Aluminum toxicity: The relevant role of the metal speciation

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Biological investigations on aluminum are inevitably complicated by the hydrolysis of the metal compounds in aqueous solutions at physiological pH. In the vast majority of experimental protocols, Al(III) was mostly administered under ill-defined pH conditions and/or as an ill-defined mixture of various chemical species. In an attempt to evaluate and understand the biological effects of Al(III) with the aim to establish new chemical and biological heuristic models a general experimental strategy based on the utilization of Al(III) compounds differently hydrolytically stable with diverse hydrophobic/hydrophilic properties was developed in our laboratory and herein briefly described.

Al(III)solution state: A heuristic model

Al(III) in water in the absence of a coordinating agent

In the absence of a coordinating agent, the Al(III) aqueous chemistry is rather simple [1] as reported in figure 1A. Aluminum salts in water produce acid solutions and they are rapidly hydrolyzed and eventually the situation is that pictured in figure 1A. When the pH value is less than 5, Al(III) is present as a hexaquo component $[Al[H_2O)_6^{3+}]$ usually represented as Al^{3+}. Other metal species as $Al(OH)^{2+}$, $Al(OH)_2^+$ and the insoluble $Al(OH)_3$ are also present in solution. When the pH is moved to physiological values, Al^{3+} precipitates as $Al(OH)_3$ which can be redissolved to $Al(OH)_4^-$ at basic pH. Practically, in a 10 mM solution of $AlCl_3$ at physiological pH, the permissible Al^{3+} is about $10^{-10.3}$ M and all aluminum soluble species are around few μmoles ($10^{-5.2}$) [1]. All other Al(III) species will form the insoluble $Al(OH)_3$.

Al(III) in water in the presence of a coordinating agent

Aluminum lactate as a "metastable" complex

When a ligand is present in solution, the scenario described in figure 1A is rather different and it appear to be more complicated in that numerous hydrolytic species are copresent in solution. Aluminum lactate [$AlLac_3$] (Fig. 1B) is largely utilized in toxicological experimentation in that it is very soluble in water (0.5 M) and apparently able to form stable solution at autogenous pH around 3.5. However, $AlLac_3$ is not expected to exist in water at neutral pH. In fact, at physiological pH $AlLac_3$ is relatively stable at concentrations up to 10^{-3} M. Diversely, below such concentration a "metastable" systems is formed [$Al^{3+}/H_2O/OH^-/Lac^-$] [2]. IR measurements of $AlLac_3$ at mmolar concentrations, demonstrate that the great majority of the ligand Lac^- exists as a hexa-aquo-hydroxo complex not coordinated to the ligand. Diversely, at autogenous pH value, Al(III) complex can survive (Data not shown).

Stable complexes of Al(III): Aluminum-maltolate and Aluminum-acetylacetonate

Aluminum-maltolate [malt = 3-hydroxy, 2-methyl,4-pyronate] [$Almalt_3$] neutral, hydrolytically stable, water soluble (6×10^{-2} M) and moderately lipophilic, and Aluminum-acetylacetonate (acac = 2,4-pentanedione) [$Alacac_3$] neutral, hydrolytically stable, scarsely water soluble (7.8×10^{-3} M) and lipophilic could be considered two paradigmatic chemical models. Figure 1C shows the solubility and speciation of Al(III) as a funtion of pH in the presence of maltolate. Al(III) exists as $Almalt_3$ in a range of pH between 6 and 9. At physiological pH, starting with a 0.1 M solution of $Almalt_3$, the Al(III) complex is present at a concentration of 10^{-2} M, in that part of it has been already hydrolyzed. Consequently, other species as $Almalt_2^+$ (10^{-4} M), $Al(OH)_4^-$ (10^{-7} M) are also formed [3]. In figure 1 (D and E) is reported the calculated domain stability for the "metastable" $AlLac_3$ and the stable complex $Almalt_3$ as a funtion of the metal ion analytical concentration and the pH values. The two pictures are rather different for the two aluminum compounds in that the second complex, which is more hydrolytically stable, can better survive to the hydrolysis.

Aluminum speciation and some biological effects speciation dependent

Now we will report three paradigmatic cases observed in our laboratory that could clarify the connection between Al(III) speciation and biological effects.

