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Toxicokinetic Study of Rat Intestinal Brush Border Membrane Enzymes Following *In Vitro* Exposure to Lead and Vanadium

K. Gupta, R. K. Upreti, A. M. Kidwai

Biomembrane Laboratory, Industrial Toxicology Research Centre, Post Box No. 80, Lucknow-226 001, India

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The intestinal brush border membrane is highly specialized plasma membrane responsible for digestive and absorptive functions and their closed vesicles retain the original orientation of the membrane (Klip et al.,1979). Exposure of gastrointestinal tract to lead and vanadium through food and water is inadvertantly possible. Vanadium is an essential element present in the living organism in trace amount but is toxic when introduced in excessive dose to animals and humans (Jandhyala and Hom, 1983). In atmosphere, lead comes from a wide variety of natural and anthropogenic sources. In children the toxic effect of this metal is due to ingestion of lead containing materials like paper, pencil etc (Pirkle et al.,1985). These heavy metals also have high affinity to remain bound to mammalian tissues and have rich capacity to combine with specific biochemical ligands such as sulphydryl, amino, carboxyl, and phenoxy groups as well as imidazole residues (Nordberg 1976). These properties can influence the structure function relationship of enzymes. The effect of lead and vanadium have mainly been focussed on brain, kidney and heart membrane ATPase. However, the effect of these metals on intestinal brush border membrane enzymes have not been investigated extensively. The present study was undertaken to investigate the in vitro effect of these heavy metals on the activities of rat intestinal brush border membrane enzymes.

MATERIALS AND METHODS

Male albino rats weighing 100-120g were procured from animal breeding facility of Industrial Toxicology Research Centre, Lucknow and maintained on standard pellet diet and water ad libitum. Prior to sacrifice, animals were fasted overnight with free access to

Correspondence to: A. M. Kidwai

drinking water. Intestinal brush border membrane (BBM) was prepared as described by Forstner et al., (1968). Alkaline phosphatase, $Ca^{+2}+Mg^{+2}$-ATPase, glutamyltranspeptidase, disaccharidases and acetylcholinesterase were assayed according to Weiser (1973), Hidalgo et al(1983), Boesterli and Zbinden (1979), Dahlqvist (1964) and Hestrin (1949), respectively. Protein was estimated according to the method of Lowry et al (1951). To carryout toxicokinetic studies, appropriate amounts of brush border membrane proteins (10-30 ug protein) were pre-incubated with varying concentrations of lead acetate and ammonium vanadate (NH_4VO_3) at 37°C for 5 min and enzymes were assayed. Determination of inhibitor constant (Ki) was carried out by the method of Dixon (1953). All chemicals used were of analytical grade.

RESULTS AND DISCUSSION

Figure 1 shows the inhibitory effect of lead on BBM enzymes. All enzymes of intestinal brush border membrane viz. $Ca^{+2}+Mg^{+2}$-ATPase, sucrase, γ-glutamyltranspeptidase and acetylcholinesterase were found to be inhibited significantly with the exception of alkaline phosphatase. Inhibition of enzymes was more or less concentration dependent. Alkaline phosphatase showed only 20% inhibition at the highest concentration tested. Sucrase and γ-glutamyltranspeptidase were more sensitive to lead induced inhibition as compared to $Ca^{+2}+Mg^{+2}$-ATPase and acetylcholinesterase. On the other hand, vanadium treatment revealed significant inhibition of $Ca^{+2}+Mg^{+2}$-ATPase and alkaline phosphatase activities (Fig.2). Inhibition of sucrase and acetylcholinesterase activities were not observed over the vanadium concentrations ranging from 1mM-40mM.

Inhibition constant (Ki) for brush border membrane enzymes was determined following the method of Dixon (1953). Specific activities of various enzymes were determined as a function of lead and vanadium concentration using two fixed substrate concentrations. Values were plotted according to Dixon (i/v Vs i) and Ki values determined. The pattern of the plot for alkaline phosphatase and $Ca^{+2}+Mg^{+2}$-ATPase indicated that the inhibition caused by vanadium was non-competitive type (Fig.3). However, in case of lead the inhibition of $Ca^{+2}+Mg^{+2}$-ATPase was competitive type (Fig.4). On the other hand, sucrase, γ-glutamyltranspeptidase and acetylcholinesterase (Fig.4) were inhibited non-competitively by lead. Table-1 summarises the concentration of lead and vanadium required for 50% inhibition of maximum activities (I_{50}) of brush

