Betaine reverses toxic effects of aluminium: Implications in Alzheimer's disease (AD) and AD-like pathology

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Impairment in T-maze performance of adult rats caused by aluminium (Al^{3+}) and the reversal of it by betaine, a methyl donor, was studied. Besides, conformational change in the secondary structure of β-amyloid peptide ($A\beta$) brought about by the addition of Al^{3+} in vitro, was also studied using CD-spectroscopy. The organismal study proved that betaine is effective in restoring the memory loss caused by Al^{3+} possibly through augmentation of choline levels, as betaine is involved in the synthesis of choline. The CD-spectra recorded indicate loss of α-helical content of the peptide ($A\beta$) caused by the addition of Al^{3+}, which was reversed to some extent by the addition of betaine. Betaine may thus prevent/stop the progression of plaque formation seen during the initial stages of Alzheimer's disease (AD), and AD-like pathology as the loss of secondary structure of $A\beta$ is suspected to be an early event in the aetiopathology of AD/AD-like perturbations caused by Al toxicity. Betaine, a natural product occurring in beetroot (Beta vulgaris), and a by product in the process of manufacturing beet sugar may thus prove efficacious in the treatment of diseases involving dysfunctions of cholinergic system leading to memory loss.

HARRINGTON et al's[1] recent findings based on immunochemical analysis of frontal cortex from 15 dialysis cases, 5 Alzheimer's disease (AD) patients and 6 control cases are consistent with a role for aluminium (Al) in the development of AD-like pathology in patients subjected to prolonged Al exposure. Earlier studies of Wisniewski and Wen-Gy[2] on the pathology of AD and Al-induced encephalopathy and others indicate that even though Al does not cause AD neuropathology, under certain conditions cognition can be affected when Al enters the brain. They suggest therefore that for individuals with renal failure or undergoing dialysis or individuals with a damaged blood–brain barrier, the

intake of Al should be controlled, since Al is capable of overcoming the blood–brain barrier[3].

Although the debate about the role of Al in the etiology of AD continues, the neurotoxic effects of Al are well established in humans as well as animals. Jope and Johnson[4] have studied the neurochemical responses to chronic oral Al administration in rats. The selective cognitive impairment demonstrated in the adult rats was attributed to the significant neurochemical consequences among which are changes in cytoskeletal protein phosphorylation states, and concentration. While Al continues to be a strong suspect in the pathophysiological cascade of events leading to AD, undoubtedly there is ample justification for the ongoing search for antidotes to Al-toxicity.

Treatment of persons suffering from AD with desferrioxamine, a trivalent ion chelator, has shown results in slowing down the progression of this disease, presumably by removing Al and/or other chelated substances[5]. At the molecular level, we have shown recently[6], that the secondary structure of β-amyloid ($A\beta$) undergoes conformational change in the presence of Al, which can be reversed by borate. β-peptide is derived from in vivo proteolysis of a larger amyloid precursor protein (APP). What prompted us to undertake this study is the fact that the secondary structure of $A\beta$ is increasingly being suspected to be an early site of aetiopathology of AD/AD-like perturbations caused by the Al toxicity.

There are studies to support a current theory that impaired methylation may occur by different mechanisms in several neurological and psychiatric disorders[7], including dementia. Preliminary studies indicate that methyl donors, such as S-adenosylmethionine, may improve cognitive function in patients with dementia, possibly through re-myelination. We report here, our results on the impairment in T-maze performance of adult rats induced by Al and the antidotal effect of betaine, a methyl donor. We have also examined if betaine could reverse the conformational change induced

by Al in β-amyloid peptide (1–40). Implications of the results arising out of these two approaches, (i) at the organismal level, and (ii) at the molecular level have been discussed in the context of AD and the AD-like pathology brought about by Al-toxicity.

Materials and methods

Experiment 1: The effect of aluminium chloride and the antidotal effect of betaine (Sigma) on the learning ability of rats were tested, using T-maze in this experiment. Laboratory-bred Wistar-albino rats (both male and female), housed in individual cages with food and water ad-lib were used. Adult rats of the age of 60 to 80 days of more or less the same weight were used. As far as possible litter mates were used in each group. Food pellets were kept at the end of one of the short arms of the T-maze as a reward. Individual rats were allowed to explore from the end of the long arm so as to reach the reward. The move towards the side of the reward was considered a positive response. Generally, ten trials were given in a 30 min duration, and the cumulative percentage of positive score was noted for each rat. Experiments were carried out between 18.00 and 20.00 h of the day. 24 h prior to testing, food was withdrawn from the cage. The following groups were tested.

Group A: Control for Al-injected group: Rats were injected with 0.1 ml of 0.9% saline into the cerebrospinal fluid (CSF). Their learning score was obtained, on the fourteenth day following the injection of saline, the vehicle for Al chloride used in group B.