The erythrocytic model

Rabbit is know as a sensitive animal to aluminum intoxication. Using rabbit erythrocytes, as a toxicological model, we have seen relevant morphological effects related to the metal speciation. As it can be seen in the figure 2 treatment of cells with $Alacac_3$, $Almalt_3$ or $AlLac_3$, three different effects are observed. $Alacac_3$ (Fig. 2C), neutral, hydrolytically stable

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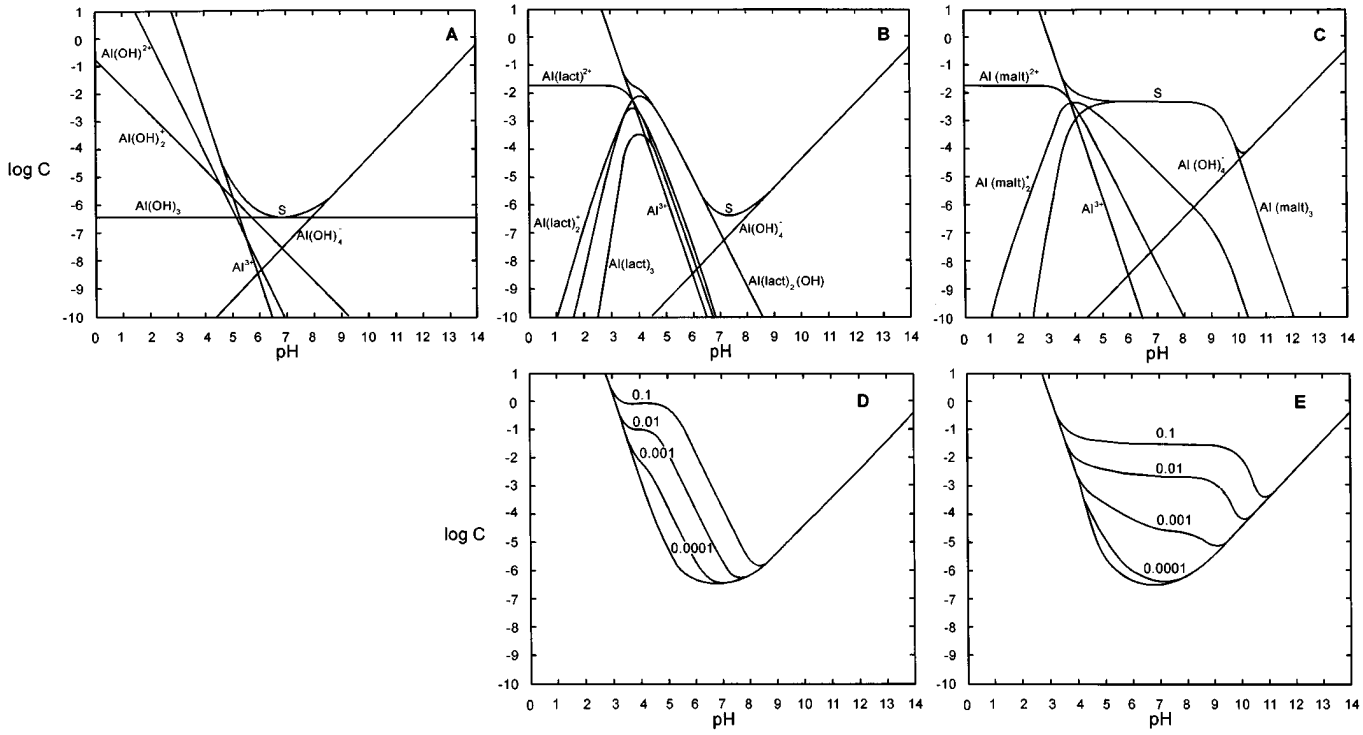

Figure 1. A) Calculated solubility and speciation diagram for Al(III) in water in the absence of ligands different from H_2O and OH^-; B) Solubility and speciation of Al(III) as function of pH in the presence of lactate; C) Solubility and speciation of Al(III) as function of pH in the presence of maltolate; D) Calculated domains for the existence of $AlLac_3$ as a function of metal analytical concentration and of pH; *t* = 25 °C; E) Calculated domains for the existence of $Almalt_3$ as a function of metal analytical concentration and of pH; *t* = 25 °C.

and lipophilic complex forms a morphological modification known as echinoacanthocytes, observed also as in some neurological pathologies; $Almalt_3$ (Fig. 2D), neutral hydrolytically stable and moderately lipophilic forms another morphological modification called echinocytes. Finally, $AlLac_3$, hydrolytically "metastable" and hydrophilic does not produce morphological modifications has observed in figure 2B.

