Membrane composition can influence the rate of Al³⁺-mediated lipid oxidation: effect of galactolipids

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In the first part of the present study we investigated the effects of pre-natal and early postnatal exposure of mice to high levels of dietary Al³⁺ on myelin lipid composition and lipid oxidation. We found: (1) a significantly higher (104%; $P < 0.01$) content of brain myelin galactolipids in the high-Al³⁺ group than in controls, and, (2) a significant correlation ($r^2 = 0.70$; $P < 0.01$) between the concentration of myelin galactolipids and TBARS (2-thiobarbituric acid-reactive substances) content, a parameter of lipid oxidation. Based on these results, we evaluated in an *in vitro* model (liposomes) whether galactolipids could affect the capacity of Al³⁺ to stimulate Fe²⁺-initiated lipid oxidation, and whether this effect could be due to the promotion of changes in membrane physical properties (membrane phase separation and rigidifi-

cation). The presence of galactolipids (10–40 mol%) in the liposomes caused a concentration-dependent increase in the stimulatory effect of Al³⁺ on Fe²⁺-induced TBARS production, and on the ability of Al³⁺ to induce phase separation and membrane rigidification. The capacity of Al³⁺ (10–100 μ M) to induce lateral phase separation in liposomes composed of phosphatidylcholine/phosphatidylserine/galactolipid (36:24:40, molar ratio) was correlated significantly ($r^2 = 0.99$; $P < 0.001$) with the stimulatory action of Al³⁺ on Fe²⁺-induced TBARS production. We propose that the high content of galactolipids found in myelin from Al³⁺-intoxicated mice could favour Al³⁺-induced changes in membrane physical properties, with the subsequent acceleration of lipid oxidation rates.

INTRODUCTION

Aluminium, which can be found in high concentrations in the environment, is a neurotoxic metal which that has been proposed to be a causative agent of several neuropathologies [1,2]. The exposure of mice, during development and early adulthood, to high levels of dietary Al³⁺ (1000 μ g of Al³⁺/g, compared with 3 μ g/g in the control diet) [3] was associated with increases in tissue Al³⁺ concentrations, and changes in several neurobehavioural parameters.

Studies from this and other laboratories have demonstrated that, while Al³⁺ is a non-redox metal, it can stimulate Fe²⁺-initiated lipid and protein oxidation in biological membranes and in liposomes [4–7]. Investigations into the mechanisms involved in the effect of Al³⁺ on Fe²⁺-induced lipid oxidation have indicated that Al³⁺ can promote the formation of clusters of negatively charged phospholipids, with the result that the mobility of the acyl groups of phospholipids is restricted [8,9]. We have speculated that these changes in membrane physical properties subsequently influence the rate at which Fe²⁺-initiated lipid oxidation occurs.

Interestingly, when studying membranes isolated from rat brain, we observed that the pro-oxidant effect of Al³⁺ was more pronounced in myelin membranes than in synaptic, mitochondrial or microsomal fractions [7]. A pro-oxidant effect of Al³⁺ was also noted in mice fed on high-Al³⁺ diets during pre-natal and early postnatal development; animals exposed to this insult had higher than normal levels of lipid oxidation products [7]. The above is consistent with the fact that myelin has a high relative content of lipids (lipid/protein, 70:30, w/w), thus creating an environment in which Al³⁺-induced changes in membrane physical properties would be favoured.

In the present study we investigated the effect of chronic exposure to high levels of dietary Al³⁺ on myelin lipid com-

position. Based on a finding of a markedly elevated galactolipids content of galactolipids in the myelin isolated from mice in the high-Al³⁺ group, in a subsequent series of studies we evaluated in an *in vitro* model whether galactolipids could affect the capacity of Al³⁺ to stimulate Fe²⁺-initiated lipid oxidation, and whether this effect could be through a promotion of changes in membrane physical properties.