Group B: Aluminium injected: Rats were injected with aluminium chloride (4 mg/kg body weight) dissolved in 0.1 ml of 0.9% saline, into the CSF at brain stem level, using a microsyringe (used for injecting insulin) under ether anesthesia. After fourteen days, they were put through the T-maze to obtain their learning score.

Group C: Rats of this group were injected with aluminium chloride and their learning score was obtained on the thirteenth day following the injection, but without administering betaine.

Group D: Aluminium-injected and betaine treated: Rats of this group were given betaine (Sigma Chemicals) (15 mg/kg body weight/day) dissolved in 25 ml of drinking water for thirty days, beginning one day after injecting aluminium chloride into the CSF. Their learning score was obtained after thirty days of daily oral ingestion of betaine. The average daily consumption of water was assessed to be 25 ml per rat through actual measurement over a period of seven days prior to the experiments.

In all the experiments, whether it was aluminium chloride solution or saline only a single dose was injected into the CSF.

Experiment 2: Interaction of β-amyloid peptide (Aβ 1–40, Sigma Chemicals) with Al^{3+} and the effect of betaine on this interaction: The paradigm was the same as reported by us earlier[6]. Circular dichroism (CD) studies were carried out using Aβ (33 μM); Aβ + AlCl$_3$ (300 μM); and Aβ + AlCl$_3$ + betaine (30 μM).

The β-amyloid peptide fragment 1–40 has the sequence, Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-(C$_{194}$H$_{295}$N$_{53}$O$_{58}$S) with a molecular weight of 4329.8 and peptide content of 84%, and is soluble in water (1 mg/1 ml). The Aβ was dissolved in Tris-HCl buffer (10 mM) at pH 7, to obtain the stock concentration of 194 mM. The peptide solution was dissolved in TFE buffer mixture for CD.

CD spectra were recorded using a JASCO 500 spectro-polarimeter equipped with a 501 N data processor. The instrument was calibrated with 10-d-camphor sulphonic acid. Sensitivity used was 1 mm/degree/cm. The average of four repeat scans was used to arrive at molar ellipticity[6].

Results

Experiment 1: The results in terms of percentage of positive response, i.e. learning ability of the rats to reach the reward, are represented through histograms in Figures 1 and 2. Statistical validation of the significance of difference among the groups was carried out using ANOVA test. The T-maze experiment clearly established that the AlCl$_3$ injected into the CSF does impair the
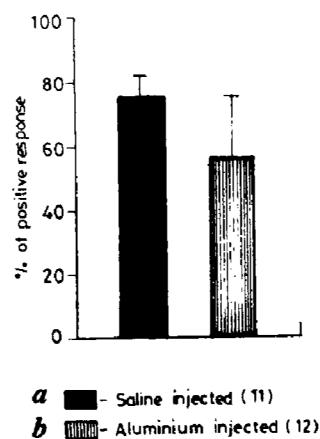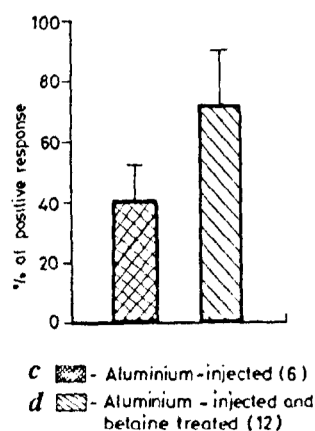

Figure 1. Learning ability (percent positive response, as inferred from the T-maze test;) *a*. control group i.e. saline-injected group; and *b*. aluminium chloride-injected group. The test was performed 14 days after the injection (F = 4.23; df = 2, 33; P < 0.05).

performance of rats in T-maze in learning to reach the site of the reward. Oral administration of betaine for a period of thirty days following the injection of aluminium chloride reverses this impairment to a significant degree and more importantly, there is no such reversal of impairment in the group where betaine was not administered. Furthermore, there seems to be a more pronounced effect of aluminium chloride after thirty days of injection when compared to fourteen days, in that the score was lower after thirty days (Figure 2).

