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GLUTATHIONE DEPLETION AND *IN VITRO* LIPID PEROXIDATION IN MERCURY OR MALEATE INDUCED ACUTE RENAL FAILURE

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Abstract—Nephrotoxic acute renal failure was experimentally induced in male rats by s.c. application of mercuric chloride and i.p. administration of maleate, respectively. Mercuric chloride and maleate are known to enhance the formation of free radicals and peroxides, which presumably overload the cell's natural elimination mechanisms for these highly reactive intermediates. In addition, a reduction in activities of superoxide dismutase, catalase and glutathione-peroxidase, enzymes responsible for the protection of cells against peroxidative action of superoxide anions and hyperperoxides was found. In both models of acute renal failure, enhanced lipid peroxidation in kidney homogenates *in vitro*, monitored as malondialdehyde production, was observed. Furthermore, $HgCl_2$ and maleate may react with free SH-groups and thus lead to a depletion of glutathione in tubular cells. Indeed, renal cortical contents of reduced and oxidized glutathione were drastically diminished. These results suggest that alterations in membrane integrity, possibly caused by peroxidative processes, can be considered the cause underlying the well-known disturbances in renal function commonly observed during the initiation phase of $HgCl_2$ and maleate induced acute renal failure.

Little is known about the possible quantitative interconnections between the eliciting events of acute renal failure and the subsequent morphologic and metabolic changes. Acute renal failure has been categorized sofar into two general groups based upon the hypothetical causes: nephrotoxic and ischemic. The first group is associated with ingestion of heavy metals like mercury, chromium or platinum, with organic solvents like carbon tetrachloride, ethyleneglycol or with aminoglycoside antibiotics. The second group is usually found as a consequence of crush injury, burns or hemorrhage. The experimental models developed to simulate the clinical situation are controversial with respect to pathogenesis of acute renal failure.

The investigation performed followed two major routes. On the one hand it was tried by means of morphologic techniques to assess the chain of damages, their localization within the kidney and their severity in dependence of the type of injury [1–4]. On the other hand, the pathophysiologic mechanisms preceding or accompanying morphological damages were carefully analyzed. The functional parameters studied in this context were changes in total and regional blood flow [5–7], glomerular filtration [3], intratubular urine flow and pressure [8] and finally the handling of electrolytes [9]. Few attempts, however, have been undertaken to obtain more information on early changes of renal biochemical function and the combined morphological changes. In a recent study [4] it has been shown that the nephron cell damage associated with acute renal failure induced by either nephrotoxic or ischemic insults is characterized by morphologic changes mainly confined to transport-related structures of the proximal nephron. These changes observed are a reduction of luminal, basolateral and mitochondrial inner membrane surface areas after $HgCl_2$ intoxication and a reduction of only mitochondrial inner membranes following maleate [4]. The reduction of luminal membrane is further paralleled by a decreased activity of alkaline phosphatase, that of the basolateral membrane by a decrease of $(Na^+ + K^+)$-ATPase activity and that of the mitochondrial inner membrane by a lowered cytochrome *c* oxidase activity [4]. This latter finding is in addition substantiated by a diminished mitochondrial phosphorylative capacity. These results indicate a loss of membrane mass, which would correspond to the functional changes during the early phase of mercury and maleate induced acute renal failure, namely a reduction of glomerular filtration rate and an increase of fractional sodium excretion and urine output. Therefore, the question for the mechanisms leading to membrane loss must be put forward. Such a loss of membrane material can most likely be caused by disintegration of membranes via peroxidation or hydrolysis of their lipid moiety.

The present investigation was therefore aimed at obtaining proofs for evidence of possible peroxidative processes in $HgCl_2$ or maleate injured rat kidneys.

MATERIALS AND METHODS

Experimental acute renal failure was induced in male Sprague–Dawley rats with body weights ranging from 250 to 280 g by s.c. injection of $HgCl_2$ (4 mg/kg body wt) and i.p. application of maleate (200 mg/kg body wt), respectively. Toxin effects were investigated 6, 12 and 24 hr after $HgCl_2$ and 2 hr after maleate administration.

Animals were anesthetized with Inactin (Byk–Gulden, 120 mg/kg body wt) and the kidneys

removed after they had been perfused blood free with ice-cold Tris–buffer (pH 7.4 at 0°), minced with scissors and homogenized at 0°. The crude homogenate was assayed for superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (EC 1.11.1.9), glutathione reductase (EC 1.6.4.2) and catalase (EC 1.11.1.6).