EXPERIMENTAL

Materials

Brain phosphatidylcholine (PC) and phosphatidylserine (PS) were obtained from Avanti Polar Lipids Inc. (Birmingham, AL, U.S.A.). Bovine brain galactocerebroside were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 2-([6-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoyl-1-hexadecanoyl-*sn*-glycero-2-phosphocholine (C₆-NBD-PC) and 1,6-diphenylhexa-1,3,5-triene (DPH) were obtained from Molecular Probes Inc. (Eugene, OR, U.S.A.). Aluminium(III) potassium sulphate was obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.).

Animals

NIH mice (seven animals per group) were fed on purified diets containing either 3 or 1000 μ g of Al³⁺/g (3 Al and 1000 Al diets respectively), as aluminium lactate, from day 0 of pregnancy. The diets and animal housing have been previously described in detail [10]. Mice fed the 1000 Al diet are not characterized by overt signs of toxicity or growth retardation [3]. At birth the number of pups was adjusted to eight per litter. Litters were weaned at postnatal day 21, and the pups were continued on the same diets as their mothers. At postnatal day 40, mice

Abbreviations used: TBARS, 2-thiobarbituric acid-reactive substances; PC, phosphatidylcholine; PS, phosphatidylserine; GL, galactolipids; C₆-NBD-PC, 2-([6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoyl-1-hexadecanoyl-*sn*-glycero-2-phosphocholine; DPH, 1,6-diphenylhexa-1,3,5-triene.

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were killed by an overexposure to carbon dioxide and their brains were quickly excised and placed at 4 °C. Brains from all mice in a given litter were pooled.

Subcellular fractionation

Brain myelin membranes were isolated as described by Lapetina et al. [11]. Protein concentration in the myelin fraction was measured according to Lowry et al. [12] using BSA as a standard.

Lipid determination

Lipids were extracted from myelin as described by Folch et al. [13], and the contents of phospholipids [14], galactolipids [15] and cholesterol [16] were determined in the washed lower phase.

Liposome preparation

Galactolipids [17] and phospholipid [18] purity was checked by TLC. Aliquots of stock solutions of PC and PS in chloroform, and of galactolipids in chloroform/methanol (2:1, (v/v) solution), were dried under high vacuum in a Buchi Rotavapor for 15 min and under a N₂ stream for a further 15 min. The dried lipids were resuspended (2.5 mg/ml) in 20 mM Tris/HCl/140 mM NaCl buffer (pH 7.4), vortexed for 1 min, and incubated at 45 °C for 10 min under N₂. The preparations were sonicated by three cycles of 45 s at 80 W in a Branson 250 sonifier (Branson Ultrasonics Corp., Danbury, CT, U.S.A.). The average diameter of the liposomes, determined by fluorescence microscopy, was similar among in the different populations and corresponds to that of intermediate vesicles. The percentage of galactolipids present in the outer leaflet of the liposomes was determined by oxidizing the galactose moiety with NaIO₄ [19] and subsequent derivatization with 2,4-dinitrophenylhydrazine. Galactolipids were similarly distributed in both leaflets in vesicles containing 10, 20, 30 and 40 mol % galactolipids.

Evaluation of lipid oxidation

Liposomes composed of PC/PS (60:40, mol/mol) containing increasing amounts of galactolipids (0–40 mol %) were incubated (0.25 mg of lipid/0.5 ml) for 90 min at 37 °C in the presence of Al³⁺ (10–100 µM) with or without the addition of 25 µM FeSO₄. The FeSO₄ stock solution (2.5 mM) was prepared in Milli-Q water immediately before addition. Lipid oxidation was evaluated at the end of the incubation as 2-thiobarbituric acid-reactive substances (TBARS) formation.

