBV). Eight complementary behavioral tests were performed at 45, 90, 135, 180 and 270 days after injections, in order to assess the levels of activity, anxiety and depression, short-term memory, muscular strength, pain, and locomotor coordination. Dose-response study showed a decrease in both activity and anxiety levels in mice injected with the two lowest doses of $Al(OH)_3$. At higher doses, no significant differences were observed with $Al(OH)_3$ alone but muscular strength was impaired at D135 in mice injected with the HBV vaccine. Further investigations are needed to understand low dose effects and antigen adsorption on $Al(OH)_3$ neurotoxicity.

Fluorescent nanodiamonds as a relevant tag for the assessment of alum adjuvant particle biodisposition

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Lack of specific staining makes difficult the assessment of low quantities of alum adjuvant particles in tissues. We explored the feasibility of coupling alum (Alhydrogel®) with fluorescent nanodiamonds (fNDs), that have specific and perfectly photostable fluorescence, to form AluDia complex. Physicochemical characteristics of AluDia particles were comparable to those of the whole reference vaccine (anti-hepatitis B vaccine). *In vivo*, AluDia injection was followed by prompt phagocytosis and particles remained easily detectable thanks to their specific signal in the injected muscle, draining lymph nodes, spleen, liver and brain. *In vitro*, fNDs have low toxicity on NSC-34 cells and AluDia showed similar cell toxicity to alum alone. Expectedly, AluDia elicited autophagy and allowed highly specific detection of alum in autophagosomes.
We show that nanodiamond technology is able to overcome the limitations of previously used organic fluorophores, appearing thus as a choice methodology for studying distribution, persistence and long-term neurotoxicity of alum adjuvants.

Elucidating upon the cellular uptake of aluminium based adjuvants and the amyloidogenic $A\beta_{42}$ peptide 'antigen' in a monocytic THP-1 cell line.

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Aluminium based adjuvants (ABA) are aluminium salts dispersed in water to form suspensions or gels of hydrated colloid particles that consist of nano-sized primary particles in micron-sized aggregates of varying size. ABA are included in human vaccinations to potentiate the efficacy of weak antigens and subsequently shape the immune response. In spite of the wide-spread use of ABA, a consensus upon the mechanisms underlying their biological activity is yet to be reached [1]. The unequivocal cellular uptake of the clinically approved Alhydrogel® aluminium oxyhydroxide (AlO(OH)) adjuvant has recently been demonstrated in a T helper 1 (THP-1) cell line, with particulate AlO(OH) found localised in vesicles of around 1µm in cell cytoplasm only [2]. Using lumogallion as a fluorescent molecular probe for aluminium, the potential cellular uptake of both clinically approved and experimental ABA aims to be investigated in an *in vitro* THP-1 cell model.

Alzheimer's disease (AD) is characterised neuropathologically via the deposition of senile plaques of insoluble amyloid fibrils of the amyloidogenic peptide, $A\beta_{42}$, in a β-pleated sheet conformation. The use of amyloid as antigens in vaccines has been shown to be effective against AD and related amyloidosis pathologies [3], however the immunopotentiating properties underpinning the therapeutic use of amyloid have yet to be established. Herein the unequivocal and potential concomitant uptake of ABA and $A\beta_{42}$ as an antigen aim to be established in a monocytic THP-1 cell line. Furthermore, elucidating the effectiveness of ABA upon the resultant cellular uptake of protein and peptide antigens may prove essential for the understanding of the adjuvanticity of ABAs administered in human vaccinations.

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Aluminium treatment of THP-1 monocytic cell line induces alterations in inflammatory and proteolytic pathways

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Several studies have linked Aluminium with the development of human pathologies (e.g. breast cancer, myofasciitis, neurodegenerative diseases), probably due to the increased exposure to the aluminum salts or aluminium-based adjuvants (ABA), often used in both cosmetic products and vaccines. However, the mechanisms underlying immunologic and proliferative alterations still remain a matter of debate.
In the present study we investigated the ability of different types of aluminium salts and ABA to trigger inflammatory and proteolytic responses in THP-1 monocytic cell line. We demonstrated, by multiplex immunoassay analyses and gelatin zymography, that THP-1 cells treated with both ABA and aluminium salts showed an altered release of 27 inflammatory mediators and 9 Matrix Metalloproteinases, with different expression profiles. The identification of the biochemical pathways involved in Al-induced cell injury pave the way for the improved knowledge of Al impact in human physio-pathology.

Effect of aluminium on migration of oestrogen unresponsive MDA-MB-231 human breast cancer cells in culture.

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Aluminium has been measured in human breast tissue and may be a contributory factor in breast cancer development from its use as underarm antiperspirant. At the 10th Keele meeting, we reported that long-term exposure to aluminium could alter migratory properties of MCF-7 oestrogen-responsive human breast cancer cells suggesting a role for aluminium in the metastatic process. We now report that aluminium can also increase the migration of estrogen unresponsive MDA-MB-231 human breast cancer cells as measured using time lapse microscopy and xCELLigence technology. Since these cells lack estrogen receptor alpha (ER), the mechanism is not ER-mediated. Instead, aluminium exposure results in increased secretion of matrix metalloproteinases MMP2 and MMP9 as measured by zymography, and increased cellular MMP14 as measured by western immunoblotting. This suggests that aluminium may influence metastasis of breast cancer cells through alterations to MMPs.

