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## Effect of long-term aluminum feeding on lipid/phospholipid profiles of rat brain myelin

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### Abstract

Effect of long-term (90–100 days) exposure of rats to soluble salt of aluminum ( $\text{AlCl}_3$ ) on myelin lipid profile was examined. The long-term exposure to  $\text{AlCl}_3$  resulted in a 60 % decrease in the total phospholipid (TPL) content while the cholesterol (CHL) content increased by 55 %. Consequently the TPL / CHL molar ratio decreased significantly by 62 %. The phospholipid composition of the myelin membrane changed drastically; the proportion of practically all the phospholipid classes decreased by 32 to 60 % except for phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Of the latter two, proportion of PC was unchanged while PE increased in proportion by 47 %. Quantitatively, all phospholipid classes decreased by from 42 to 76% with no change in the PE content. However the membrane fluidity was not altered in Al-treated rats. Many of the changes we observe here show striking similarities with the reported phospholipid profiles of Alzheimer brains.

### Introduction

Loss of short-term memory marks the beginning of Alzheimer's disease (AD) and the condition ultimately leads to progressive dementia [1-7]. This involves memory loss, disorientation and impairment of judgement and reasoning [1-7]. Pathologically, abnormally high deposits of senile plaques comprising  $\beta$ -amyloid protein and, neurofibrillary tangles in specific brain regions have been reported [4,8,9]. In later stages of AD reduced levels of neurotransmitters and extensive neuronal and synaptic loss are the common biochemical features [2,3,6,10-13]. Specifically, there is a selective loss of acetylcholine releasing neurones in the basal forebrain, hippocampus and

cortex [12,13]. Impaired cholinergic function in AD has been correlated with loss of memory [2,6,10,12].

Amongst the various hypotheses concerning AD [2,7,14-16], the membrane hypothesis [7,16] and the one implicating aluminum (Al) as a possible environmental etiologic factor [7,15,17-22] are of considerable interest. Neurotoxicity from exposure to Al is known to result in impairment of learning memory and cognition function both from clinical observations and from animal experiments [5,14,15,17,23]. Crapper et al. reported that the concentrations of Al in the brains of AD patients were significantly high [23]. Long-term administration of soluble

salt of Al to rats worsens their learning ability together with diminished cholinergic function and the rats become lethargic [14,15,17,23]. Role of Al intoxication in neurodegenerative diseases has been recently emphasized [18,24-29].

Earlier studies from our laboratories have shown that prolonged treatment with  $\text{AlCl}_3$  given in the diet caused significant impairment of energy metabolism in the rat brain mitochondria [19]. In parallel studies, we also noted that this treatment resulted in decreased proportion and content of phospholipid classes in the rat brain microsomal and synaptic plasma membranes [30,31]. Importance of myelin membrane for insulation is well documented [32]. It was therefore of interest to find out if prolonged treatment with  $\text{AlCl}_3$  can affect the myelin lipid profile. The findings of these investigations are summarized in the present communication. The results of our present studies show that indeed the prolonged exposure to  $\text{AlCl}_3$  resulted in significant changes in content and composition of phospholipid classes and in cholesterol content of the rat brain myelin. It is possible that this altered lipid / phospholipid content and composition could affect the insulation properties of the myelin. The finding may thus have some bearing on loss of short-term memory in Alzheimer's disease.

## Materials and Methods

### Chemicals

Silica gel G was purchased from E. Merck, Germany and 1,6 diphenyl-1,3,5 hexatriene (DPH) was purchased from Sigma, U.S.A. All other chemicals were of analytical – reagent grade and were purchased locally.

### Animals and treatment with Al

Adult male albino rats (100–120 g, 6–7 week old) of Charles-Foster strain were given in their diet 100 mg of  $\text{AlCl}_3$ /kg body weight /day for 90 to 100 days [19,30,33]. The animals were weighed every week and accordingly the dose of  $\text{AlCl}_3$  was adjusted on weekly basis. The animals in control group were given equivalent amounts of NaCl. The regimen for Al treatment is described in detail in [30]. We have earlier shown that under these conditions, compared to controls, in the experimental group the Al body burden is about 2.2 times higher throughout the experimental period [30].