For determination of TBARS in liposomes [20], incubations were stopped by the addition of 0.1 ml of 4% (w/v) butylated hydroxytoluene in ethanol. SDS [0.25 ml of a 3% (w/v) solution] was added to 0.5 ml of the incubation mixture and, after mixing, 0.5 ml of 1% (w/v) 2-thiobarbituric acid in 0.05 M NaOH and 0.5 ml of 25% (v/v) HCl were added. Samples were heated for 15 min in boiling water, and TBARS were extracted in 3 ml of n-butanol. After centrifugation at 700 g for 10 min, the fluorescence of the butanol layer was measured at 555 nm (excitation wavelength 515 nm) in a Kontron SFM-25 spectrophotofluorimeter (Kontron Instruments S.P.A., Milan, Italy). TBARS values are expressed as malondialdehyde equivalents. Stimulation of lipid oxidation was calculated as:

$$\text{Stimulation} = (\text{TBARS with Al}^{3+} + \text{Fe}^{2+}) / (\text{TBARS with Fe}^{2+}) \quad (1)$$

In liposomes, TBARS were calculated based on phospholipid

content. TBARS content in myelin membranes was determined as described by Fraga et al. [21].

Phase separation

Membrane lipid phase separation was evaluated using a fluorescence technique as described by Hoekstra [22]. PC/PS liposomes (total lipid concentration: 30 µM) containing increasing amounts of galactolipids (0–40 mol %) and 5 mol % of the fluorescent probe C₆-NBD-PC, suspended in 20 mM Tris/HCl/140 mM NaCl buffer (pH 7.4), were preincubated for 5 min at 25 °C. Al³⁺ (10–100 µM) was added, and the lateral phase separation was evaluated 2 min after the addition of the cation by the decrease in fluorescence intensity which that occurred due to quenching. Fluorescence (excitation at 468 nm; emission at 530 nm) was measured in a Kontron SFM-25 spectrophotofluorimeter; fluorescence quenching [*Q* (%)] was calculated as:

$$Q (\%) = 100 \times (I_0 - I_i) / I_0 \quad (2)$$

where *I*₀ and *I*_i are the fluorescence at 530 nm in the absence and in the presence respectively of Al³⁺.

Lipid packing

Al³⁺-induced changes in membrane fluidity were evaluated using the fluorescent probe DPH. Liposomes comprising PC/PS (60:40, mol/mol) containing increasing amounts of galactolipids (0–40 mol %) and 1 mol % of DPH in a lipid concentration of 0.5 mg/ml were preincubated for 5 min at 37 °C. After addition of 10–100 µM Al³⁺, liposomes were incubated for 5 min at 37 °C with continuous stirring. Fluorescence polarization was measured in a Kontron SFM-25 spectrophotofluorimeter (excitation 360 nm; emission 450 nm) with temperature control. The order parameter (*Sp*) was calculated as described by van Blitterswijk et al. [23].

Statistics

One-way analysis of variance (ANOVA test) and estimates of correlations were performed using Statview 512+ (Brainpower Inc., Calabasas, CA, U.S.A.).

RESULTS

Lipid composition in Al³⁺-intoxicated mice

Mice in the control and high-Al³⁺ groups had similar body and brain weights at postnatal day 40 (results not shown). Myelin from the 3 Al and 1000 Al groups was isolated and the concentrations of the major lipids were determined (Table 1). The brain myelin phospholipid concentration was significantly higher

Table 1 Lipid composition of brain myelin from control and Al³⁺-intoxicated mice

Lipids from brain myelin were extracted as described in the Experimental section, and the concentrations of proteins, phospholipids, galactolipids and cholesterol were measured in the extracts. Results are shown as means ± S.E.M. (*n* = 6). Significant difference compared with control: **P* < 0.05 (*t* test).

	3 µg of Al ³⁺ /g	1000 µg of Al ³⁺ /g
Protein (mg/g wet tissue)	5.9 ± 0.4	5.9 ± 0.3
Phospholipids (µmol/g wet tissue)	3.3 ± 0.4	4.5 ± 0.4*
Cholesterol (µmol/g wet tissue)	29 ± 2	29 ± 2
Galactolipids (µmol/g wet tissue)	1.9 ± 0.4	3.9 ± 0.5*

Figure 1 Correlation between galactolipid concentration and TBARS production in myelin from mice fed diets containing 3 or 1000 μg of Al^{3+} /g

Brain myelin from control and high- Al^{3+} mice was isolated, and the concentration of lipid peroxidation products (TBARS content) and the galactolipid content were determined as described in the Experimental section.

