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A Long-Term Toxicological Investigation on the Effect of Tris(maltolate)Aluminum(III) in Rabbits

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ABSTRACT

The toxicity of iv injected hydrophilic aluminum complex tris(maltolate)aluminum(III) was studied in New Zealand white rabbits for a period of time ranging from 5 to 63 wk. Animals were injected 3–5 times a week with 1 mL of 7.5 m*M* $Al(malt)_3$ and one rabbit with a dose 10 times higher after 14 wk of treatment. Autopical examination was performed on all animals. Chemoclinical analysis (glucose, urea, creatinine, cholesterol, bilirubin, alanin aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltransferase, LDH, CK, total protein, triglycerides, and Ca^{2+}) gave no variation in treated animals with respect to the control. The toxicological data show a moderate systemic general toxicity at doses far higher than those used in similar previous experiments using $Al(acac)_3$ (acac = 2,4 pentanedionate), a hydrolytically stable and more lipo-

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philic aluminum(III) complex (*1*). The diversity of behavior is discussed in terms of metal speciation as well as respect to the thermodynamic and kinetic properties of the two complexes in aqueous solution. The toxicological model presented here emphasizes that neutral, water compatible aluminum(III) complexes are to be considered as promising tools for toxicological experiments providing biological models of human pathologies.

Index Entries: Aluminum(III); maltol; acetylacetone; metal complexes; rabbits.

INTRODUCTION

In previous papers, we have presented our novel approach for testing the in vivo and in vitro toxicological effects of Al^{III} (*2*) as well as our relevant results (*1,3–5*). Our chemical and biological model of Al^{III} toxicity is based on the administration of lipophilic (or hydrophilic), Al^{III} complexes of differing hydrolytic stabilities with the aim of employing metal toxins bearing precise chemical identity at pH 7.5, and characterized by tailored solubility properties (*6*).

$Al(acac)_3$ (acac = 2,4 pentandionate) is a lipophilic and hydrolytically stable complex ($\beta = 10^{+22.3}$) (*7*), which exhibits a remarkable cardiotoxic potency in rabbits (iv) (total doses: 250 μmol/kg body wt) (*1,5*). It is worth pointing out that our original expectation with this toxin was to produce novel in vivo neurological effects (*8*), but the cardiotoxicity of $Al(acac)_3$ turned out to be so pronounced to make long-term experimentation in rabbits rather questionable.

On the basis of this observation, we decided to move to $Al(malt)_3$ (malt = 3-hydroxy-2-methyl-4-pyronate or maltolate) (*9*), which is hydrolytically stable as Al $(acac)_3$ ($\beta = 10^{22.5}$ (*10*), but definitely hydrophilic in character (*6*) and expected by us to be less cardiotoxic than $Al(acac)_3$, and perhaps more suitable for producing long-term neurological effects in rabbits.

We devised toxicological experiments aimed to strictly parallel (in terms of administration protocol and doses) those carried out with $Al(acac)_3$ and just at the end of our experiments, a paper by Bertholf et al. (*11*) prompted us to publish our own data. These authors found that iv injection of fairly high total amounts (2.25–6.75 mmol) $Al(malt)_3$ produce significant lesions in the liver and kidney of the rabbit with concomitant accumulation of aluminum. Neurotoxic effects are very limited as well as metal accumulation in the brain. Heart tissue results unaffected and chemoclinical indexes appear to be practically normal. Our toxicological data based on the administration of doses generally lower than those employed by these authors, specifically confirm that $Al(malt)_3$ is definitely noncardiotoxic and some novel chemical data obtained in these laboratories on the kinetic stability of $Al(malt)_3$ in aqueous media strongly suggest that the chemical identity of the employed toxin is likely to be

preserved in the bloodstream of the experimental animals, at the analytical concentration employed both by Bertholf et al. (*11*) and by us.

MATERIALS AND METHODS

Animals

Young adult (3.0–4.0 kg) New Zeland White male rabbits were used in this study. Rabbits were housed in individual cages, received ad libitum water, were fed a standard rabbit chow (Italiana Mangimi Milano), and were maintained under the light–dark conditions of an ordinary pound. Rabbit wt was systematically controlled, and no important changes different from those expected from the normal growth were observed. Three rabbits were kept as controls. Rabbits were observed for signs of motorial deficits. Rabbit number is a code related to our pound.

Reagents

Al(malt)$_3$ was prepared and characterized (elemental analysis, IR spectrum in nujol mull) according to Finnegan et al. (*9*). Aluminum(III) solutions (7.5 or 60 m*M*) were prepared under sterile conditions by dissolution of weighed amounts of Al(malt)$_3$ in known volumes of sterile physiological solution (pH about 7).