Experiment 2: As the Figure 3 *a* indicates, the CD spectrum of the Aβ peptide is characteristic of predominantly α-helical conformation with prominent trough at 204 nm with a shoulder at 220 nm. Addition of Al^{3+} to the Aβ peptide (1–40) used in the study resulted in the loss of α-helical content of the peptide as Figure 3 *b* indicates. Addition of betaine (30 µM) to Aβ + Al^{3+} restored the trough at 204 nm and the shoulder at 220 nm (Figure 3 *c*), as far as the qualitative profile of the CD curve was concerned, although the magnitude of both remained below that of pure peptide.
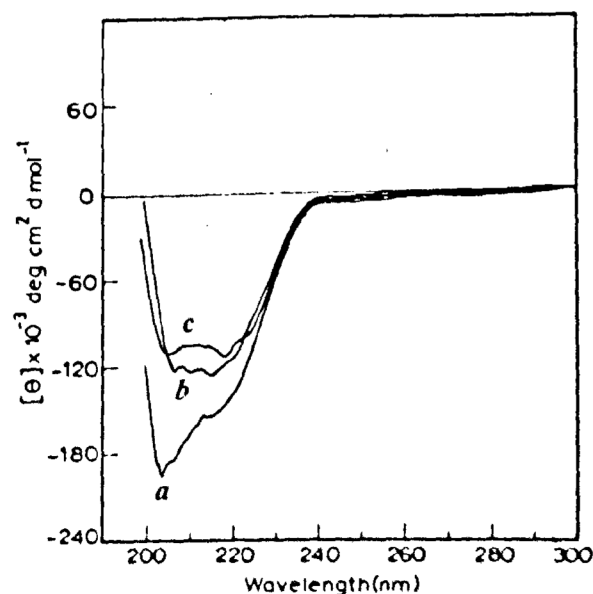
Discussion

Al has been recognized as a neurotoxic agent acting as a risk factor in AD and other neuronal dysfunctions[8,9]. The toxicity may arise in promoting aggregation of the phosphorylated neurofilament subunits[10,11], the tau protein, the principal component of the paired helical filaments of neurofibrillary tangles[1,12] and the amyloid-β peptide composing senile plaques. Elderly patients have shown higher brain concentration of Al irrespective of whether they have AD or not. The highest concentration was found in hippocampus and lowest in corpus callosum.
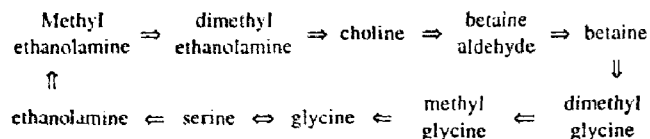
Recently, we proposed a plausible mechanism that could explain the induction of multimeric tau species by Al between 24 and 37°C (ref. 13). While these changes are considered involutive morphological phenomena, there are functional phenomena in which alterations are caused. The latter includes alterations in energy metabolism, in protein synthesis, and in the neurotransmitter metabolism. Currently it is assumed that the neurons most affected seem to be primarily those of the ascending cholinergic activating systems, whose somas are situated in Meinert's basal nucleus (Mbn). It is important to note that certain neurons are affected in most of the degenerative diseases (the high-risk neurons). These neurons could be affected by toxic and environmental factors at some stage in the cascade of aetiological events[14]. It follows hence that remedial measure(s) can be attempted at different levels, which may include morphological/functional restoration of the loss.

The significantly poor score of T-maze performance, exhibited by the Al-injected group indicates impairment of memory function. It is significant to note that the amygdaloid complex may play an important role in memory function as well as in AD[15,16]. It is also known that AChE-containing neurons are among the most vulnerable cells in AD[17]. This has prompted the recent, increasing attempts at the cholinergic therapy by way of using cholinergic inhibitors in the treatment of AD. The rationale behind this approach is the restoration of cholinergic balance, through elevation of ACh levels and augmentation of the function of the remaining ACh receptors. Though there is some temporary improvement after anti-ChE treatment, the side effects due to peripheral

c ▨- Aluminium-injected (6)
d ▧- Aluminium - injected and betaine treated (12)

Figure 2. Learning ability (percent positive response); *c*. aluminium chloride-injected group, and *d*. the aluminium chloride-injected + betaine-treated group. The test was performed 30 days after the injection. (F = 64.13; df = 2, 16; *P* < 0.05). Numbers in parentheses are the number of rats in each group. Values are mean ± SD.

Figure 3. CD Spectra of; *a*. Aβ (1–40) (33 µm); *b*. Aβ (1–40) + Al^{3+} (300 µm); *c*. Aβ (1–40) + Al^{3+} + betaine (30 µm).

ACh stimulation can sometimes be severe[17]. In the light of these reports it is extremely significant that betaine, in the present experiment, has been effective in restoring the memory function in the Al^{3+}-injected rats. Betaine, which occurs in beet root (*Beta vulgaris*) may lead to the synthesis of choline, as is shown below[18]:

Methyl ethanolamine $\Rightarrow$ dimethyl ethanolamine $\Rightarrow$ choline $\Rightarrow$ betaine aldehyde $\Rightarrow$ betaine

$\Uparrow$ $\Downarrow$

ethanolamine $\Leftarrow$ serine $\Leftrightarrow$ glycine $\Leftarrow$ methyl glycine $\Leftarrow$ dimethyl glycine