Preliminary results and status of the study: The use of antiperspirants containing aluminium-salts and its relation to breast cancer

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The aetiology of breast cancer is likely multifactorial and involves genetic and environmental factors. Previous epidemiologic studies investigating the relationship between breast cancer and underarm antiperspirant use showed conflicting results. Recent publications suggest that breast cancer is linked to the use of antiperspirants containing aluminium-salts, which are associated with oxidative stress, proliferation and DNA double-strand breaks. We designed a hospital-based case-control study including a questionnaire and a biochemistry part. The antiperspirant use, concentration of aluminium in biosamples (breast tissue, serum, urine) and clinical/life-style data are compared between a group of 200 women suffering from breast cancer and 200 age-matched controls without breast cancer. Biosamples are taken from patients undergoing a mastectomy (cases) or a mamma reduction surgery (controls). To date, we have collected questionnaires from 220 women and biosamples from 60 women. We present challenges and lessons learned from study implementation as well as preliminary results from the questionnaire part.

A pilot study measuring aluminium in bone in Alzheimer's and reference subjects: work in progress

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Key Words: Neutron activation analysis, Aluminum, Alzheimer's disease, Bone

Aluminum, being the most abundant metal in the earth's crust, is widely distributed in the environment, and is therefore, taken up by human body predominantly through ingestion and inhalation. Although not considered an essential element, it can be toxic. The body retains only very small amounts of consumed aluminum which is mostly stored in the bones. There has been an ongoing debate about the correlation between excess amounts of aluminum and the late onset on Alzheimer's disease (AD). The most common method for measurement of aluminum in the bone is through biopsy which discourages subject participation. We have developed a system for non-invasive measurement of aluminum using in vivo neutron activation analysis. A subject's hand is placed in a beam of thermalized neutrons available through McMaster University's linear accelerator for 45 seconds which activates the elements with significant neutron capture cross-section while keeping the dose to the subject at the minimal level. The reactions of interest are $^{27}Al(n,\gamma)^{28}Al$ and $^{48}Ca(n,\gamma)^{49}Ca$. The subject's hand is then placed in the detection system which is composed of 9 NaI (Tl) detectors arranged in a 4π geometry. The ^{28}Al emits a single gamma ray of energy 1.78 MeV with a half-life of 2.35 minutes. The ^{49}Ca decays with a half life 8.72 minutes and emission of a 3.08 MeV gamma ray. The data acquisition system records 10 cycles of one minute counts which are then analyzed and the areas under the Al and Ca peaks are calculated. The results are reported as ratio of Al to Ca in order to eliminate the variations in beam parameters and geometry as well as the physical variations among the subjects such as size of the hand and bone structure.

This pilot study has been done on subjects diagnosed with AD as well as control subjects, all of whom are 60 year or over. All subjects, and in the case of AD subjects, their caretakers, have given informed consent. This study aims for 15 AD and 15 control subjects. Currently, based on 15 AD and 9 control subjects, the unweighted mean value for the control group is 0.97±2.22 μgAl/gCa (inverse variance weighted mean 3.69±1.22 μgAl/gCa) and for the AD subjects is 12.15±12.22 μgAl/gCa (inverse variance weighted mean 9.97±1.11 μgAl/gCa). The difference between the mean of the AD group and the mean of the control group is 11.18±12.42 μgAl/gCa, with a p-value of 0.01 achieving significance at the 1% level for the 22 degrees of freedom available.

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Aluminium and bone: new clinical circumstances where aluminium deposition occurs in the calcified matrix of bones.

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Several decades ago, aluminium encephalopathy associated with osteomalacia has been recognized as the major complications of chronic renal failure in dialyzed patients. Removal of Al from the dialysate has led to a disappearance of the disease. However, Al deposit occurs in the hydroxyapatite of the bone matrix in some clinical circumstances. We have encountered Al in bone in case of increased intestinal permeability (coeliac disease) or in case of prolonged administration of Al anti-acid drugs. A co-localisation of Al with iron was also noted in cases of hemochromatosis and drepanocytosis. Corrosion of prosthetic implants composed of grade V titanium (an alloy containing 6% Al and 4% vanadium) was also observed in a series of hip or knee revisions. Al was identified in bone matrix studied undecalcified and stained by the mordant blue technique, a highly specific stain allowing the detection of ~15-27 x 10^{-6} % confirmed by wavelength-dispersive spectroscopy.

Study of aluminium in cancellous and compacted bone of human: factors effect

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It is becoming increasingly important to assess the risk to bone tissue from exposure to aluminium of environmental and occupational origin. It is well known, that the processes of bone remodelling are active throughout the lifespan, and therefore can be an indicator of metal accumulation in bone tissue from long-term chronic exposure. Spongy bone metabolism is more active than cortical bone and depends on many factors such as age, diet and health status. Many elements have a significant impact on bone metabolism. The toxic effects may be revealed after many years of exposure or may appear suddenly.

The aim of the study was to determine the content of aluminium in the proximal femur bone tissue (cancellous and compacted bone) of patients undergoing total hip replacement for osteoarthritis using GF-AAS analytical technique. The interdependencies among aluminium and its correlations depended on factors including age, gender, place of residence, tobacco consumption, alcohol consumption, exposure to environmental pollution, physical activity, and type of degenerative change were investigated by statistical and chemometrics analyses.