Test of Hydrolitic (Meta)stability of Al(Malt)$_3$ Aqueous Solutions at $1 \times 10^{-4}M$ and $5 \times 10^{-5}M$ Concentrations

The theoretically expected speciation of Al(malt)$_3$ in water at Al concetrations in the 50–100 μ*M* range, (i.e., comparable to those in the rabbit body after injection of 7.5–75 μmol of Al(III) (25–250 μ*M*)), was evaluated by UV spectrophotometry in the 250–350 nm range at 37°C. In fact, Al(malt)$_3$ exhibits a strong charge-transfer absorption band (ε about 25000*M*/cm) at 340 nm, which disappears when Al(malt)$_3$ is completely hydrolized into Al(OH)$_3$ and Hmaltol. Simple monitoring of this UV spectrum with the time reveals that ca. 78% and 60% of the initial UV absorption attributable to metallorganic ring is still observed in solution after 170 h at 100 and 50 μ*M* complex concentrations, respectively. In contrast with this apparent (meta)stability, thermodynamic calculations based on the known formation constant of Al(malt)$_3$ ($\beta = 10^{22.5}$ (*10*) lead to the expectation of quantitative hydrolysis of Al(malt)$_3$ in such metal concentration range.

Venous Access and Injection

Injections were carried out through the rostral auricolar vein. Rabbits were injected 3–5 times/wk with 1 mL of 7.5 m*M* Al(malt)$_3$ solution,

with the exception of rabbit 51, for which the dose was increased 10 times after 104 injections.

Necropsy

Rabbits were killed by a letal injection of Ketalar (Parke-Davis) (3 mL). Autoptical examination was performed on all animals. After macroscopic examination, samples of brain, cerebellum, heart, liver, kidney, spleen, and lung were examined microscopically after 24–48 h fixation in 10% buffered formalin. Sections were stained with ematoxylin-eosin and brain was also examined with Bodian's stain.

Chemoclinical Analyses

Urea, glucose, creatinine, total cholesterol, total bilirubin, alanine aminotransferase (ALAT/GPT), aspartate aminotransferase (ASAT/GOT), alkaline phosphatase, gamma glutamyltransferase (GGT), lactic dehydrogenase (LDH), pyruvate kinase (CK), total protein, triglycerides, and Ca^{2+} were determined by means of Boehringer (Mannheim) kits.

RESULTS AND DISCUSSION

The administration time schedule is schematically depicted in Fig. 1. The histological findings, total Al^{III} doses, and total treatment times are collected in Tab. 1. Chemoclinical data (*see* parameters reported in Methods) measured in serum samples from all investigated rabbits gave normal figures (*12*).

Metal doses were chosen to make possible a direct dose-effect comparison with the effect of $Al(acac)_3$ (*5,1*). Rabbit 48 was sacrificed owing to the occurrence of a paralysis to both the posterior legs. Rabbit 49 died spontaneously, most probably owing to a pulmonary infection. Both these animals received total Al^{III} doses largely greater than those uptaken by the $Al(acac)_3$-treated rabbits (*1,5*), so that the relevant autoptical evaluations are to be considered reliable and relevant to the specific target of this investigation. In fact, limited heart, liver, spleen, kidney effects were observed with no damage to brain and cerebellum. Chemoclinical indexes were normal, particularly those related to cardiac functionality. A very long-term experimentation with 200 μg Al^{III} doses/d turned out to be possible with rabbits 50 and 52. In their case, little damage to the liver could be documented, with practically no cardiac damage after total doses equal to 38.8 and 58.6 mg, respectively.

Rabbit 51 was treated with "normal" $Al(malt)_3$ doses (*see below*) for 154 d, before their multiplication by 10. This animal received a total dose of Al^{III} (82.8 mg) that was comparable to those normally employed by Bertholf et al. (*11*) (about 100 mg). It died under the heavier Al^{III} treatment and only distinct histological damage to kidney and spleen (as for

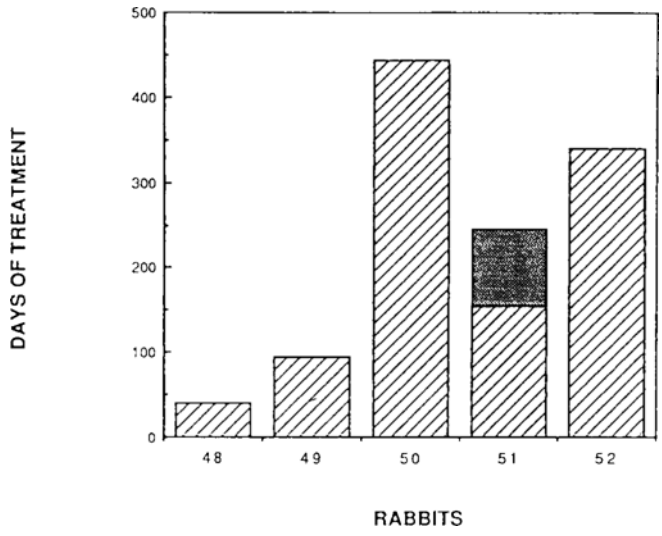

Fig. 1. Administration schedule for the investigated animals.

rabbit 49) could be observed. None of the employed rabbits gave evident signs of gross behavioral effects.