The neuroblastoma model

Toxicological studies on aluminum, utilizing cell cultures, are now increasing with respect to the experimentation *in vivo*. Murine neuroblastoma cells (N2A) represents an interesting model for the aluminum neurotoxicology. $Alacac_3$ and $Almalt_3$ (Figs. 3C and D) are highly toxic in the range of concentration between 0.1 – 1.0 mM. Moderately toxic appears to be their ligand but at higher concentrations. On the contrary, $AlLac_3$ (Fig. 3B) in the range between 0.1 and 1.0 mM results to be scarsely toxic on N2A after 48 hr of treatment. In addition, $AlLac_3$ is strongy neuritogenic in that it has been observed in figure 3B.

The Blood-brain barrier model

In mammals the neuronal environment is controlled by the regulated exchange of solute across the blood-brain barrier (BBB). Al(III) administered systemically in experimental animals has been shown to accumulate in the cerebro-endothelial cells surface. Al(III) might not only thus affect the BBB permeability in terms of free diffusion through the endothelial cell membranes, but it may selectively alter the saturable transport system [4]. In our laboratory, it has been demonstrated that Al(III) can modify the rat BBB permeability in a metal speciation-dependent fashion [5]. In fact, while $Almalt_3$ modifies the BBB permeability transiently, $Alacac_3$ produces a permanent modification and $AlLac_3$ results to be uneffective (Fig. 4).

An analytical approach to the speciation in biological samples

Growing interest concerning the bioavailability and toxicity of Al(III) resulted in the development of numerous analytical techniques to the study of Al(III) speciation. The techniques most frequently used such as spectrophotometry, ion-exchange and chelation ion-exchange chromatography enable determination of the sum of positively charged monomeric Al(III) species. In recent years investigations were directed to the development of analytical methods for the simultaneous determination of different Al species by single procedure. Among the various chromatographic techniques applied, only a cation-exchange FPLC-ICP [6] procedure enabled quantitative determination of particular monomeric Al species [$Al(H_2O)_6^{3+}$ $Al(OH)(H_2O)_5^{2+}$ and $Al(OH)_2(H_2O)_4^+$] in pure aqueous solutions, which is of great importance in investigations of Al toxicity.

