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ASPIRIN REDUCES ALUMINIUM-MEDIATED INCREASE IN LIPID PEROXIDATION IN LIVER AND KIDNEYS OF SWISS MICE

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ABSTRACT

Swiss mice fed an $AlCl_3$-mixed (20 mg/100 g body weight) diet for 10 days showed increased lipid peroxidation in liver and kidneys, but not in brain regions and the heart; the possible mechanism of action being through increased generation of reactive oxygen species (ROS). Co-administration of aspirin (15 mg/100 g body weight) through diet reduced lipid peroxidation induced by the aluminium salt. It is believed that aspirin reactive intermediates act as scavengers of ROS responsible for the lipid peroxidation.

RESUMEN

Ratones suizos alimentados durante 10 días con una dieta conteniendo $AlCl_3$ (20 mg/100 g de peso corporal) mostraron un incremento en la peroxidación de lípidos en el hígado y el riñón pero no en las regiones del cerebro y en el corazón; es posible que el mecanismo de acción responsable sea el aumento en la generación de especies de oxígeno reactivas (ROS). La coadminstración de aspirina a través de la dieta redujo la peroxidación de lípidos inducida por la sal de aluminio. Se considera que los reactivos intermediarios de la aspirina actúan como secuestradores de las ROS causantes de la peroxidación de los lípidos.

INTRODUCTION

Environmental pollution due to metal toxicity has received considerable attention during recent years. The use of aluminium in water purification, industry, food processing, medicine and in cookwares is a constant threat to human health, as the bioaccumulation of the metal may lead to metabolic interferences and many other deleterious effects (Eichhorn 1993). Besides being known as a neurotoxic agent, aluminium has been implicated in various pathological states: dementia of Alzheimer's type (Crapper *et al.* 1973, Forbes *et al.* 1995), parkinsonism dementia, amyotrophic lateral sclerosis (Perl *et al.* 1982), various bone diseases (Ott *et al.* 1982), microcryptic anaemia (Taylor 1991) and dialysis encephalopathy (Alfrey *et al.* 1976).

In view of Al's involvement in various disorders, it is necessary to study the mechanisms by which it produces toxicity and on the other hand, the actions of protective agents in counter-acting its effects. Reactive oxygen-derived free radicals (ROS) are known to cause damage to biomolecules. The polyunsaturated fatty acids of the membrane lipids are most vulnerable to their attack through peroxidative reactions. Lipid peroxidation is implicated in various pathological and toxicological conditions (Halliwell 1994; Halliwell and Gutteridge 1985). Lipid peroxidation causes damage to cell membranes and the breakdown products (lipid peroxides) are highly toxic to cellular environment. Attempts were made in the present study to verify if aluminium induces lipid per-oxidation in Swiss mice and whether a nonsteroidal antiinflam-matory drug like aspirin has a protective effect against the dam-aging action.

MATERIALS AND METHODS

Male Swiss mice (*Mus musculus*) 2 ½ to 3 months old housed in cages at room temperature (27° ± 2°C), with 12 h light, 12 h dark cycle, were fed daily on a freshly prepared diet (200 g of flour mixed with one whole egg and 1 g of common salt). They also received tap water *ad libitum* as described elsewhere (Behera and Patnaik 1979).

Mice were divided into three groups with 5-6 individuals each. Group-I (control) received the normal diet daily. While group-II was fed $AlCl_3$ (E. Merck, India Ltd., Bombay)-mixed diet (20 mg/100g body wt) daily and group-III received both

aspirin (Western Chemicals, Indore, India)-(15 mg/100g body wt) and $AlCl_3$-(20 mg/100g body wt) mixed diet.

After a 10 day treatment mice were killed by stunning. The brain, liver, kidneys and heart were dissected and suspended in ice-cold distilled water to clear the adherent materials. The different brain regions (medulla oblongata, cerebellum and cerebral hemispheres) were separated and tissues were soaked in the folds of Whatman No. 1 filter papers and weighed. A 2% homogenate of each tissue was prepared in distilled water employing a Potter-Elvehjem type of homogenizer with a teflon pestle (Remi RQ 127A, Bombay) at a medium speed for 2 min.

The lipid peroxidation potential (LPP) of the different tissues was measured by estimating the concentration of thiobarbituric acid (TBA) reactive substance (RS) as described by Sestini *et al.* (1991) with some minor modifications as described elsewhere (Jena *et al.* 1995). The TBA-RS were extracted with chloroform: glacial acetic acid (3:1 v/v) and the absorbance was measured at 535 nm in a JASCO-7800 UV/Visible Spectrophotometer (Japan). The TBA-RS were expressed as malonaldehyde (MDA) equivalent (n moles/g tissue wet wt), using the molar extinction coefficient of $1.56x10^5M^{-1}xcm^{-1}$ for MDA (Sinnhuber *et al.* 1958). Student's t-test was done to ascertain the significance of the results.

RESULTS AND DISCUSSION

LPP did not differ significantly among the brain regions in control mice, but it was higher than those of kidneys and heart. In biological systems, lipid peroxidation is influenced by various factors (Leibovitz and Siegel 1980). Moreover, tissues differ in their biochemical composition and degree of response and resistance to oxidative stress. Early observations in rat (Oshino *et al.* 1975), lizard (Jena *et al.* 1995) and fish (Radi *et al.* 1985) indicate tissue differences in LPP. The greater LPP of brain may be due to the fact that it generates more free radicals than any other organ per gram of tissue, it has a high lipid content enriched with polyunsaturated fatty acids and its antioxidant defense mechanisms are poorly developed (Reiter 1995).