The toxicological data here reported reveal a moderate systemic general toxicity of iv administered Al(malt)$_3$ to rabbits, at doses far higher than those found to be strongly cardiotoxic for Al(acac)$_3$, and we attribute this evident diversity of behavior to speciation reasons, i.e., to the lesser bioavailability of the hydrophilic toxin Al(malt)$_3$ than that of the lipophilic one, Al(acac)$_3$.

This very marked speciation effect strongly suggests that the chemical identity of both Al(acac)$_3$ and Al(malt)$_3$ has to be preserved at least for a few hours after their injection into the animals body. In fact, if the fate of the metal center after injection would be identical in both cases, no such enormous speciation effect would be observed (both Hacac and Hmaltol are toxicologically inactive). In contrast with this result, calculations based on hydrolytic stability of Al(malt)$_3$ and formation constant of Al^{III} complexes with transferrin and citrate led Bertholf et al. (*11*) to estimate that more than 98% of injected Al^{III} should be transferrin and citrate bound, so that "essentially none of the aluminum exists in the form of injected aluminum maltol". We totally disagree with this "pessimistic" estimation. Of course, we do not object to thermodynamically-based expectations, but we also expect (*13*), and we did prove this by means of in vitro experiments (*see* Methods), Al(malt)$_3$ to exhibit a remarkable hydrolytic (meta)stability at pH about 7. In fact, inspection of Fig. 2 indicates that in a 150 μM (analytical) solution of Al(malt)$_3$ (pH ca. 7), only about 10 μM Al(malt)$_3$ should be present as such, at

Table 1
Histological Findings, Total Al^{III} Doses, and Total Treatment Times for the Investigated Animals

Rabbit *n*[a]	Total Al doses, mg	Histology
48	5.4	Killed owing to a paralysis to both posterior legs
	(27 i, 40 d)[b]	Heart: mild edema
		Liver: mild inflammation of the portal tracts
		Spleen: follicular hyperplasia
		Kidney: mild pyelonephritis
		Cerebrum: normal
		Cerebellum: normal
49	9.6	Death with respiratory distress
	(48 i, 95 d)[b]	Heart: edema
		Liver: acute statis and hepatocytic necrosis
		Kidney: acute stasis
		Spleen: acute stasis
		Cerebrum: normal
		Cerebellum: normal
50	58.6	Killed
	(293 i, 443 d)[b]	Heart: very mild myocarditis
		Liver: portal and periportal round-cell inflammation
		Kidney: normal
		Lung: normal
		Spleen: normal
		Cerebrum: normal
		Cerebellum: normal
51	82.8	Deceased
	(135 i, 245 d)[b]	Heart: normal
	multiplication by ten	Liver: normal
	after 104 i, 154 d	Kidney: acute stasis
		Spleen: acute stasis
		Cerebrum: normal
		Cerebellum: normal
52	38.8	Killed
	(194 i, 340 d)[b]	Heart: normal
		Liver: very mild portal inflammation
		Spleen: normal
		Kidney: normal
		Lung: normal
		Cerebrum: normal
		Cerebellum: normal

[a]Numbers refer to pound registry.
[b]Number of injections and treatment days.