(37%; $P < 0.05$) in the 1000 Al group than in controls. Myelin cholesterol concentrations were similar in the two groups, but the content of galactolipids was 104% higher ($P < 0.01$) in the 1000 Al mice than in the controls. Myelin protein concentration was similar in the two groups.

As reported previously, the concentration of TBARS in myelin from the 1000 Al mice was significantly ($P < 0.05$) higher than in the controls. The concentration of TBARS in the myelin fraction of the control and high- Al^{3+} mice was significantly correlated with the concentration of galactolipids (Figure 1) ($r^2 = 0.70$; $P < 0.05$). The galactose-headgroup of galactolipids was not found not to interfere with the TBARS reaction under the conditions used in the assay (results not shown).

In vitro studies

Given the high levels of lipid oxidation products found in the myelin from 1000 Al mice, and the observation of a strong correlation between myelin TBARS and galactolipids concentration, the potential role of these lipids in the stimulatory effect of Al^{3+} on Fe^{2+} -initiated lipid oxidation was investigated in liposomes. Liposomes comprising PC/PS (60:40, mol/mol), or PC/PS/galactolipid (36:24:40, molar ratio), were incubated for 90 min at 37 °C in the presence of Al^{3+} (10–100 μM) and/or Fe^{2+} (25 μM). In the absence of Fe^{2+} , the TBARS concentration was not influenced by Al^{3+} addition either in the presence or in the absence of galactolipids. When 25 μM Fe^{2+} was added, the concentration of TBARS was 17.2 ± 0.7 and 17.2 ± 2.0 nmol of TBARS/mg of phospholipid for PC/PS (60:40) and PC/PS/galactolipid (36:24:40) liposomes respectively. The stimulation of Fe^{2+} -initiated TBARS production was strongly influenced by Al^{3+} concentration in both liposome systems. In the presence of Fe^{2+} and Al^{3+} , the concentration of TBARS was significantly higher in PC/PS/galactolipid (36:24:40) liposomes at concentrations 50 and 100 μM compared with PC/PS (60:40) liposomes at Al^{3+} concentrations of 50 and 100 μM (Figure 2A). At 50 μM Al^{3+} , the stimulation of TBARS production was a 29% higher in the PC/PS/galactolipid (36:24:40) liposomes compared with PC/PS (60:40) liposomes; with 100 μM Al^{3+} it was 56% higher. There was a significant correlation ($r^2 = 0.98$; $P < 0.001$) between the concentration of galactolipids and the stimulatory effect of 100 μM Al^{3+} on Fe^{2+} -initiated TBARS production (Figure 2B).

Figure 2 Effect of the presence of galactolipids on Al^{3+} -mediated stimulation of Fe^{2+} -initiated lipid peroxidation

(A) Liposomes composed of PC/PS (60:40, mol/mol) (●) or PC/PS/galactolipid (molar ratio 36:24:40) (○) were incubated in the presence of Al^{3+} (10–100 μM) and Fe^{2+} (25 μM) for 90 min at 37 °C. After incubation, lipid oxidation was evaluated by measuring the content of TBARS, as described in the Experimental section. Stimulation of TBARS production stimulation was calculated according to eqn. (1). Results are shown as means $\pm$ S.E.M. of four independent experiments. * indicates a significant difference from the value obtained in PC/PS liposomes ($P < 0.01$). (B) Correlation between the stimulatory effect of Al^{3+} (100 μM) on Fe^{2+} -initiated TBARS production and membrane galactolipid concentration.

Figure 3 Lateral phase separation induced by galactolipids

Liposomes composed of PC/PS (60:40, mol/mol) containing increasing various amounts of galactolipids (10–40 mol%) and 5 mol% $\text{C}_6\text{-NBD-PC}$ were incubated for 5 min at 25 °C, and the fluorescence intensity was measured. Fluorescence quenching (Q) was calculated according to Equation. (2). Results are expressed as means $\pm$ S.E.M. of four independent experiments.