The sample consisted of 96 patients operated on for total hip replacement (THR). Bone tissue condensation at the superolateral part of the femoral head and cysts in femoral head and neck were present. Directly after acquisition, the spongiest bone was separated from the femoral heads under sterile conditions. Samples were cut from the head and neck of the femur. Age on the day of the surgery ranged from 25 years to 91 years (mean 64 years). The use of femoral heads in the investigations was permitted by the Bioethical Committee of the University of Medical Sciences in Poznan (Poland).

The study confirmed significant differences in aluminium content in cancellous and cortical bone. The significant positive correlation between the content of aluminum in the femoral head and neck was observed. The meaning difference in the content of aluminum in the femoral head between the patient before and after the age of 60 was confirmed.

Impact of aluminium on human fertility

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A deterioration of human semen quality has been observed over recent decades. A possible explanation could be an increased exposure to environmental pollutants, including aluminium.
We present a study on 62 patients showing that the mean aluminium concentration in human semen was high: 339 µg/L. Patients with oligozoospermia exhibited a statistically higher concentration than others. Cytological analysis showed the presence of aluminium within spermatozoa.
These results have to be completed by investigating the link between aluminium exposure and its potential impact on male and female fertility. We will recruit couples performing In Vitro Fertilization (IVF); collect informations about their aluminium occupational and every-day life exposure; sample semen, follicular fluids and immature oocytes in which aluminium concentration will be measured. This will be compared with fertility parameters (semen quality, fertilization rate, IVF success rate; clinical pregnancy rate and take home baby rate) to estimate the potential impact of aluminium on fertility.

Aluminium and silicon in human sweat

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Despite knowing very little about the implications to our health, there are now myriad publications stating that aluminium is not safe, as well as questioning its bioaccumulation in the body despite signalling toxic effects [1]. Increasing attention is now being given to methods of removing aluminium from the body. Prior research indicates that the most likely path for aluminium excretion is in urine [2], research has shown that we excrete aluminium in our urine at around 100µg per day [3]. The excretion of aluminium from the human body has been seen to be significantly larger in sweat than urine, reaching levels of 5000µg per 24 hours, strongly suggesting that perspiration is the major route of excretion of systemic aluminium [3]. Recent data has shown that silicon may provide protection for humans against a potentially burgeoning exposure to biologically available Al [4]. In this study, sweat was collected from each individual using a qualified sweat collection pad after a 30 minute period of moderate exercise. Once baseline aluminium and silicon concentrations are established, the participants then consume 1L orthosilicic acid rich mineral water an hour before exercise. The corresponding concentrations can then be compared to determine whether the intake of a silicon rich fluid affected aluminium excretion rates. Herein we are testing the hypothesis that silicon-rich mineral waters can be used as non-invasive methods to reduce the body burden of aluminium.

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Sea vegetables and aluminum

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Sea vegetables are attracting much attention as a healthy food. Various kinds of brown sea vegetables such as wakame, kombu, mozuku and hijiki have been taken daily in Japan. Sea vegetables contain dietary fibers such as alginate, laminaran and fucoidan. Alginate contains a carboxyl group in each monosaccharide moiety and fucoidan consists of fucoses partly with sulfate groups. These functional groups in the dietary fibers bind with aluminum ions in addition to metal ions such as sodium and calcium.

Aluminum contents in brown sea vegetables are very high. One of the highest aluminum contents in foods was hijiki. However, we did not observe any increase of aluminum absorption after taking foods made from hijiki. From these points of view absorbable aluminum accessible to specific organs through blood stream should be measured and discussed. Total aluminum intake has no mean with respect to the influence of aluminum on health.

DEVEXI Health Analytics Research Database for Aluminum Exposure Research

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DEVEXI is powerful, intuitive, widely-accessible and cost-effective health research database of medical, dental, pharmaceutical, environmental and behavioral data enabling groundbreaking studies never before possible to: improve quality of health care delivery, identify best practices, and increase successful, cost-effective outcomes. The initial DEVEXI product will consist of 23 years of de-identified medical and dental records of over 10 million subscribers seamlessly integrated with additional layers of data on contributing factors to health including environmental impact, socio-economic impact, behavioral impact and much more. This will be accomplished by connecting the dots across disparate health and medical databases, creating a sophisticated query engine below the surface and a simple, intuitive user interface and graphical output capability above the surface to create a revolutionary approach to health and medicine.
Data mining research using DEVEXI on aluminum and human health topics can include long term health outcomes after exposure to aluminum adjuvants, parenteral nutrition, aluminum containing drugs, dental materials and other over the counter and prescription medicines used by Medicaid recipients.

Aluminium and health: a century of controversies, research and regulation

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Aluminium is an ambivalent material. Like a Russian doll, within a single word, we find different meanings. First the notion of alloy, aluminium as a metal is rarely used pure. Second, we use the same word for the metal (as in pan) or for non-metallic forms (as in food additives). For consumers, the metal is tangible, and if the salts are invisible, the growth of their usage has been spectacular since the interwar period.
The emergence of science in the 18th century triggered a research movement in several stages. In the 19th century, aluminium is seen as a healthy kitchen material, an ideal alternative to hazardous materials such as copper. From the late 19th to the 1930s, appears a first controversy in the US, then a different one in Europe. Finally, the post-war period sees a change in the perception of the problem that becomes globally considered, and involves both research and regulation perspectives.