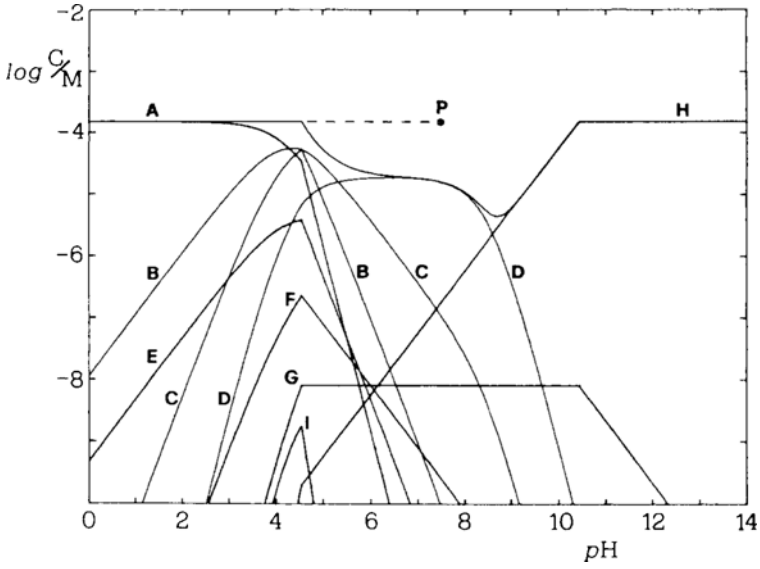

Fig. 2. Calculated speciation diagram for a 150 μ*M* solution of Al(malt)$_3$, as a function of pH. (A = Al^{3+}, B = $Al(malt)^{2+}$, C = $Al(malt)_2^{+}$, D = $Al(malt)_3$, E = $Al(OH)^{2+}$, F = $Al(OH)_2^{+}$, G = $Al(OH)_3$, H = $Al(OH)_4^{-}$, I = $Al_3(OH)_4^{5+}$, coordinated water molecules are omitted). Point P refers to a 150 μ*M* Al^{III} solution, in which the species Al(malt)$_3$ is found to survive, thanks to its kinetic (meta)stability toward hydrolysis. In fact, the thermodynamically expected concentration of Al(malt)$_3$ would be ca. 10 μ*M*.

this pH value, the other two "important" species in solution being $[Al(malt)_2(H_2O)_2]^{+}$ and $[Al(OH)_4]^{-}$, both at concentrations lower than 1 μ*M*. A simpler presentation of these concepts can be seen in Fig. 3, which shows clearly that the proportion of Al(malt)$_3$ existing in solution with respect to that actually dissolved (i.e., Al^{III} analytical concentration) decreases quite markedly under $10^{-3}M$.

In contrast with these predictions, our data prove that 50 and 100 μ*M* solutions of Al(malt)$_3$ the aluminum maltolate metallorganic ring survive long time to hydrolysis at 37°C at pH about 7.

In view of the dissociative character of the mechanism that regulates the ligand substitution reactions inside the coordination sphere of Al^{III} complexes, hydrolytic (meta)stability does imply a general *kinetic resistance to changes inside the metal coordination sphere*, i.e., kinetic reluctance of the metal center to undergo (thermodynamically predicted) complexation, e.g., by transferrin and excess citrate in plasma.

In conclusion, we confirm (*1,3,5*) that the toxicity of Al^{III} is dramatically affected by the nature of its coordination sphere (speciation), and

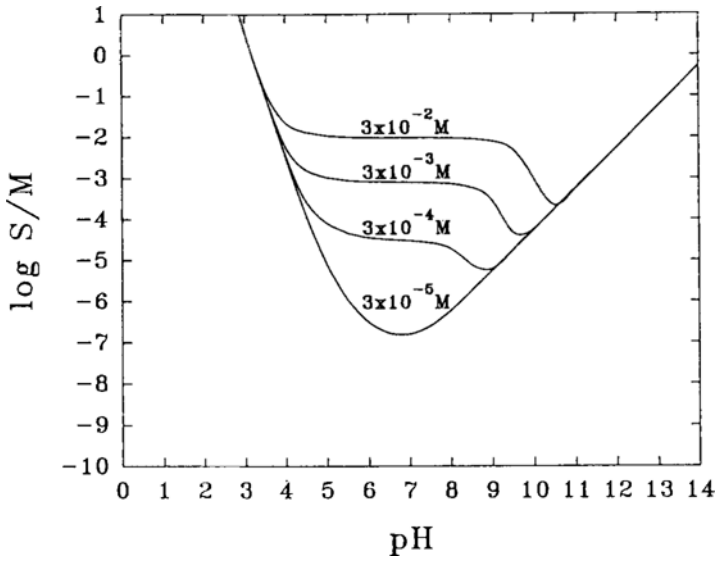

Fig. 3. Solubility diagram of $Al(OH)_3$ in the presence of various concentrations of Hmaltol. It is apparent that the pH window that enables $Al(malt)_3$ to exist as such, becomes narrower as the initial concentration of the complex decreases. The -logS figure corresponding to the plateaux furnishes the concentration of $Al(malt)_3$, expected on thermodynamic grounds, for any initial Al^{III} concentration.

that artificial metal toxins have to be tailored by taking into account their lipophilic-hydrophilic character.

Moreover, we wish to strongly emphasize that neutral, water-compatible, Al^{III} complexese are to be considered interesting and promising tools for toxicological experimentation directed at obtaining biological models of human pathologies. In fact, also at concentration levels at which thermodynamic calculations lead one to expect extensive speciation changes, kinetic (and experimentally verifiable) conditions may ensure the survival of the administered molecular species, which is a requisite for obtaining reliable biological effects.

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