Given the above results, the influence of galactolipids in the membranes on the capacity of Al^{3+} to induce lateral phase separation was investigated. Liposomes consisting of PC/PS (60:40), or PC/PS/galactolipid (36:24:40), and containing

Figure 4 Effect of galactolipids on Al^{3+} -mediated lipid lateral phase separation

Liposomes composed of PC/PS (60:40, mol/mol) containing galactolipids at the following concentrations: 0 mol% (●), 10 mol% (□), 20 mol% (■), 30 mol% (▲) or 40 mol% (○) and 5 mol% C_6 -NBD-PC were incubated in the presence of Al^{3+} (10–100 μM) for 2 min at 37 °C. Lateral phase separation was evaluated as described in the Experimental section. Results are shown as means $\pm$ S.E.M. of four independent experiments.

Figure 5 Correlation between Al^{3+} -mediated stimulation of Fe^{2+} -initiated lipid peroxidation and Al^{3+} -mediated lateral phase separation in PC/PS/galactolipid liposomes

The effects of Al^{3+} (10–100 μM) on lipid lateral phase separation and on Fe^{2+} -initiated TBARS production were evaluated in liposomes composed of PC/PS/galactolipid (molar ratio 36:24:40), as described in the Experimental section. Data are taken from Figures 2(A) and 4.

5 mol% of the fluorescent probe C_6 -NBD-PC, were incubated for 5 min at 25 °C, and the decay in the fluorescence intensity was measured. Figure 3 depicts the quenching of C_6 -NBD-PC in the liposomes as a function of galactolipid concentration (10–40 mol%) after 5 min incubation, when the fluorescence decay reached a plateau. In the absence of galactolipids, no fluorescence quenching was registered during the period of the assay. At 10–40 mol% galactolipids, Al^{3+} (10–100 μM) caused a dose-dependent (10–100 μM) increase in the quenching of C_6 -NBD-PC fluorescence (Figure 4). Al^{3+} -mediated fluorescence quenching was significantly higher ($P < 0.001$) in galactolipid-containing liposomes than in PC/PS (60:40) liposomes, even at the lowest Al^{3+} concentrations (10 μM). The capacity of Al^{3+} (10–100 μM) to stimulate Fe^{2+} -induced TBARS production in PC/PS/galactolipid (36:24:40) liposomes was significantly correlated ($r^2 = 0.99$; $P < 0.001$) with the ability of the cation to induce lateral phase separation in the same experimental system (Figure 5).

Figure 6 Dependence of membrane fluidity on galactolipid concentration in liposomes: effect of Al^{3+}

Liposomes composed of PC/PS containing various amounts of galactolipids (10–40 mol%) were incubated for 5 min at 37 °C in the absence (●) or in the presence (○) of 100 μM Al^{3+} . The membrane order parameter (S_p) was calculated from the fluorescence polarization of DPH. Results are shown as means $\pm$ S.E.M. of five independent experiments. * indicates a significant difference from the value obtained in the absence of Al^{3+} ($P < 0.01$).

To evaluate whether the presence of galactolipids affects membrane fluidity, the order parameter (S_p) of liposome membranes was determined by measuring the fluorescence polarization of DPH. PC/PS (60:40) liposomes containing increasing amounts of galactolipids (10–40 mol%) and 1 mol% DPH were incubated at 37 °C, and fluorescence polarization was measured when the kinetics had reached a plateau (5 min). The order parameter (S_p) was significantly higher ($P < 0.001$) in the galactolipid-containing membranes; this effect was dependent on the concentration of galactolipids (Figure 6). At 20 mol% galactolipids the S_p was a 36% higher than the value obtained in the absence of galactolipids. There was a significant correlation ($r^2 = 0.99$; $P < 0.001$) between the S_p and the lateral phase separation in liposomes containing galactolipids (0–40 mol%).

Finally, to characterize further the effect of Al^{3+} on membrane rigidity, Al^{3+} (10–100 μM) was added after the preincubation step, and the samples were incubated for an additional 5 min at 37 °C. As shown in Figure 6, the presence of Al^{3+} was associated with a significant ($P < 0.01$) rigidification of membranes at a galactolipid concentration of 10 mol%. When the galactolipid concentrations were 20 mol% or higher, an effect of Al^{3+} on membrane rigidification was not observed.