Aluminum-induced, NF-kB-mediated pro-inflammatory signaling and amyloidogenesis in human brain microglial cells and tissues – multiple and highly interactive neurotoxic effects

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In human brain beta-amyloid precursor-protein (βAPP) is processed by tandem beta-gamma secretase cleavage into 42 amino acid amyloid (Aβ42) peptides, whose progressive accumulation is one distinguishing feature of Alzheimer's disease (AD) neuropathology. Aβ42 over-abundance and inability of brain cells to effectively phagocytose and clear them contribute to the pro-inflammatory and innate-immune pathology of AD. Aluminum has the remarkable capability to: **(1)** aggregate Aβ42 peptide monomers into higher order, more neurotoxic oligomeric structures; **(2)** impair the cellular machinery responsible for Aβ42 monomer phagocytosis and clearance; **(3)** up-regulate pro-inflammatory NF-kB and interleukin 1β (IL-1β) secretion; and **(4)** activate important elements of the NLRP3-inflammasome, a multiprotein complex and key component of the innate-immune surveillance system. Many of these aluminum-triggered neurotoxic actions are mediated by a family of CNS-resident pro-inflammatory microRNAs. This paper will assess these functionally overlapping, neurotoxic properties of aluminum on pathogenic pro-inflammatory signaling and innate-immune system deficits in the aging CNS.

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provide post-mortem human brain and retina for study. Research on the structure and function of aluminum, amyloidogenesis, NF-κB and miRNA expression, speciation and complexity in AD brain and retina 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), NIH NIA Grant AG038834 (WJL), NIH NEI Grant NEI EY006311 (WJL) and Research to Prevent Blindness (RPB). 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.

Colocalization of aluminium and iron in neurodegenerative diseases: In the case of Alzheimer's and Parkinson's disease.

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There is increasing evidence that metal-induced oxidative stress plays a pivotal role in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). We investigated the colocalization of aluminium (Al) and iron (Fe) in the nuclei of nerve cells in the brains of AD and PD patients. Fe is a transition metal capable of generating hydroxyl radicals that can oxidize nuclear DNA. Al has been reported to facilitate an iron-mediated oxidative reaction. In this study, elements contained in the brains of patients and in those of age-matched controls were examined by scanning electron microscopy combined with energy-dispersive X-ray spectroscopy (SEM-EDS).

AD is characterized by the degeneration of nerve cells in the temporal lobe and hippocampus. SEM-EDS analysis demonstrated elevated levels of Al and Fe in the nuclei of nerve cells located at these lesion sites. On the other hand, in the non-demented control brains, the levels of Al and Fe in the neuronal nuclei were significantly lower than those in the brains of AD patients. Al and Fe colocalized in the neuronal nuclei may play a key role in the pathogenesis of AD by inducing oxidative damage to nuclear DNA.

PD is the disease whereby nerve cells localized in the substantia nigra pars compacta selectively degenerate. In contrast to the brains of AD patients, very high levels of Al and Fe were detected in the nuclei of nerve cells in the substantia nigra in control brains by SEM-EDS analysis. Meanwhile, the levels of Al and Fe in neuronal nuclei of the brains of PD patients were markedly lower than those in control brains. The present results showed that the levels of Al and Fe in neuronal nuclei of the substantia nigra markedly decreased during the progression of PD. The hypothesis that might be applied to the pathogeneses of AD and PD will be discussed.

Aluminium intoxication and long treatment with chelation therapy

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We have previously shown that many patients affected by neurodegenerative diseases and bearing aluminium (Al) intoxication had beneficial effects by chelation therapy with calcium disodium ethylene diamine tetraacetic acid (EDTA) (1). Such therapy was further improved by daily treatment with the antioxidant Cellfood (2). In the present study we have examined the effects of long-time treatments with both EDTA and Cellfood.

Materials and Methods
Patients have been subjected to the chelation test to show possible Al intoxication. Indeed, they were invited to collect the urine samples before and after the intravenous treatment with the chelating agent EDTA 2 (g/10mL diluted in 500mL physiological saline). EDTA was intravenously slowly administered (the infusion lasted about two hours) to the patients. The time of urine collection following chelation lasted 12 h. Recovered urine samples were then processed for their Al content using the inductively coupled plasma mass spectrometry (ICP-MS) test. Patients who revealed Al intoxication (expressed in μg per g creatinine) were subjected to EDTA chelation therapy once a week for ten weeks, indeed once two weeks for other six or twelve months. At the end of treatment (a total of 22 or 34 chelation therapies), Al levels and clinical symptoms of patients were evaluated.

Results
Our results showed that Al intoxication was significantly reduced by EDTA treatments and clinical symptoms were improved. These results were further ameliorated by daily Cellfood treatment.
In conclusion, the obtained data suggest the use of chelation therapy in subjects affected by Al intoxication who have developed neurological diseases.

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Can 5-hydroxy-2-(hydroxymethyl)pyridin-4(1*H*)-one tautomer cure aluminium overload diseases?

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Deferiprone (3-hydroxy-1,2-dimethylpyridin-4(1*H*)-one) related chelators were extensively studied in the recent years, but not their tautomers. Unfortunately, because their tautomers, having additional negative charge on nitrogen atom, result in much stronger ligands for metal ions.
In these last months we synthesized the 5-hydroxy-2-(hydroxymethyl)pyridin-4(1*H*)-one molecule and studied its aluminium coordination ability with the use of different techniques: potentiometry, NMR and ESI-MS.
We extended our studies performing biological experiments on rats and we obtained promising results.