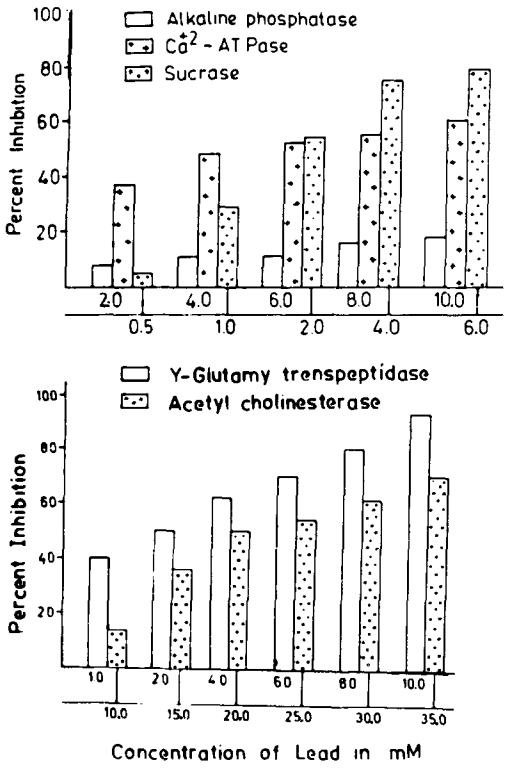

Figure 1. In vitro inhibition of intestinal brush border membrane enzymes by lead.

border membrane enzymes as well as the values of inhibitor constants as determined from Dixon plots.

The present investigation indicated that most of the brush border membrane enzymes were inhibited by lead in a dose dependent manner with the exception of alkaline phosphatase, which was less effected even by the higher concentrations of lead. Lead is known as potent inhibitor of Na^+-K^+-ATPase. It also effects the cation pump by interaction with calcium ion. Studies have also indicated that lead may substitute for calcium in the activation of phosphodiesterase by calmodulin (Goldstein and Ar 1983). It was also observed in the present study that lead inhibited intestinal brush border Ca^{+2}+Mg^{+2}-ATPase in a competitive manner. Similar inhibitory pattern for brain mitochondrial ATPase have been observed by Holtzman et al,(1978). The I_{50} values of lead for Na^+-K^+-ATPase in eel electrophax organ occured at 4 uM (Siegel and Fogt 1977), and for brain ATPase 55 uM. Conversely, in case of intestinal Ca^{+2}-Mg^{+2}-ATPase a high I_{50}

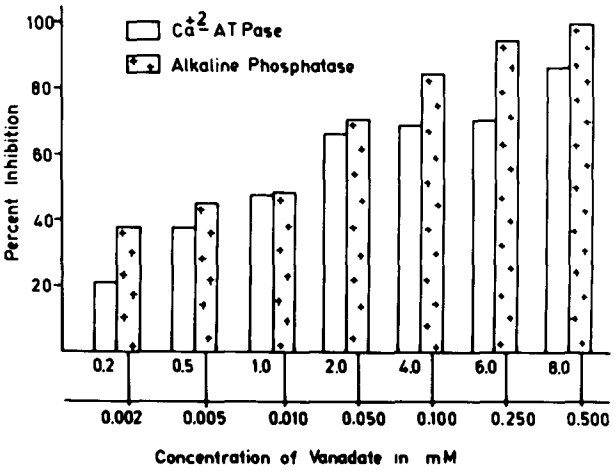

Figure 2. In vitro inhibition of intestinal brush border membrane enzymes by vanadate.

value (4.0 mM) was observed. However, lower concentrations of lead (50-300 uM) also revealed 22-30% inhibition of Ca^{2+} -Mg^{2+}-ATPase activity (data not shown) which is sufficient to influence the Ca^{2+}-transport of intestinal cells. Alkaline phosphatase was not significantly inhibited by lead. Kuliszewska and Nicholis (1985) have shown that kidney brush border alkaline phosphatase activity was enhanced by the treatment of lead. The sensitivity of the same class of enzyme may also vary to a single inhibitor. The variation in sensitivity may be due to the variation in requirement of a specific enzyme. Inhibition of intestine brush border membrane enzymes by lead represent a classical example. The inhibition of intestinal sucrase, ϑ -glutamyltranspeptidase and acetylcholinesterase was non-competitive type. It is generally accepted that lead inhibits most enzymes with functional sulphydryl group (Nordberg 1976). Lead has high affinity to remain bound to mammalian tissues. It can bind to membrane and may thus alter BBM permeability and intrupt substrate transport through membrane.

It is well known that meta vanadate is a potent inhibitor of Na^{+} -K^{+} -ATPase(Cantley et al. 1978; Bond and Hudgins 1980). It has also been shown that vanadate acts as a non- competitive inhibitor. Later on, Haffar et al (1988) observed that vanadate inhibited ileal ATPase nonspecifically. Vanadium is known to induce conformational changes in phosphoenzyme ion transpor-