### Isolation of myelin

At the end of the treatment period, the animals were killed by decapitation and their brains were quickly dissected out and kept in beakers containing chilled (0 to 4°C) 0.25 M sucrose. Isolation of myelin from 20 % (w/v) homogenates was according to the procedure of Burgyone and Rose [34], as described [30,35], which is based on discontinuous sucrose density gradient centrifugation. Briefly,

after the removal of nuclei and cell debris at  $600 \times g$  for 10 min., the combined mitochondrial-synaptosomal-myelin fraction was sedimented by centrifugation at  $10,000 \times g$  for 10 min. The resulting pellet was then subjected to hypotonic lysis using 5 ml of 5 mM Tris-HCl buffer pH 8.1. After incubation at 0°C for 30 min., the lysate was mixed with 5.0 ml of 80 % (w/w) sucrose, transferred to a centrifuge tube of a Beckman SW 28.1 rotor and was carefully overlaid with 10.0 ml of 28.5% (w/w) sucrose, followed by 8.0 ml of 10% (w/w) sucrose. The tubes were then subjected to centrifugation at  $60,000 \times g$  for 1.5 hr. The myelin fraction banding at the top of the gradient was carefully removed, resuspended in 0.25 M sucrose and re-sedimented by centrifugation at  $100,000 \times g$  for 40 min. in a TFT80 rotor. The resulting pellets were suspended in 0.25 M sucrose to give a final protein concentrations of 2–3 mg / ml. All operations were carried out at 0–4°C.

The isolated myelin fraction showed only negligible  $\text{Na}^+$ - $\text{K}^+$ -ATPase activity [35].

### Lipid analysis

Extraction of total lipids [36], and estimations of cholesterol and phospholipid phosphorus were by the procedures described [37,38]. The phospholipid classes were separated by thin layer chromatography [39]. The detailed procedures have been described earlier [30].

### Membrane fluidity measurements

Measurements of membrane fluidity were carried out at 25°C in a Shimadzu RF 5000 spectrophotofluorimeter using 1,6 diphenyl-1,3,5 hexatriene (DPH) as the probe as described in details earlier [30].

Protein estimation was according to the method of Lowry *et al.*, [40].

Statistical evaluation of the data was by Student's 't'-test.

## Results

Data in Table 1 show that long-term exposure of rats to Al resulted in 60% decrease in the total phospholipid (TPL) content of the myelin membrane while the cholesterol (CHL) content increased by 55%. Consequently the molar ratio of TPL / CHL decreased significantly. However, the yield of myelin protein was almost comparable in the two groups and was around 7–8 mg protein / g brain (wet wt.). The results thus emphasize that long-term exposure to Al brought about significant qualitative and quantitative changes in lipid composition (Table 1) without affecting myelin protein content.

We then analyzed the phospholipid composition of the myelin membrane. These data are given in Table 2. Our

**Table 1: Effect of long-term Al feeding on the total phospholipid and cholesterol content of myelin membrane in the rat brain.**

Animals	TPL ( $\mu\text{g}/\text{mg}$ protein)	CHL ( $\mu\text{g}/\text{mg}$ protein)	TPL/CHL (mole : mole)
Control(6)	1357.4 $\pm$ 136.4	785.1 $\pm$ 36.5	0.86 $\pm$ 0.07
Al-fed(8)	824.7 $\pm$ 95.8*	1222.1 $\pm$ 104.9**	0.33 $\pm$ 0.04***

The rats weighing 100–120 g were given in their diet 100 mg  $\text{AlCl}_3$  / kg body weight / day for 90–100 days [19,30,33] as described in the text. Results are given as mean  $\pm$  SEM of the number of observations indicated in the parentheses. \*  $p < 0.01$ , \*\*  $p < 0.005$ , \*\*\*  $p < 0.001$ .

**Table 2: Effect of long-term Al feeding on phospholipid composition of myelin membrane in the rat brain.**

Phospholipid Class	Phospholipid composition (% of total)		Change (%)
	Control(24)	Al-fed(18)	
Lyso	5.85 $\pm$ 0.61	3.53 $\pm$ 0.28*	-39.7
SPM	9.29 $\pm$ 0.52	6.31 $\pm$ 0.38**	-32.1
PC	27.63 $\pm$ 1.51	26.12 $\pm$ 0.78	-5.5
PS	6.18 $\pm$ 0.78	3.28 $\pm$ 0.29*	-46.9
PI	8.96 $\pm$ 0.83	3.58 $\pm$ 0.46**	-57.7
PE	37.48 $\pm$ 1.00	55.20 $\pm$ 1.58**	+47.3
PA	5.33 $\pm$ 0.38	2.20 $\pm$ 0.33**	-58.7

Experimental details are as described in the text and in Table 1. Results are given as mean  $\pm$  SEM of the number of observations indicated in the parentheses. \*\*  $p < 0.002$ , \*  $p < 0.001$ .