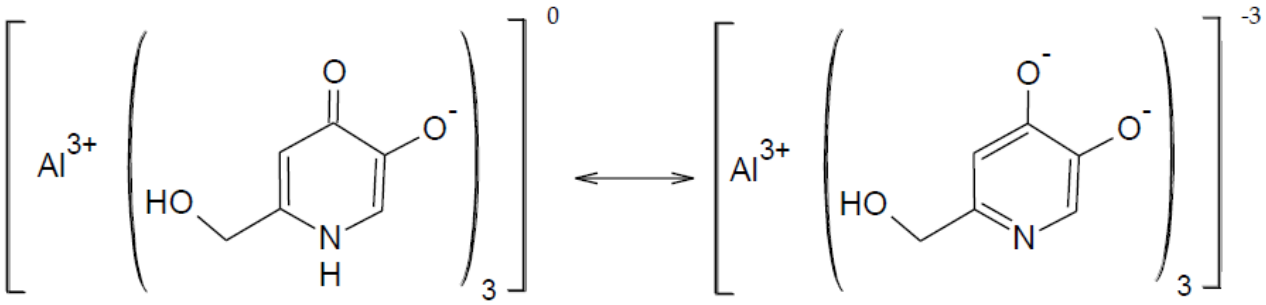

Figure - 5-hydroxy-2-(hydroxymethyl) pyridin-4(1*H*) tautomer complexes with aluminium.

Clinical features and functional brain imaging in patients with persisting aluminium hydroxide-induced macrophagic myofasciitis (MMF)

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Macrophagic myofasciitis (MMF) is characterized by specific muscle lesions assessing abnormal long-term persistence of aluminium hydroxide within macrophages at the site of previous immunization. Affected patients mainly presenting with diffuse arthromyalgias, chronic fatigue, and cognitive dysfunction. Representative features of MMF-associated cognitive dysfunction include dysexecutive syndrome, visual memory impairment and left ear extinction at dichotic listening test. Most patients fulfil criteria for non-amnestic/dysexecutive mild cognitive impairment but some patients display features suggestive of hippocampus involvement. Remarkable correlations were found between cognitive impairment and SPECT brain imaging in MMF patients, with hypoperfusion predominating in the hippocampus, posterior areas, cingulum and corpus callosum. Most MFM patients demonstrated a typical pattern of hypometabolism on FDG-PET imaging, involving precunei, posterior temporo-occipital regions, internal occipital regions (lingual, fusiform gyri), amygdalo-hippocampal complexes and cerebellar culmen.

Aluminum-Induced Entropy in Biological Systems

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Because of the abundance of aluminum in the Earth's crust, it was regarded as harmless before its use in products became prevalent. Humans are exposed to aluminum from food, water, medicinals, vaccines, cosmetics, and industrial exposure. However, Al is toxic to living systems and has no physiological role in biological systems. Beginning with the biophysics of water, disruptions progress from biophysical processes to macromolecules crucial to living processes. Aluminum induces exogenous interfacial water stress by disrupting hydrogen bond cooperativity and the quantum coherence of water essential for life. Al disrupts biological self-ordering, energy transduction, and signaling systems, thus increasing biosemiotic entropy. It injures cells, circuits, and subsystems and can cause catastrophic failures ending in death. Al forms toxic complexes with other elements. Al negatively impacts the central nervous system in all species studied including humans. CNS disorders in humans are sensitive indicators of the Al toxicants.

Predictors of costs of care in aluminium related dementia: a stress simulation hypothesis

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We are living in an “aluminium (Al) age” with increasing Al bioavailability, contributing significantly to Al body burden. Al accumulates and is stored predominantly in the lungs, bones, liver, kidneys and brain. Based on available data on the worldwide economic impact of dementia, aim of the study is to understand how Al may impact societal costs of dementia and how this may affect families, health and social care services. The basic design is a societal, gross cost of illness study in which costs are aggregated to World Health Organization publicly available data and World Bank income groupings. Since prevalence data that involve Al as a main variable are few, dementia in patients undergoing haemodialysis is used as a model. The total estimated worldwide cost of dementia was US$ 604 billion in 2010. 5-8% of cases undergoing haemodialysis will develop dementia: it means US$ 30.2-48.3 billions. Only by investing now in research and cost-effective approaches can soon to come societal costs be anticipated and managed.

Plants Do Some Surprising Things to Deal With Toxic Aluminum in the Soil!

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Abstract: Acid soils comprise up to 50% of the world's potentially arable lands, and are a major limitation to worldwide crop production. On these acid soils, when soil pH values drop to pH 5 or lower, Al^{3+} ions are solubilized into the soil solution from aluminosilicate clay minerals. These Al^{3+} ions are quite toxic to plant roots, inhibiting root growth and development. This in turn reduces crop yields due to drought and mineral nutrient deficiencies resulting from stunted and damaged root systems. Hence aluminum (Al) toxicity greatly limits crop yields especially in the tropics and sub-tropics where many developing countries reside and food security is most tenuous. Significant genetic variation in plant tolerance to Al toxicity has been identified and exploited for many years by plant breeders to generate crops with increased Al tolerance. Over the past 20 years, plant biologists have also mined this same genetic variation in Al tolerance in combination with recent technological advances in molecular biology, genomics and physiological research approaches to identify a number of plant Al tolerance genes and their associated physiological/biochemical mechanisms. Plants have developed a surprisingly sophisticated array of responses to deal with toxic Al ions in the soil. This includes the exclusion of Al^{3+} ions from the growing root tip via Al sensing and response mechanisms that trigger the release of Al-chelating organic compounds from the root. Furthermore, it appears that plants can modify their root cell wall to minimize toxic effects of Al in the cell wall of dividing and expanding root cells in the root growth zone. Finally, evidence from recent research suggests that plants can employ membrane transporters working in concert with endogenous Al-chelating compounds to sequester and detoxify Al ions that enter the plant symplasm. Some of this fundamental research is being translated for use in molecular breeding programs for the generation of more Al tolerant crops that have increased yields on acid soils in the tropics and subtropics.