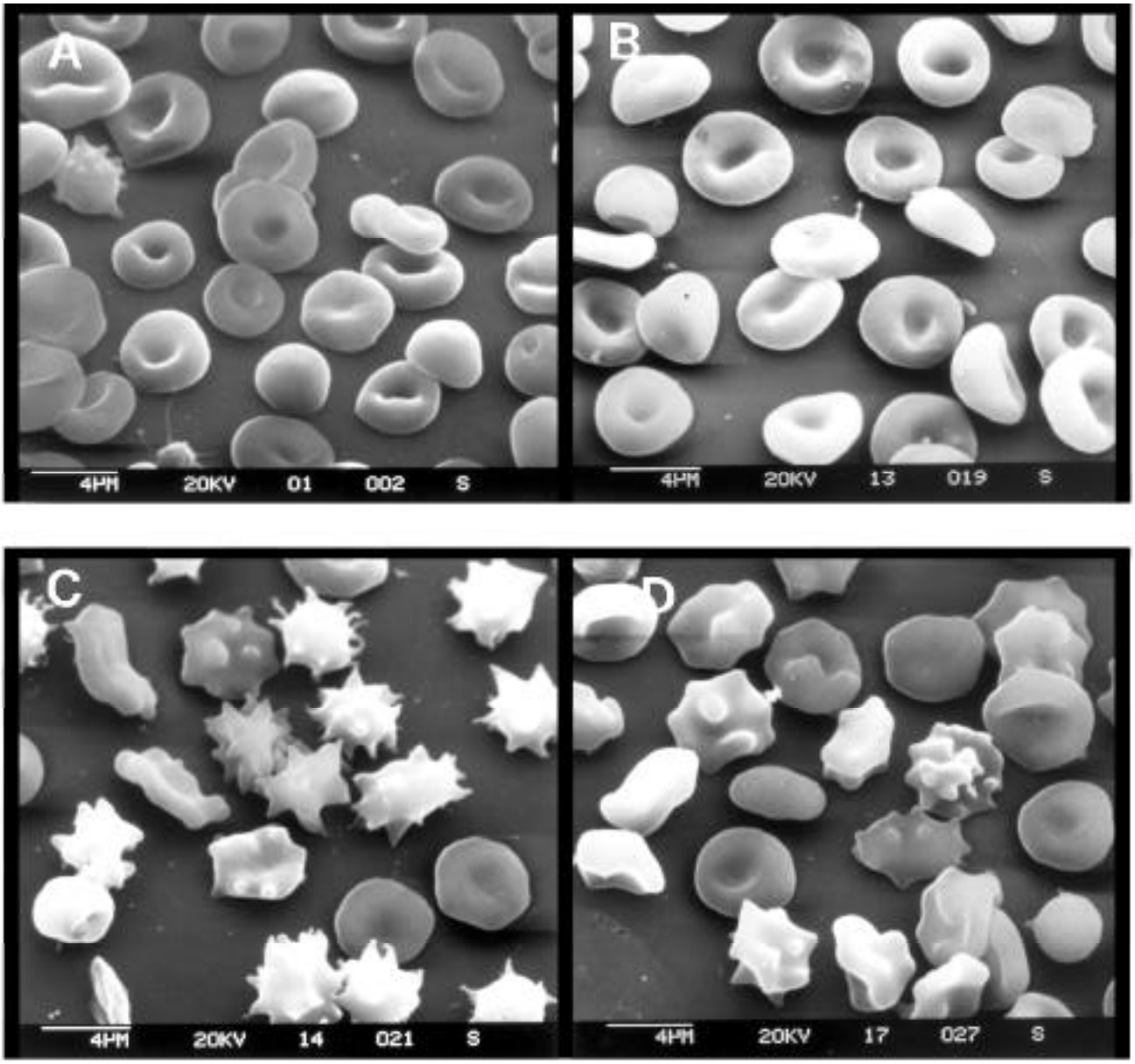

Figure 2. Scanning electron microscopy of rabbit erythrocytes. Differential effects produced by: A) Control; B) $AlLac_3$; C) $Alacac_3$; D) $Almalt_3$.

Protein and low molecular weight organic complexes of Al, as well as hydroxy-Al species, play an important role in biological systems. Al-citrate and $Al(OH)_4^-$ are presumed to be the most active species in terms of Al bioavailability, at physiological pH, and its transport through cell membrane [7]. Due to its biological importance, the distribution of Al-citrate in biological samples was frequently investigated by computer simulation studies. On the basis of known thermodynamic equilibrium constants, aluminum speciation was calculated in blood plasma and in the gastrointestinal tract [8]. There have been many experimental studies performed on the binding of aluminum to serum constituents applying microultrafitration, various chromatographic techniques and gel electrophoresis. The reported data indicated that transferrin is the main high molecular weight aluminum complex [9], while Al-citrate was presumed to be the predominant low molecular weight serum species. Speciation of Al-citrate was investigated employing various chromatographic techniques. The species was identified, but the recoveries for Al-citrate were moderate and the methods were not suitable for quantitation of Al-citrate in biological samples. Bantan et al. [10] performed a systematic study on the quantitative determination of Al-citrate and some other negatively charged low molecular weight aluminum complexes by FPLC separation of Mono Q HR 5/5 strong anion-exchange column with ICP-AES detection, using $NaNO_3$ as eluent. The procedure developed enables quantitative determination of Al-citrate over a wide pH range (from 3.5 to 11.0). At pH values higher than 8.0, $Al(OH)_4^-$ can be identified, but quantitatively determined only at pH 11.0. Development of reliable and quantitative analytical methods for speciation of $Al(OH)_4^-$ at physiological pH values is still under development. The applicability of the above techniques for speciation of Al-citrate in the majority of biological samples was limited due to its