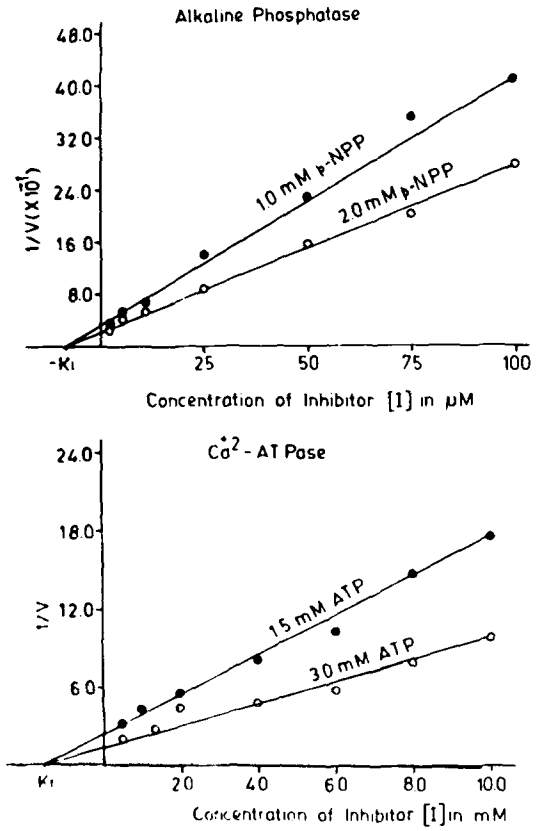

Figure 3. Dixon plots of rat intestinal brush border membrane enzyme kinetics as a function of vanadate concentration.

ting ATPase and the formation of two dimentional crystaline arrays of Ca^{+2}-ATPase molecules in sarcoplasmic reticulum membrane residues (Dux and Martonosi 1983). The present findings showing the inhibition of Ca^{+2}+ Mg^{+2}-ATPase of intestinal BBM in a noncompetitive manner is also in agreement with the previous findings and suggest a generalised mode of vanadium action. The effect vanadium on alkaline phosphatase from different sources have been studied. The inhibitor constant of vanadate on plasma membrane alkaline phosphatase of rat mesentrie and human liver have been reported to be 1.5 uM and 65 uM, respectively, using p-nitrophenylphosphate as substrate. Similarly, the inhibitor constant (Ki) for BBM alkaline phosphatase was found to be 8.7 uM. Present findings, in general, suggest atleast a partial blocking of enzymes active

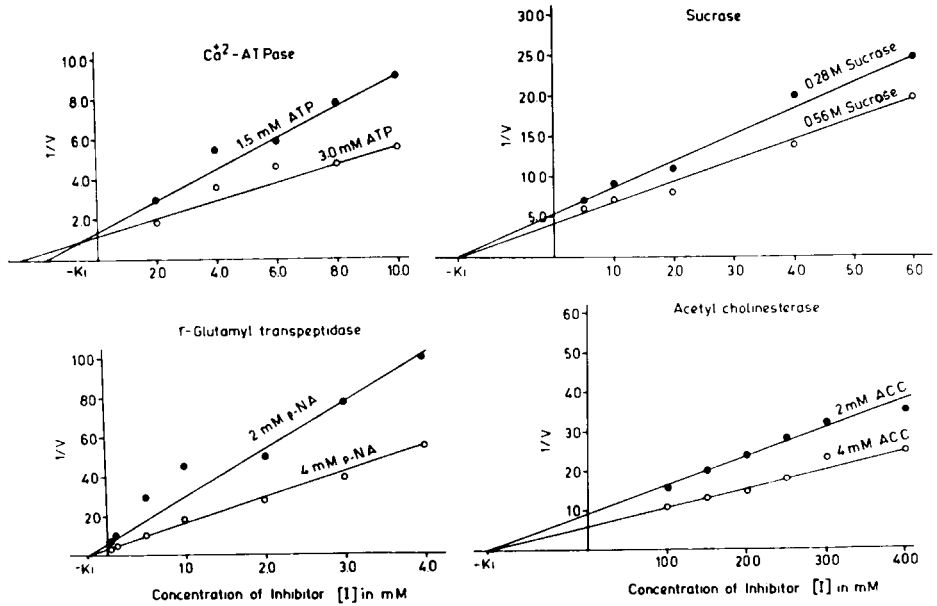

Figure 4. Dixon plots of rat intestinal brush border membrane enzyme kinetics as a function of lead concentration.

Table 1 I_{50} and Ki values of lead and vanadium for rat intestinal brush border membrane enzymes.

Enzymes	Lead		Vanadium	
	I_{50} (mM)	Ki (M)	I_{50} (mM)	Ki (M)
Alkaline phosphatase	N.D.	-	0.01	$8.7X10^{-6}$
$Ca^{+2}+Mg^{+2}$-ATPase	4.0	$0.6X10^{-3}$	1.00	$1.6X10^{-3}$
Sucrase	2.0	$1.7X10^{-3}$	N.D.	-
γ-glutamyl-transpeptidase	2.0	$0.25X10^{-3}$	N.D.	-
Acetylcholinesterase	20.0	$10.3X10^{-3}$	N.D.	-

N.D. - Not detectable.

sites due to these metals which inturn is responsible to cause the depletion of enzyme activities. Most of these BBM enzymes are also involved in the movement of important intermediates including glucose and amino acids across the BBM. Therefore, the inhibition of these enzymes by lead/vanadium can also bring about deleterious effects in the intermediary metabolism.

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