Application of ion selective electrodes and optical sensors for the detection of aluminium (III) ions in clinical samples.

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Quantitative analysis of the free ion concentration within a single cell is highly critical to the advancement of accurate disease screening and personalised medicine. Therefore, a colour changeable polymer membrane based sensor has been proposed for the highly selective and sensitive determination of Al(III) ions in cellular environment. The sensing layer of the probe consists of lumogallion derivative covalently attached to the polymer backbone. This prevents the diffusion of fluorophore from the membrane bulk into the sample allowing sensing process to take place. The red optode film on reaction with Al(III) ions turns orange and the intensity of the developed colour is found to be directly proportional to the activity of Al(III). The ligand-aluminium complex formation is also accompanied by the change in phase boundary potential that directly corresponds to the concentration changes within the measured sample. The proposed sensor shows great promise to both on-chip or direct cell measurements with high precision, sensitivity, specificity and simplicity. This poster identifies the challenges associated with the development of optical and potentiometric technologies for cellular Al(III) detection.

Experimental reproduction of ovine ASIA syndrome: From symptoms to genomics

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Ovine autoimmune/inflammatory syndrome induced by adjuvants (ASIA syndrome) is a new entity linked to the repetitive inoculation of aluminium-containing adjuvant vaccines. It consists on an acute phase, with severe neurological symptoms and a chronic phase, characterized by weakness, cachexia and tetraplegia. We are undertaking an experimental work to study and explain the syndrome. A total of 63 lambs will be used, distributed in three different flocks, representing different environmental conditions and production systems. Lambs in each flock (n=21) will be divided in three groups (n=7): a) control, b) aluminium adjuvant and c) vaccinated with commercial aluminium-adjuvanted vaccines. The work, intended to cover a period of no less than 15 months, will include a comprehensive array of studies including clinics, mental status, biopathology, serology, immunopathology, and genomics, among others. Accumulation of aluminium in central nervous system and other tissues will be measured at the end of the experiment.

Effects of aluminium on miR29 and beta-amyloid (1-42) in rat brain

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Abstract The abnormal generation and deposition of β-amyloid (Aβ) are hallmark features in the brains of Alzheimer's disease patients and animals administered with aluminum. BACE1 cleavage is a role rate-limiting enzyme for Aβ formation. Some studies have suggested that mi29 may adjust BACE. In the present study, we investigated the modulation of Aβ deposition and the level of BACE1 and miR29 in aluminium-maltolate-treated (0, 15, 30, 45 mmol/Kg body weight via intraperitoneal injection) experimental rats. We measured Aβ1-42 in the cortex and hippocampus in rat brains using ELISA. BACE1 were determined using western blotting and RT-PCR analyses,then miR29 were detected by RT-PCR. These results indicated that aluminium-maltolate induced deposition of the Aβ1-42 both in the cortex and hippocampus. Accordingly, BACE1 showed significant increase in gene and protein. And, miR29a, miR29b, miR29c decreased significantly. Furthermore,miR29a,b1 correlated with BACE1 intently, although no correlation were observed between miR29b2, miR29c1, miR29c2 and BACE1. Taken together, these results suggest that miR29a, miR29b1, through adjusting BACE1, may correlate to the abnormal deposition of Aβ by aluminium in rat brains.

Keywords Aluminium; β-amyloid; BACE1; miR29

Effect of aluminium on DNA damage and DNA repair in MCF10A immortalised non-transformed human breast epithelial cells

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Use of underarm aluminium-based antiperspirant salts may be a contributory factor in breast cancer development. At the 10th Keele meeting, there was a report that aluminium could cause anchorage-independent growth and double strand DNA breaks in MCF10A immortalised non-transformed human breast epithelial cells. We now report that exposure of MCF10A cells to aluminium also causes DNA damage as measured in a Comet assay. Furthermore, long-term (19-22 weeks) exposure to aluminium chloride or aluminium chlorohydrate results in loss of expression of the breast cancer susceptibility genes BRCA1 and BRCA2 which are key genes in repair of DNA in breast cells. Alterations to expression of other DNA repair genes will be discussed. If aluminium can both damage DNA and compromise DNA repair systems, then there is the potential for aluminium to impact on breast carcinogenesis.