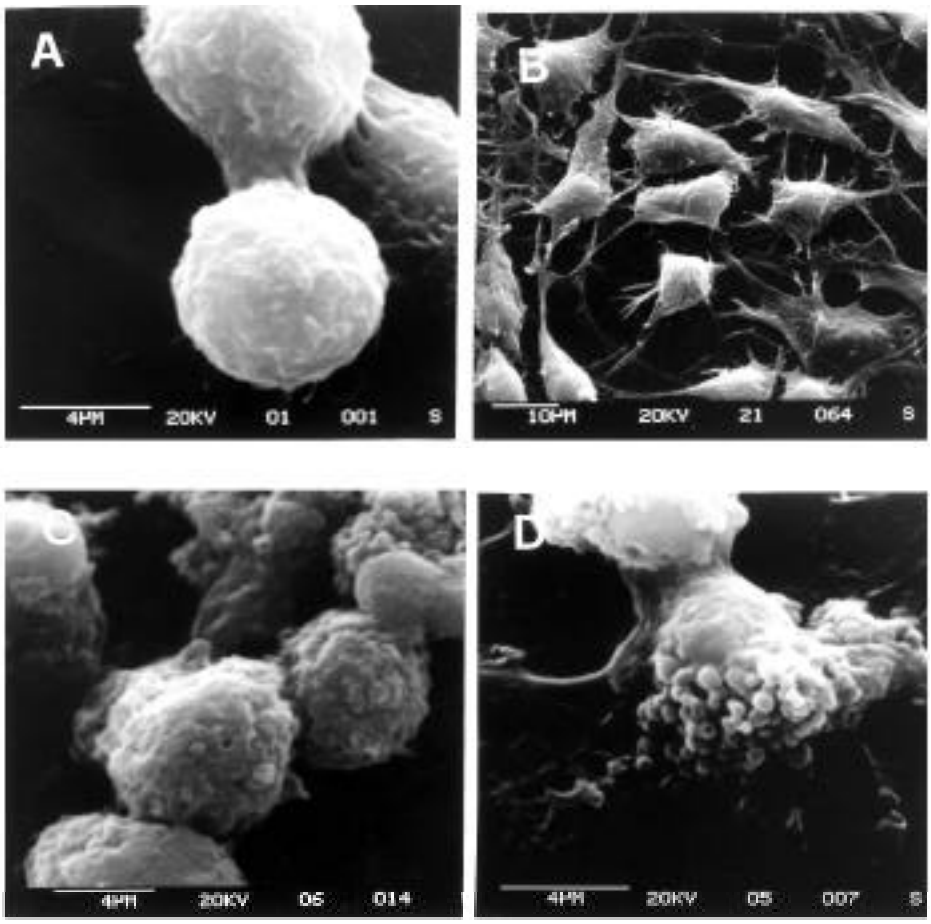

Figure 3. Scanning electron microscopy of neuroblastoma cells. Different morphological effects produced by B) $AlLac_3$, C) $Almalt_3$ and D) $Alacac_3$ with respect to the control A).