DISCUSSION

We reported previously that high levels of lipid oxidation products occur in the myelin fraction isolated from brains of mice at postnatal day 40 that had been chronically exposed to high levels of Al^{3+} , while the TBARS content in whole brain homogenates was not affected [7]. As would be expected, Al^{3+} was present in the myelin fraction obtained from both groups of mice; there was a tendency ($P < 0.07$) towards higher concentrations of Al^{3+} in the 1000 Al group [7].

In the present study, using the same model of Al^{3+} intoxication, we characterized myelin lipid composition. The concentrations of myelin phospholipid and galactolipid were significantly higher (37% and 104% respectively) in the 1000 Al mice than in the controls; cholesterol concentrations were similar in the two groups. Significantly, a strong correlation was found between the

myelin galactolipid concentration and the myelin TBARS concentration, suggesting that the higher content of galactolipids in the brain myelin from the 1000 Al mice brain myelin could be involved in an increased susceptibility of myelin to lipid oxidation.

Galactolipids are present in the myelin membrane in appreciable amounts [24]. Although myelin has a high cholesterol content, Johnston and Chapman [25] demonstrated that some of the lipids in myelin are present in the gel phase at physiological temperatures. Myelin galactolipids have long-chain fatty acids, mainly of 22–26 carbon atoms, while phospholipids have shorter acyl chains. The difference in fatty acid chain length produces interdigitations between lipid molecules [26]. Boggs et al. [27] proposed that, at low membrane galactolipid concentrations, bilayers occurs in a non-interdigitated gel phase. As the galactolipids concentrations increases, they can interdigitate with surrounding phospholipids, causing defects in the membrane surface [27]. While brain phospholipids are highly unsaturated, making them good substrates for lipid oxidation, galactolipids have saturated fatty acids that are not susceptible to oxidation. Thus, one might predict that there should be a decreased rate of lipid oxidation in galactolipid-rich membranes. That this is not the case for the Al^{3+} -intoxicated animals suggests that the galactolipid enrichment must be altering the physicochemical properties of the membrane to a significant extent.

In order to investigate whether the presence of galactolipids at high concentrations can produce an enhancement of the stimulation by Al^{3+} of Fe^{2+} -initiated lipid oxidation, we performed a series of *in vitro* studies. For this purpose, liposomes composed of PC/PS (60:40, mol/mol) and containing brain galactolipids in the range 10–40 mol % were used, and Fe^{2+} -induced TBARS productions was evaluated in the presence of variable amounts of Al^{3+} . Galactolipids did not affect the formation of TBARS caused by Fe^{2+} alone. However, at 50 and 100 μM Al^{3+} the stimulation of Fe^{2+} -induced TBARS production was significantly higher in PC/PS/galactolipid (36:24:40) liposomes than in PC/PS (60:40) liposomes. We observed a positive correlation between the concentration of membrane galactolipids and the stimulation of Fe^{2+} -initiated TBARS production mediated by 100 μM Al^{3+} . The differences in the effects of Al^{3+} and Fe^{2+} on the stimulation of TBARS production in the presence of galactolipids could be due to the different mechanisms through which these metals act. While Fe^{2+} is an oxidant due to its redox capacity, Al^{3+} is a pro-oxidant due to its ability to promote changes in the organization of the bilayer which makes the membrane more susceptible to an oxidative stressor such as Fe^{2+} [9]. We propose that galactolipids enhance Al^{3+} -mediated changes in membrane physical properties that facilitate the propagation of lipid oxidation.