Micro Crystalline Tyrosine (MCT): its use as a depot adjuvant in allergy immunotherapy, future perspectives and applications

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Allergen-specific immunotherapy commonly consists of administering a long-course programme of subcutaneous injections using preparations of relevant allergens (up to 70 injections / year / 5 yrs). Historically, the adjuvant of choice has always consisted of aluminium salt preparations (1.25 mg Al^{3+} per shot). Aluminium adjuvants have been consistently demonstrated to induce IgE which is clearly an unwanted and potentially adverse effect in any IgE-mediated disease, such as allergy. Since aluminium in immunotherapy is marketed and described as a depot adjuvant - a suitable depot carrier/adjuvant should support the immunogenic effect of specific immunotherapy without causing unwanted side effects.

In light of the growing number of toxicological considerations surrounding aluminium accumulation, its use in immunotherapy is a current hot topic of discussion between authorities, the public and academic/industrial representatives. Other endogenous and biodegradable adjuvant systems have or are being developed but difficulties in achieving regulatory approval without having extensive mode-of-action and safety studies make it costly and time-consuming to bring market. When evaluating the profile of an adjuvant for possible new applications very few adjuvants can match the extremely comprehensive cohorts that are available for aluminium adjuvants in terms of records of efficacy and safety profiles. A natural, alternative, adjuvant – Micro Crystalline Tyrosine (MCT) - exists and has been marketed as a depot adjuvant, in registered allergen-specific immunotherapy products, for a number of decades. To date, >4.3 injections of MCT have been administered in Germany alone since 2005, including >1 million in children. We present the adjuvant properties of MCT in animal and human studies together with an assessment of its safe use in immunotherapy (including repeat-dose parenteral toxicity, genotoxicity, local tolerance studies and pharmacovigilance). A summary of the range of investigational data (unpublished) as well as published literature reports will be presented.

An array of *in vitro* and *in vivo* studies demonstrate that MCT has ideal adjuvant properties comprising a unique adsorptive power for proteins, enhancement of IgG antibody induction with no stimulatory effect on IgE antibody level (unlike aluminium adjuvants) and action as a short-term depot adjuvant; as well as being a natural, metabolised and biodegradable alternative to aluminium. In more recent studies, results from immunized CBA mice, administered (intralymphatically) with MCT and aluminum-based allergen-adjuvant preparations are presented. Here, blood was collected 2-3 weeks after the last injection and

analysed for anti-PLA2/anti-OVA IgG2a, - IgG1 and – IgE. In a separate rat model, radiolabelled (^{14}C) MCT was calculated as having a half-life of approximately 48 hours at the subcutaneous injection site. The current data, its use and record in subcutaneous immunotherapy and lack of findings of toxicological concern supports the safe and effective use of MCT as a depot mediator with work ongoing to further unravel its adjuvant (Th_1) potential through further mode-of-action studies across the allergy model. A proposal to apply this into a wider context using bacterial and viral candidates is currently being assessed with the possibility of using recombinant antigen +/- Aluminium and +/- MCT to assess and compare basic mechanistic and immunogenic profile in diseased animal models to consider the propensity of MCT as a novel adjuvant in different model systems.

The fate of L-Tyrosine: What happens at the injection site?

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L-Tyrosine, a biologically available amino acid, is currently being used as an alternative to aluminium adjuvants in subcutaneous allergy immunotherapy. Although a number of studies report some of its properties and advantages as a depot adjuvant, its exact mechanisms of action remain unknown. Clarification of the processes that occur at the injection site after vaccination would provide a good starting point and better insight into the role L-Tyrosine plays after subcutaneous administration.
The aim of this poster is to summarize the possible pathways in which tyrosine potentiates the immune response at the injection site and subsequent downstream mechanisms.
A comparison of the action and properties of L-tyrosine with commonly used aluminium adjuvants will be made whilst exploring the various ways in which L-Tyrosine could be metabolised by the human body.

The use of antiperspirants containing aluminium-salts and its relation to breast cancer: Methods and implementation of biospecimen sampling

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Recent publications suggest that breast cancer is linked to the use of antiperspirants containing aluminium-salts. We designed a hospital-based case-control study (n=400) including a questionnaire and a biochemistry part. Biosamples are taken from patients undergoing a mastectomy (n=100 cases) or a breast reduction surgery (n=100 controls) and will be analysed in two independent laboratories (Keele and Innsbruck). The tissue is sampled respectively directly at the operating theatre during the breast reduction surgery or at the macro-diagnosis of the mastectomy supplement. The tissue is taken from three spots alongside the transect from the upper outer quadrant (axilla) to the upper inner quadrant (medial). The distances between sample spots and distances to the tumour are documented. Additionally urine and serum samples are collected from both groups. To date, we have collected biosamples from 60 interviewed women. In summary a set of 1000 biospecimen will be analysed by graphite furnace atomic absorption spectrometry.

Aluminium in human brain tissue: Measurement and imaging.

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Preliminary data are presented regarding the presence of aluminium in human brain tissue from donors who have died from familial Alzheimer's disease. The application of the fluor, lumogallion, to the identification and localisation of aluminium in human brain tissue will be outlined and its optimisation demonstrated.

Aluminium is deposited in the calcified matrix of bone exostoses.

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Exostosis (or osteochondroma) or is the most common benign bone tumour encountered in children and adults. Exostoses may occur as solitary or multiple tumours (in the autosomal syndromes of hereditary multiple exostoses). Exostoses are composed of cortical and medullary bone covered by an overlying hyaline cartilage cap. We have searched iron and Aluminium in the matrix of cortical and trabecular bone of 10 patients with exostosis. Perl's staining (for iron) and mordant blue (for aluminium) were used on undecalcified sections of the tumours. Al was detected in all the tumours as linear bands deposited by the osteoblasts. Iron was detected in 8 out of the 10 patients as linear bands in the same locations. This advocates for a disturbed metabolism of osteoblasts in exostosis similar to that observed in case of hemochromatosis. Al and/or iron are two metals actively substituted to calcium in hydroxyapatite crystals of the bone matrix.