It is possible that as in AD, the Al^{3+} may affect the function of cholinergic neurons, thereby leading to perturbations in learning and memory in rats which might be corrected by betaine administration. The oral ingestion of betaine might eventually lead to the augmentation of the choline levels. The fact that a transmitter is most markedly depleted in those AD brain regions where the plaque density is highest may suggest that certain constituents of the plaque, such as β-amyloid, may cause degeneration of the local fibres[15]. In the light of this the reversal of the loss of α-helix conformation in the β-peptide, caused by Al^{3+}, by betaine assumes significance. If highly-pleated beta structure represents dysfunctional/toxic element in an amyloid fibril, the reversal to whatever extent of such conformation in the $A\beta$ is desirable. It was suggested that the degeneration of cortical cholinergic afferents from the neurons of the Mbn is an important feature in the pathogenesis of neuritic plaques. There is a strong correlation between the number of neuritic plaques in neocortical areas and the loss of neurons in the Mbn, giving rise to cholinergic innervation of the affected cortical areas[19].

Betaine may prevent/stop the progression of plaque formation on one hand, and may augment levels of choline on the other, in case of AD and AD-like pathology caused by Al^{3+} which involves dysfunctions of cholinergic systems and the consequent memory loss. Interestingly, betaine was suggested as therapeutic alternative in the treatment of homocystinuria. After betaine therapy no disturbances were observed in the hepatic, renal, and bone marrow functions nor were there any clinically relevant side effects[20].

Conclusion

Conformational change in the secondary structure, in terms of loss of α-content in $A\beta$, is suspected to be an early step in the cascade of events leading to the formation of amyloid plaques, which might eventually manifest in cholinergic dysfunctions and memory loss in AD/AD-like pathology caused by Al toxicity. Betaine restores to some extent the lost α-content in $A\beta$ *in vitro*. Memory loss inferred through T-maze tests in the Al-injected rats was also reversed by betaine. There was no such improvement in the T-maze scores of rats injected with Al but with no oral betaine administration. It is therefore suggested that betaine may prevent formation/stop progression of amyloid plaques by $A\beta$ on the one hand, and augment choline levels on the other, thereby restoring memory functions.

1. Harrington, C. R., Wischik, C. M., Mc Arthur, F. K. et al., *Lancet*, 1994, **343**, 993–997.
2. Wisniewski, H. M. and Wen-Gy., *Ciba Found. Symp.*, 1992, **169**, 142–154.
3. Senitz, D. and Bluthner, K., *Zentralbl. Allg. Pathol. Pathol. Anat.*, 1990, **136**, 329–336.
4. Jope, R. S. and Johnson, G. V., *Ciba Found. Symp.*, 1992, **169**, 254–262.
5. Newman, P. E., *Med. Hypothesis*, 1992, **39**, 123–126.
6. Ramakrishna, T., Vatsala, S., Madhav, T. R., Sreekumar, E., Ramesh, J. and Easwaran, K. R. K., *Alzheimer's Res.*, 1997, **3**, 223–226.
7. Bottiglieri, T., Hyland, K. and Reynolds, E. H., *Drugs*, 1994, **48**, 137–152.
8. Doll. R., *Age Aging*, 1993, **232**, 138–153.
9. Meiri, H., Banin, E., Roll, M. et al., *Prog. Neurobiol.*, 1993, **40**, 89–121.
10. Leterrier, J. F., Langui, D. and Probst, A., *J. Neurochem.*, 1992, **58**, 2060–2070.
11. Hollosi, M., Shen, Z. M., Perczel, A. and Fasman, G. D., *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 4902–4906.
12. Scott, C. W., Fieles, A., Sygowski, L. A. and Caputo, C. B., *Brain Res.*, 1993, **628**, 77–84.
13. Madhav, T. R., Vatsala, S., Ramakrishna, T., Ramesh, J. and Easwaran, K. R. K., *Neuroreport.*, 1996, **7**, 1072–1076.
14. Toledano-Gasca, A., *Pharmacopsychiatry*, 1988, **21**, 17–25.
15. Benzing, W. C., Mufson, E. J. and Armstrong, D. M., *Brain Res.*, 1993, **625**, 125–138.
16. Emre, M., Geula, C. and Mesulam, M. M., *J. Comp. Neurol.*, 1993, **336**, 117–134.
17. Kasa, P., Rakonczay, Z. and Gulya, K., *Progress Neurobiol.*, 1997, **52**, 511–535.
18. Oser, B. L., (ed.) *Hawk's Physiological Chemistry*, Tata McGraw Hill Publishing Co., New Delhi, 14th edn, 1971, pp. 687
19. Arendt, T., Bigl, V., Tennstedt, A. and Arendt, A., *Neurosci. Lett.*, 1985, **14**, 1–14.
20. Montero-Brens, C., Dalmau-Serra, J., Cabello-Tomas, M. L. et al., *An. Esp. Pediatr.*, 1993, **39**, 37–41.