Guillermina Girardi · María Mónica Elias

Evidence for renal ischaemia as a cause of mercuric chloride nephrotoxicity

Received: 17 October 1994/Accepted: 5 January 1995

Abstract The present study was undertaken to investigate if the source of oxidative stress and the renal injury produced by mercuric chloride could be renal ischaemia. Verapamil Vp was used because it was described that calcium channel blockers protect cells from nephrotoxicants and from ischaemia. Vp (75 μg/kg, i.v.; 30 min before HgCl₂ injection) prevented mercuric chloride renal injury observed 1 h post-HgCl₂ injection as measured by clearance techniques. Vp also prevented the diminution of non-protein-sulfhydryls (NPSH) and the increased lipid peroxidation (LPO) induced by HgCl₂ in renal tissue. Hg²⁺ toxicokinetic alterations were not observed in Vp plus HgCl₂ treated rats, nor was Vp ability found as a free radical scavenger in renal tissue homogenates. The results described in this study give some evidence for the role of renal ischaemia in the production of oxidative stress, generating LPO and functional and morphological renal injury described in mercuric chloride treated rats.

Key words Mercuric chloride · Nephrotoxicity · Ischaemia · Verapamil · Oxidative stress

Introduction

The possible mechanisms involved in the development of mercuric chloride acute renal failure and the subsequent morphologic and metabolic changes remain a central concern of biochemical toxicology. Many authors favor lipid peroxidation (LPO) as a cause of cell death in mercury induced nephrotoxicity (Gstraunthaler et al. 1983). Data from our laboratory have shown that renal mercuric chloride toxicity developed with increased LPO tissue levels (Girardi and Elias 1991). LPO development in mercuric chloride treated rats seems not to be due to a deficiency in antioxidant enzymatic activity (Girardi and Elias 1994).

Other work from our laboratory suggested that mercuric chloride promotes a significant diminution in glomerular filtration rate and in renal blood flow (Girardi et al. 1989). Hypoxia or ischaemia due to vasoconstriction has been proposed as a mechanism by which several toxicants induced nephrotoxicity (Commandeur and Vermeulen 1990). Ischaemia could be a significant source of free radicals (Féher et al. 1987), and thus could promote LPO and induce renal failure (Weinberg 1991; Molitoris 1992).

The present study was undertaken to examine whether mercuric chloride produces renal ischaemia and thus promotes oxidative stress as measured by LPO. To this end, a calcium channel inhibitor such as verapamil (Vp) was used to prevent the renal vasoconstriction observed in mercuric chloride treated rats. Vp has a protective effect in ischaemic renal failure (Goligorsky et al. 1985; Harris et al. 1988; Almeida et al. 1992). Vp has also been described as a reactive oxygen species scavenger (Yoshioka et al. 1990; Tong Mak et al. 1992), and this Vp capacity was also tested in vitro using kidney homogenates, incubated with mercuric chloride.

Materials and methods

Animals

Adult male Wistar rats (300–350 g) were used in all experiments. Animals were housed in rooms with controlled temperature (21–23°C) and light (0600–1800 hours). They were maintained on a standard diet and water ad libitum.

In vivo experiments

Vp (Isoptino, Hoechst Lab-Argentina) was administered to rats in two different doses (25 and 75 μg/kg body wt; i.v.) 30 min before HgCl₂ injection (5.0 mg/kg body wt, s.c.).
The following experimental groups were studied: control rats ($n = 10$); Hg rats ($n = 12$) which received a single dose of HgCl$_2$ as described above; Vp$_{25}$ + Hg rats ($n = 4$) receiving Vp (25 µg/kg body wt, i.v.) followed after 30 min by a single dose of HgCl$_2$ and Vp$_{75}$ + Hg rats ($n = 5$) receiving Vp (75 µg/kg body wt, i.v.), followed after 30 min by a single dose of HgCl$_2$. Verapamil doses were reported as effective in preventing acute renal failure in experimental renal ischaemia in isolated perfused rat kidney (Schrier 1991).

Control rats treated with Vp$_{25}$ ($n=4$) or Vp$_{75}$ ($n=4$) were also used.

Experimental procedure

Rat renal function was assayed by clearance techniques performed 1 h after HgCl$_2$ treatment. Clearance studies have the ability to measure if verapamil effects on mercure chloride effects on both vascular and tubular structures. The animals were anaesthetized with thiopental (70 mg/kg body wt, i.p.). The femoral vein and femoral artery were cannulated (P.E. 50) and a bladder catheter was inserted through a suprapubic incision. Animals were maintained in restraining cages throughout the experiments. A solution containing D-mannitol (5 g%), inulin (1 g%) and p-aminohippuric acid (PAH) (0.3 g%) was infused at a rate of 4.5 ml/h. 45-min equilibration period elapsed (5 g%).

Renal function in HgCl$_2$-treated rats pretreated with Vp

Rats treated with Vp$_{25}$ and Vp$_{75}$ did not show any differences with respect to control rats, either in haemodynamic parameters, or in tubular function, LPO levels and NPSH renal content (see legend to Fig. I and Table 1). Mercuric chloride treatment promotes renal failure as previously reported (the data are collected in Fig. 1). Vp$_{25}$ + Hg rats showed glucose and potassium renal handling which was similar to control animals, while GFR, RPF, Fe%Na and Fe%H$_2$O remained impaired and similar to Hg rats values. On the other hand, Vp$_{75}$ + Hg rats showed haemodynamic renal function as measured by GFR and PRF and tubular functions which were not different from control animals (Fig. 1). Both Vp$_{25}$ and Vp$_{75}$ pretreatment avoided the impairment in NPSH and LPO renal levels observed in Hg rats (Fig. 2 and Table 1).

Toxicokinetic study in Vp$_{75}$ + Hg-treated rats

No differences were observed in toxicokinetic parameters between Hg and Vp$_{75}$ + Hg treated rats, at least during the short period of time studied (Table 2). Mercury renal content in Vp$_{75}$ + Hg rats was not different from Hg rats. This suggests that Vp did not modify...