SOD activity was assayed according to Fong *et al.* [10] in the 'cytosolic fraction' of the crude kidney homogenate obtained by centrifugation at 15,000 *g* for 2 min to remove nuclei and mitochondria. The reaction medium consisted of 0.05 mole/l potassium phosphate (pH 7.5) containing 0.1 mmole/l hypoxanthine, cytochrome *c* (0.13 mg/ml, Sigma Type III) and 10 μl homogenate corresponding to approximately 25 μg protein. The reaction was started by adding 10 μl xanthine oxidase (4 U/ml) and the increase of optical density monitored at 550 nm and 37° in a double-beam spectrophotometer against a blank without homogenate. One unit of enzyme activity was defined as the amount of SOD required to inhibit the rate of reduction of cytochrome *c* by 50% [11].

Selenium and non-selenium dependent GSH-peroxidase [12], GSSG-reductase [13] and catalase [14] were assayed from the crude kidney cortex homogenate by established methods.

For determination of total kidney cortex glutathione (GSH + GSSG), 1 g tissue was homogenized in 10 ml of 10% perchloric acid. Protein was removed by centrifugation and total glutathione was determined in the neutralized supernatant according to Brehe and Burch [15].

Lipid peroxide formation was measured in kidney homogenates as malondialdehyde (MDA) formed and quantified by the thiobarbituric acid (TBA) test [16]. In detail, 1 g cortex wet weight of control and injured kidneys was homogenized in 20 ml Tris–buffer as described and incubated in 100 ml Erlenmeyer flasks at 37° in a shaking water bath. After addition of cumene hydroperoxide in a final concentration of 10 mmole/l, aliquots were taken from the homogenate and analyzed for MDA-formation according to Slater and Sawyer [17]. Aliquots of the clear supernatant, obtained after precipitation of homogenate samples with 10% (w/v) trichloroacetic acid, were mixed with an equal volume of 0.67% (w/v) thiobarbituric acid and heated in a boiling water bath for 10 min. After cooling, extinction of the samples at 535 nm was determined. Protein was assayed by the method of Lowry *et al.* [18], with bovine serum albumin as a standard. All reagents used were of the highest analytical grade available and were obtained from Sigma, Merck and Boehringer.

Assay results were statistically tested for significance using Student's *t*-test.

RESULTS

Superoxide dismutase, catalase, GSH-peroxidase, GSSG-reductase

The activities of SOD, catalase, GSH-peroxidase and GSSG-reductase in kidney homogenates are changed in both models of acute renal failure investigated (Fig. 1). SOD and catalase activities are significantly ($P < 0.05$) decreased 24 hr after $HgCl_2$ intoxication to 52 and 68% of control values, respectively, whereas no changes were found 12 hr after $HgCl_2$ and 2 hr after maleate administration. As compared to controls, GSH-peroxidase activity was with 75% of the control value significantly ($P < 0.05$) lower in both experimental models. The selenium and non-selenium GSH-peroxidase activities are shown (Fig. 1), assayed with cumene hydroperoxide as substrate [12]. The activity of the selenium dependent GSH-peroxidase, determined with H_2O_2 as substrate, was affected to the same extent and did not differ significantly from the decreases of activity presented in Fig. 1. GSSG-reductase showed a decrease of enzyme activity only for the mercury model after 12 hr and increased slighly 24 hr after $HgCl_2$ administration. No significant change in GSSG-reductase activity could be observed for the maleate model when compared to the control.

The absolute values of enzyme activities and total

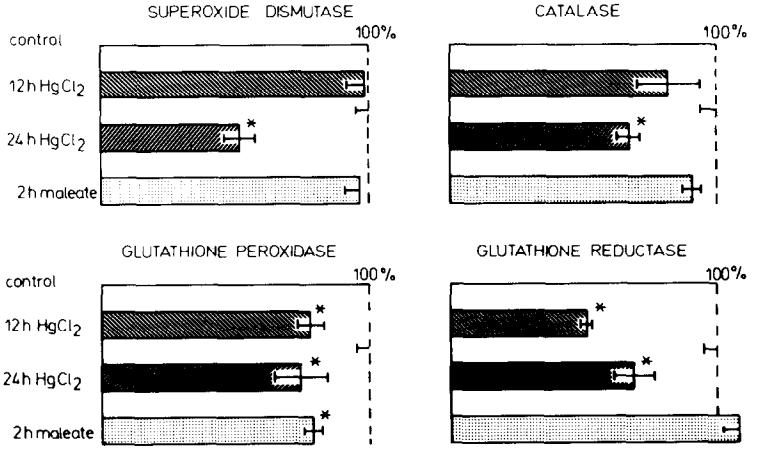

Fig. 1. Effect of $HgCl_2$ and maleate intoxication on activities of superoxide dismutase, catalase, selenium and non-selenium GSH-peroxidase, and GSSG-reductase in rat renal cortex. Enzyme activities are expressed as percentage of control values obtained from normal kidneys (for absolute data see Table 1). Each value given represents mean ± S.D. of 6 animals.* Statistically significant ($P < 0.05$) differences from control values.