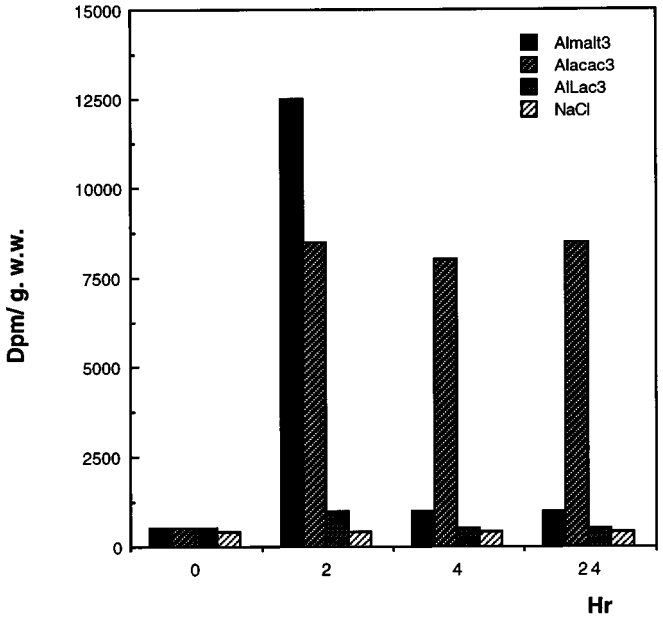

Figure 4. Blood brain-barrier permeability to sucrose ^{14}C, 2, 4 and 24 hr after injection of NaCl, 2.2 mg Al/kg as $Alacac_3$, 2.2 mg Al/kg as $Almalt_3$ and 5.0 mg Al/kg as $AlLac_3$.

moderate sensitivity. To lower the detection limits for speciation of Al-citrate to the low ng/cm^3 concentration level the choice of an appropriate eluent which would enable quanti-

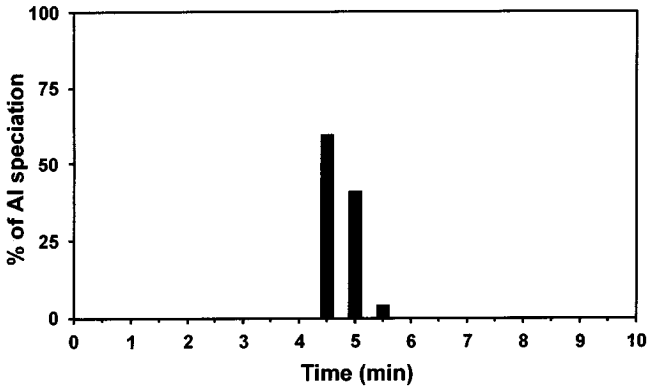

Figure 5. Typical chromatogram of Al-citrate (100 ng Al cm^{-3}, 100 fold molar excess of citric acid) at pH 7.4, separated on an anion-exchange FPLC Mono Q HR 5/5 column and detected by ETAAS with Zeeman correction. Sample volume 0.5 cm^3, aqueous NH_4NO_3 (– 4 mol dm^{-3}) linear gradient elution, flow rate 1 cm^3 min^{-1}, fraction collection 0.5 cm^3, n = 3.

tative separation of Al-citrate on a Mono Q HR 5/5 strong anion-exchange FPLC column and reliabel determination of separated species by ETAAS is of critical importance. Aqueous – 4 mol dm^{-3} NH_4NO_3 gradient elution was found to separate quantitatively Al-citrate on the column in the range 3.5 to 8.0. The main advantage of NH_4NO_3 lies in its ability to decompose quantitatively in the graphite tube during the ashing step, enabling quantitative and very reproducible determinations of separated Al species by ETAAS. A detection limit of 2 ng Al-citrate cm^{-3} was obtained, and the techniques developed applied for the quantitative speciation of Al-citrate in spiked human serum samples. A typical chromatogram for Al-citrate at pH 7.4, separated on an FPLC Mono Q HR 5/5 anion-exchange column with ETAAS detectionis presented in the figure 5. It is evident that negatively charged Al-citrate species are quantitatively eluted at this pH between 4.5 and 5.5 min with a maximum peak at a retention time of 4.5 min.

Conclusions

Molecular bases of Al(III) toxicity are far to be completely understood. Among many experimental approaches cellular models appear to be suitable candidates for a better understanding of the peculiarity of the biological effects toward the metal speciation. The study of the interaction between Al(III) and biological systems, in our view, is just at the beginning, and more interdisciplinary work is necessary to shed light on the aluminum connection with human neuropathologies such as for instance Alzheimer's disease, Parkinsons disease and others. It is rather clear that the individual susceptibility for aluminum toxicity is a key event (genetic phenotype) on the predisposition to accumulate the metal ion in terms of blood brain-barrier vulnerability, membrane phospholipis dismetabolism etc., during the life span, in relation to different environmental possibility to acquire the metal ion from different sources and external environment, food and pharmaceutical products, etc. uptake.

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