Al exposure from food contact materials made of aluminium

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Two types of Al food contact materials (FCM) were studied from the point of view of the new European test protocol published in 2013 on metals and alloys (CM/Res(2013)9). CM/Res(2013)9 recommends the use of specific simulants in function of the pH of the food to test compliancy. In order to investigate the legitimacy of these simulants, the two groups of Al-FCM were studied in contact with 5 different simulants. As a result, the stipulated simulant for acid food is questioned.
Al burden of the body was estimated from release models established with Statgraphics Centurion trial version. As a conclusion, it is estimated that regular users of this type of material (percentile99) can be exposed up to an Al burden of 100 mg/day after two hours whereas the Al burden estimated from CM/Res(2013)9 corresponds to 70 mg/day (corresponds to percentile 95).

Accumulation of aluminium in human eye tissue: a role in age-related macular degeneration?

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Age-related macular degeneration (AMD) is the leading cause of blindness in developed nations, characterised by formation of drusen deposits between Bruch's membrane (BM) and retinal pigment epithelium (RPE). Multiple genetic and environmental risk factors have been identified for AMD, but whether metal ions accumulate within BM with age has not been extensively studied. We analysed eyes from 77 human donors (11-88 years) and quantified the level of metal ions in BM by ICP-MS. Although there wasn't a significant age-related increase in the level of aluminium, we identified a population of older (non-AMD) donors with a high level of this metal ion. Walton staining localised aluminium to BM and RPE (and drusen in AMD donors) and preliminary gene expression analysis of the RPE cell layer indicates that SOD2 correlates with the amount of aluminium present in the adjacent BM. This suggests that aluminium may contribute to pathogenic oxidative stress in AMD.

Small sensory fiber neuropathy in patients with aluminium hydroxide-induced macrophagic myofasciitis (MMF)

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Patients with aluminium hydroxide-induced MMF mainly complain of musculoskeletal pain, chronic fatigue, and neurocognitive dysfunction. The mechanisms underlying chronic pain in MMF patients are unknown. To deepen understanding of MMF-associated pain, we investigated small sensory nerve fibre involvement in 39 MMF patients by using DN4 scale for neuropathic pain, laser evoked potentials (LEP) and intraepidermal nerve fibre density (IENFD) quantification on skin biopsy. Symptoms were suggestive of neuropathic pain (DN4≥4/10) in 30/39 (67%) patients. DN4 score was 5.05±2.06 (mean±SD). LEP were performed in 22/39 and showed small fibre neuropathy (SFN) in 7/22 (32%). All patients with abnormal LEP had neuropathic pain (DN4≥4/10). Skin biopsy and IENFD quantification was performed in 5 patients. All of them had distal IENFD values below the 50th percentile according to age and gender. In one patient, IENFD was below the 5th percentile and consistent with definite small sensory fibre neuropathy.

Do we need a holistic understanding about the effects and working mechanism of aluminium

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Aluminium, the third most abundant element on earth, is never found in nature as metal, but only in its mineral compounds in the trivalent form. From solution Al precipitates in the neutral pH range (pH 5 – 7) as aluminium hydroxide, Al(OH)3. Aluminium is not known to have any positive function in any form of life. The negative influences seem ever more obvious.

Aluminium has a major role in the fish and forest deaths caused by acid rains dissolving aluminium from the earth. **Fish**: When the acid aluminium-containing water (pH < 5, Al > 50 ug/l) enters the gills of the fish, aluminium hydroxide, precipitating in the neutral milieu, kills the fish by suffocation. **Tree:** the mechanism is obviously basically the same; the Al(OH)3 precipitate prevents transport of nutrients and water, and the tree dries out.

A lot of Al, natural and man-made, enters the human body. The main pathways are the lungs, digestion and skin. A small part of the Al remains in the body. The health effects of Al received publicity for the first time with news about deaths of dialysis patients caused by aluminium in the water. These problems have then been overcome by removing Al from the dialysate. There are a number of other diseases where aluminium has or is suspected to have a role, such as Alzheimer's, autism, ADHD, Parkinson's and breast cancer.

Several doctors recommend prudence. In the words of Dr. John McDougall: "Based on present information prudent action would be to avoid all sources of ingestable and inhaled aluminium. Those who fail to heed this advice will serve as "guinea pigs" for the human experiment that may eventually prove the presence or absence of serious health effects of aluminium." The list of man-made sources of aluminium is long.

It seems that the basic behaviour of Al is always the same: in the neutral life-sustaining pH range Al is bound in its compounds and does not interfere. In water, it dissolves in acid (pH<5) or basic (pH>8) milieu, and precipitates as aluminium hydroxide in the neutral range. The positive and negative effects of aluminium are due to this hydroxide precipitation. **Positive:** Water and wastewater treatment, antiperspirants, buffered aspirins, adjuvants… **Negative:** acid rain and fish & forest kills; blood: aluminium coagulates and produces clots. In the worst case, the clots lead to death; Al is distributed with blood to all parts of the body, for instance the brain (Alzheimer's!)…

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