**Table 3: Effect of long-term Al feeding on phospholipid content of individual phospholipids of myelin membrane from the rat brain.**

Phospholipid Class	Phospholipid content ( $\mu\text{g} / \text{mg}$ protein)		Change (%)
	Control(24)	Al-fed(18)	
Lyso	79.46 $\pm$ 8.18	28.74 $\pm$ 2.79*	-63.8
SPM	125.86 $\pm$ 9.83	52.86 $\pm$ 4.69*	-58.0
PC	376.08 $\pm$ 28.18	216.56 $\pm$ 15.77*	-42.4
PS	84.36 $\pm$ 9.65	26.69 $\pm$ 2.72*	-68.4
PI	121.74 $\pm$ 10.89	29.61 $\pm$ 3.63*	-75.7
PE	506.86 $\pm$ 33.36	456.06 $\pm$ 33.18	-10.0
PA	71.39 $\pm$ 6.13	18.04 $\pm$ 2.41*	-74.7

Experimental details are as described in the text and in Table 1. The contents of individual phospholipid classes was computed from the TPL content and composition for the respective sample. Results are given as mean  $\pm$  SEM of the number of observations indicated in the parentheses. \*  $p < 0.001$ .

values for the control group are in close agreement with those reported by others [32,41]. Thus phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are the major phospholipids (37 and 28 % respectively) of myelin while the other components ranged from 5 to 9%. Al treatment resulted in generalized decrease (32 to 60% decrease) in all the phospholipid classes except for PC and PE. Of the latter two the PC was unchanged while the PE component had increased by 47%. The quantitative data

on contents of individual phospholipid classes are given in a Table 3, which show that prolonged Al treatment resulted in a generalized decrease in the content of all the phospholipid classes from 42–76% except for PE which was unchanged. Measurements of membrane fluidity however revealed that in spite of significant compositional changes in the lipid/phospholipids content and composition (Tables 1,2,3), the membrane fluidity was not altered in the Al-treated rats. Thus the values for the

fluorescence polarization (P), fluorescence anisotropy (r), limited hindered anisotropy ( $r_{\infty}$ ) and order parameter (S) were:  $0.254 \pm 0.008$ ,  $0.186 \pm 0.006$ ,  $0.148 \pm 0.008$  and  $0.612 \pm 0.006$  respectively for the controls; these values did not differ significantly in Al-treated animals (Table 3).

## Discussion

From the foregoing results it is clear that Al-treatment resulted in significant reduction in the phospholipid content accompanied by major compositional changes, which is consistent with membrane hypothesis of AD [2,16]. According to this hypothesis, in order to make up for the choline deficiency, the neurons try to extract choline from choline containing phospholipids. This results in the disruption of cell membranes and ultimately in neuronal cell death [2]. From the data presented (Tables 2 and 3), it is clear that this decrease occurred in both sphingomyelin (SPM) and PC with the effect being more pronounced on the former component. In related studies we have observed that in the synaptic plasma membranes also the content of SPM decreased significantly in Al-treated rats, while the effect on PC component was of lesser magnitude [30,31]. Taken together, the results would suggest that PC is relatively more important for membrane function than SPM and that choline for neurotransmitter synthesis may be extracted in the first instance from SPM component. The results thus complement the membrane hypothesis of AD [2]. The other interesting feature of our observation is a decrease of greater magnitude in the contents of the acidic phospholipids viz. phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidic acid (PA), while content of the major basic phospholipid i.e. PE did not change (Tables 2 and 3). Decrease in the PI in the brains of the AD patients has already been reported [42-44]. The net result of the compositional changes we observe here (Tables 2 and 3) would be the altered charge distribution in the myelin membrane. The phospholipids are known to be asymmetrically distributed in the two membrane leaflets [45]. Thus the net decrease in the negative charges and relatively lesser decrease in the positive charges that we find here could have major influence on the insulation properties of the myelin membrane. One more interesting feature is the decrease in the lysophospholipids (Lyso) and PA which is indicative of decreased phospholipid turnover. We have noted earlier, similar pattern for rat brain synaptic plasma membranes and microsomes [30]. In this connection, it is of interest to note that in the brains of AD patients the lysophospholipase activity increased significantly [42,44], and lysophospholipid acyl transferase activity increased [42,44]. This will correlate well with our observation on decreased Lyso content (Tables 2 and 3).

The result of our present studies, taken together with our earlier observations on synaptic plasma membranes and microsomes [30] suggest that long-term exposure to Al specifically alters the brain lipid/phospholipid metabolism and/or their transfer to various membrane systems. Al may affect these processes by various mechanisms such as creating energy deficiency [19], forming a complex with ATP where the Al-ATP complex is energy compromised [18], or by affecting the functions of various enzymes [15]. Role of Al-ATP complex in  $Ca^{2+}$  mediated excitotoxicity and neurotoxicity will ultimately results in neuronal cell death is well recognized, as is the requirement of phospholipases for  $Ca^{2+}$  [18]. Al is known to replace metal ions in many enzyme systems [15] which in turn could influence the lipid/phospholipid metabolism. Additionally we have shown earlier that prolonged exposure to Al resulted in decreased rates of substrate oxidation in rat brain mitochondria. In particular, the cytochrome oxidase activity decreased significantly [19]. A similar decrease in cytochrome oxidase activity in the brains of AD patients has been reported [46].