In Al-fed mice, brain (medulla oblongata, cerebellum and cerebral hemispheres) and heart LPP did not differ from those of control values. The same parameter showed a significant increase in liver and kidneys ($p< 0.02$, + 82.3% and $p< 0.001$, + 55.2% respectively) of Al-fed mice (**Table I**). The Al toxic effects on biochemical parameters of animal tissues are not consistent. Such inconsistency is due to the Al administration procedures (oral, through diet, drinking water, intracranial injection etc.), the dose of the salts used and the duration of the treatment. It has been suggested that, like other metal ions, Al may cause an increased pool of substances capable of promoting the formation of free radicals (Forbes *et al.* 1995, Evans *et al.* 1992, Fraga *et al.* 1990). Chainy *et al.* (1991) reported that Al is capable of inducing lipid peroxidation *in vitro* in crude cerebellum homogenates of chick when this tissue was incubated with 100 and 500 μM of $Al_2(SO_4)_3$. But chicks given $Al_2(SO_4)_3$ (200 mg and 400 mg/kg body wt) for 15 days orally did not show any change in endogenous lipid peroxides of cerebellum. Our results in brain regions of mice also indicated that $AlCl_3$ ingestion did not alter LPP. In spite of the possibilities, the lack of effect of Al on brain LPP cannot solely be due to ineffective Al absorption as liver and kidneys showed a significant effect. It is possible that the transport of the metal ion to the brain of young mice and its retention are inadequate to induce a significant effect as reported for young rabbits (Yokel 1989). Moreover, the $AlCl_3$ dose administered and the duration of the treatment could be accounted for the lack of neurotoxic effect.

Co-administration of aspirin reduced the LPP of liver ($p<0.05$, -38.2%) and kidneys ($p<0.001$, –21.9%). The effects on brain regions and heart were not statistically significant (**Table I**). Nonsteroidal antiinflammatory drugs (carboxylic acids which include the acetylated form-called aspirin, pyrazolones and oxicams) inhibit the activity of cyclooxyge-nase, an enzyme that produces lipid peroxides and prostaglandins (PGG_2 and PGH_2) by oxygenation and cyclization of arachidonate in cell membrane. Some of these drugs (e.g. phenylbutazone) are also known to act as ROS scavengers (Rang and Dale 1991). Piroxicam and indomethacin inhibit the generation of superoxide anion by disrupted cells preparations and sodium salicylate has a modest effect (Weissman 1991). In mouse brain, ethanol induced lipid peroxidation is attenuated by tenoxicam but piroxicam does not have such effect. The mechanism of action of the nonsteroidal

TABLE I. EFFECT OF ALUMINIUM AND CO-ADMINISTRATION OF ASPIRIN ON LIPID PEROXIDATION IN BRAIN REGIONS, LIVER, KIDNEYS AND HEART OF SWISS MICE

Tissue	TBA-RS as MDA equivalent (n moles / g tissue wet wt)						
	Control	p	% change	Aluminium-fed	p	% change	Aspirin + Aluminium-fed
MO	78.5 ± 3.5 (5)	NS	+ 9.1	85.7 ± 9.5 (5)	NS	+ 5.1	90.1 ± 4.2 (6)
CBM	90.4 ± 4.7 (5)	NS	– 2.8	87.8 ± 7.2 (5)	NS	– 5.2	83.2 ± 5.1 (6)
CH	75.5 ± 2.9 (5)	NS	– 0.6	75.0 ± 7.4 (5)	NS	+ 13.8	85.4 ± 5.2 (6)
L	70.6 ± 7.9 (5)	<0.02	+ 82.3	128.7 ± 18.3 (5)	<0.05	– 38.2	79.5 ± 7.3 (6)
K	57.7 ± 4.8 (5)	<0.001	+ 55.2	89.6 ± 3.8 (5)	<0.001	– 21.9	69.9 ± 2.5 (6)
H	58.8 ± 6.5 (5)	NS	+ 22.9	72.4 ± 7.3 (5)	NS	– 6.5	67.6 ± 1.8 (6)

MO = medulla oblongata; CBM = cerebellum; CH = cerebral hemispheres; L = liver; K = kidneys; H = heart. The values are mean ± SEM. The numbers in parentheses indicate the number of animals used. NS = not significant at p level of 0.05.

antiinflammatory drugs listed under the oxicam group appears to have intrinsic differences (Pérez-Tapia *et al.* 1996), since hepatic lipid peroxidation induced by ethanol is known to be inhibited by piroxicam (Zentella de Piña *et al.* 1992). Salicylates are also believed to chelate metal ions (Cu^{2+}, Al^{3+}, Fe^{3+}) by forming complexes (Flower 1979). Copper-salicylate complex has been shown to react rapidly with superoxide anion thus suggesting a ROS scavenging mechanism for aspirin (De Alvare *et al.* 1976). Aspirin also stabilizes membranes (Miller and Smith 1966). Both renal and hepatic mitochondria are known to convert salicylate to reactive intermediates, 2,3 dihydroxybenzoic and 2,5 dihydroxybenzoic acids (Kyle and Kocsis 1986). These acids especially, 2,5 dihydroxybenzoic acid upon autooxidation, are converted to trihydroxybenzoic acids which are potent ROS scavengers (Capelle *et al.* 1992). Our results are similar to those of Zentella de Piña *et al.* (1993). These authors used a number of nonsteroidal antiinflammatory drugs including aspirin and inhibited ethanol induced lipid peroxidation in the rat liver. We believe that the aspirin induced LPP reduction in liver and kidneys of mice following induction of the parameter through Al treatments is regulated by more than one mechanisms.

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