In the PC/PS/galactolipid liposomes, a spontaneous lipid lateral phase separation was found, which showed a dependence on galactolipid concentration. This finding is in agreement with Ruocco et al. [28] who, working with membranes composed of dipalmitoyl PC and galactolipids at concentrations up to 23 mol %, reported that these membranes are in a gel phase at 37 °C. When the concentration of galactolipids in the membrane is higher than 23 mol %, gel and crystal phases can co-exist at the same temperature, producing the formation of two different domains: one, a dipalmitoyl PC-rich gel with approx. 23 mol % of galactolipids entrapped, and the second in a crystal phase composed of galactolipids [28]. In our membrane model a similar process could occur, although the lipids used for the experiments are from bovine brain, and thus the composition of acyl chains with respect to length and degree of unsaturation is variable. The spontaneous lateral phase separation caused

by the presence of galactolipids should not have major consequences on the rate of lipid peroxidation, since, in the galactolipids-enriched clusters that are formed, the saturated fatty acid chains are not substrates of oxidation. In fact, in the absence of Al^{3+} , the rate of Fe^{2+} -induced lipid oxidation was similar in the liposomes containing from 0 and 40 mol % galactolipid.

In the PC/PS/galactolipid (36:24:40) liposomes, Al^{3+} significantly increased lateral phase separation compared with its effect on PC/PS (60:40) membranes, indicating that Al^{3+} could enhance the natural formation of lipid domains in the membrane caused by the presence of high concentrations of galactolipids. The capacity of Al^{3+} to promote lateral phase separation in the PC/PS/galactolipid (36:24:40) liposomes was strongly correlated with the ability of the cation to stimulate Fe^{2+} -initiated TBARS production in these liposomes. This result indicates that lateral phase separation is one of the mechanisms involved in the observed stimulation of lipid oxidation. Al^{3+} , which has a high affinity for PS, could act by forming rigid clusters of PS in the phospholipid-enriched domains, generated by the presence of galactolipids in the membrane, and thus facilitate the propagation of lipid oxidation [9].

Brain galactolipids increase the transition temperature of PC-containing membranes [27]. In the PC/PS (60:40) liposomes, the presence of galactolipids (10–40 mol %) caused an increase in the membrane order parameter calculated from DPH fluorescence polarization. Similarly, van Blitterswijk et al. [29] demonstrated that the presence of sphingomyelin caused an increase in the fluorescence polarization of DPH in phospholipids mixtures, an effect that depended on the sphingomyelin molar fraction. The membranes used in the present work do not show a neat transition temperature as evaluated by DPH fluorescence polarization (results not shown), probably due to the heterogeneity in the acyl chain composition of brain phospholipids [30]. The order parameter was positively correlated with lateral phase separation, and both effects were dependent on the galactolipid concentration in the membrane. Addition of Al^{3+} produced a significant increase in the order parameter, but this effect was observed only at the lowest concentration of galactolipids. As previously mentioned, at concentrations lower than 23 mol % galactolipid, and at 37 °C, most of the membrane is in the gel state, where galactolipids are homogeneously distributed [28] and Al^{3+} would have a maximal packing action. Based on this, the values of S_p found in the presence of Al^{3+} at 10 and 20 mol % galactolipids can be compared without complications caused by the existence of multiple lipids domains. The lack of an additional effect of Al^{3+} at 20 mol % and higher galactolipids concentrations suggests that either the rigidity of the phospholipid-enriched domains has reached a maximum and/or that, since DPH fluorescence polarization measures the overall membrane rigidity, the changes in membrane fluidity caused by Al^{3+} in the gel domain could be masked by the higher greater rigidity of the crystal domain composed of galactolipids.

Taken together, these results support the concept that galactolipids can favour the capacity of Al^{3+} to stimulate Fe^{2+} -induced lipid oxidation, facilitating Al^{3+} -mediated changes in membrane physical properties. In essence, we suggest that Al^{3+} induces in the membrane the formation of clusters of negatively charged phospholipids, where the motion of fatty acids is restricted [9]. In these clusters, the closer proximity of the phospholipids acyl chains acts to accelerate the propagation rate of the lipid oxidation process [31,32]. Galactolipids could act progressively, segregating phospholipids in domains where this mechanism would be favoured. An intriguing question which still needs to be answered concerns the mechanism that underlies the

increase in myelin galactolipids in the Al^{3+} -intoxicated mice. We suggest that the shift in galactolipid content initially represents a positive response of the animal to a pro-oxidant condition, in that the number of polyunsaturated fatty acids is reduced; however, with time, this positive response could result in adverse consequences if high concentrations of Al^{3+} persists.

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