Roth *et al.* [16] have reported that reconstituted membrane from the brain lipids of AD patients were thin due to decreased cholesterol content. This is in contrast with our observation that the cholesterol content was actually higher in Al-fed rats. (Table 1) However it may be pointed out that Roth *et al.*, [16] were reporting on the lipid profile of the whole brain regions, whereas we are dealing with purified membrane system. In related studies, we have found that the cholesterol content of the microsomes decreased after Al-treatment [30,31]. It is thus likely that overall average content of cholesterol of the whole brain might have decreased as reported by Roth *et al.* [16]; myelin may be a special case where increased cholesterol content may be a compensatory mechanism to ensure the insulation properties following significant alterations in phospholipid profiles (eg. see Tables 2 and 3). We have already shown earlier that changes in lipid/phospholipid profiles drastically impaired the synaptic plasma membrane  $Na^+$ ,  $K^+$ -ATPase activity [31] which will get compounded further due to compromised energy transduction and Al-ATP complex formation referred to above [18,19]. Additionally, the  $V_{max}$  of cerebral acetylcholinesterase decreased significantly under these experimental conditions [47]. Obviously, these factor will result in impaired signal transmission while the lipid/phospholipid changes in myelin would alter the insulation properties.

Despite the significant changes in lipid / phospholipid profiles (Tables 1,2,3), the membrane fluidity was not altered in the Al-treated group. The molar ratios of TPL/CHL, PC/PE and SPM/PE are the accepted indexes of membrane fluidity [48,49]. Thus increase in the latter two

**Table 4: Effect of long-term Al feeding on the fluidity of the myelin membrane.**

Parameter	Control	Al-fed
Fluorescence Polarization, P	0.254 ± 0.008	0.260 ± 0.003
Fluorescence anisotropy, r	0.186 ± 0.006	0.190 ± 0.002
Limited hindered anisotropy, r <sub>α</sub>	0.148 ± 0.008	0.153 ± 0.003
Order parameter, S	0.612 ± 0.006	0.622 ± 0.002

The experimental details are as given in the text and in Table 1. Fluidity parameters were determined at 25°C within 2–3 hr of myelin membrane preparation using DPH as the probe[30,47]. Results are given as mean ± SEM of 18 observations in each group.

indicates decreased fluidity, whereas the opposite is true for the TPL/CHL molar ratio [48]. From the data given in Tables 1 and 2, it is clear that the TPL/CHL molar ratio decreased which will decrease the fluidity. However the PC/PE and SPM/PE molar ratios decreased which will increase the fluidity. Thus it is possible that the two opposite effects might have counterbalanced each other and hence there is no apparent net change in membrane fluidity parameters. Interesting to note in this connection is the fact that DPH monitors only the bulk membrane fluidity [50]. Altered membrane fluidity in platelets from AD patients has been reported [43,51], which is in contrast to our present observations. However once again these authors [43,51] were measuring the fluidity of the whole cells which can not be extrapolated to purified myelin membrane system described here.

It is well recognized that cerebroside are major component of myelin [52]. It is possible that long-term Al exposure might have caused alterations even in the content of the cerebroside in the myelin. However in the present studies, we have not looked at this possibility; further investigations along these lines could provide useful information. Interesting to note in this context is the observation that Al under *in vitro* conditions increased lipid peroxidation only of the galactolipids [53].

In conclusion, results of our present studies have brought into focus several parallels in the myelin membrane lipid alterations in Al-treated rats and the AD brains [16,42,44]. Such changes in turn can affect the insulation properties leading to memory and cognition dysfunctions which is a common feature of AD [1-7]. The clues that we get from rat studies reported here suggest that it might be of interest to enquire and investigate whether similar changes occur in the myelin membranes in the AD patients.

### Abbreviations

AD, Alzheimer's disease; Al, aluminum; CHL, Cholesterol; DPH, 1,6, diphenyl-1,3,5 hexatriene; Lyso, Lyso-phospholipid; PA, Phosphatidic acid; PC, phosphatidylcholine; PE, Phosphatidylethanolamine; PI,

Phosphatidylinositol; PS, Phosphatidylserine; SPM, Sphingomyelin; TPL, Total phospholipid.

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