Table 1. Absolute data of control enzyme activities and total glutathione content in rat kidney cortex. Values are expressed as mean ± S.D. of 6 animals

Superoxide dismutase	81.5 ± 8.5 U/mg
Catalase	1373 ± 148 k/mg
Glutathione peroxidase	
with H_2O_2 as substrate	291.4 ± 28.2 mU/mg
with CuOOH as substrate	402.4 ± 31.3 mU/mg
Glutathione reductase	127.6 ± 10.4 mU/mg
GSH + GSSG	9.8 ± 1.2 nmole/mg

glutathione contents in kidneys of control animals are given in Table 1.

Kidney glutathione content

Total renal cortical glutathione (GSH + GSSG) is affected in both the mercury and maleate model of acute renal failure. No significant depletion of renal glutathione could be observed 6 hr after $HgCl_2$ administration (Fig. 2). After 24 hr mercury intoxication, however, renal cortical glutathione decreased significantly to 38% of tissue content in control animals. Two hours after maleate administration renal glutathione depletion was even more pronounced. Only 25% of the control glutathione content could be recovered.

Hydroperoxide induced malondialdehyde formation

Malondialdehyde (MDA) content in $HgCl_2$ and maleate treated kidneys did not differ from control MDA levels, determined in the crude homogenates of control and injured kidneys, which can be seen in the common starting point in Fig. 3.

Therefore, cumene hydroperoxide was added to kidney homogenates as described in Materials and Methods to induce *in vitro* lipid peroxidation and to determine the protective capacity of $HgCl_2$ and maleate injured rat kidneys against enhanced peroxidative processes. Addition of cumene hydroperoxide enhances the amount of MDA formed for control homogenates as well as for the homogenates derived from mercury and maleate treated kidneys (Fig. 3). It can be recognized that there exists a time dependent and statistically significant enhancement in MDA production upon addition of cumene hydroperoxide in cortex homogenates of kidneys obtained 24 hr after $HgCl_2$ intoxication and a high rate of lipid peroxidation in kidney cortex homogenates of maleate treated animals.

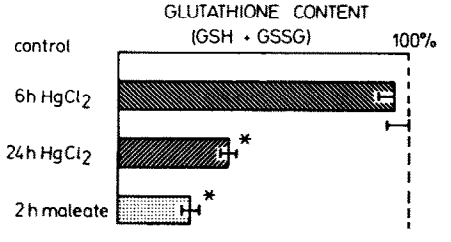

Fig. 2. Total renal cortical glutathione (reduced and oxidized) contents in $HgCl_2$ and maleate injured rat kidneys, expressed as percentage of control tissue level (for absolute values see Table 1). Results are expressed as in Fig. 1.

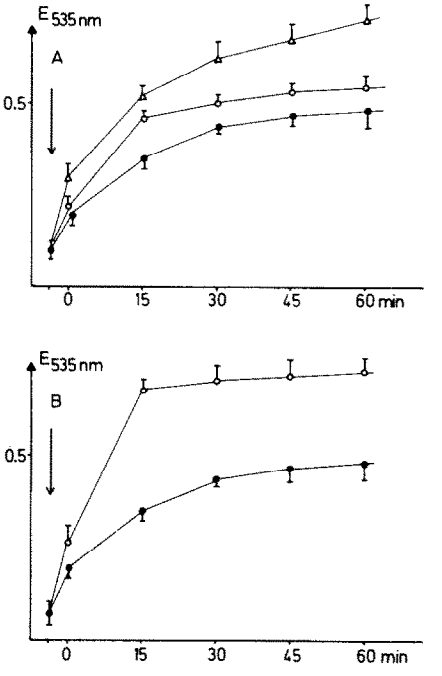

Fig. 3. Malondialdehyde (MDA) production in homogenates of normal and injured rat kidneys upon addition of 10 mmole/l cumene hydroperoxide (arrow). MDA-formation was measured at the indicated intervals after addition of cumene hydroperoxide. The values are expressed as absorption at 535 nm/g cortex wet wt (values are means ± S.D. of three separate experiments). (A) MDA-formation in control kidneys (—●—●—) and in kidneys obtained 6 hr (—○—○—) and 24 hr (—△—△—) after $HgCl_2$ administration. (B) MDA-formation in control kidneys (—●—●—) and in 2 hr maleate injured kidneys (—○—○—).

DISCUSSION

The well-known functional disturbances characteristic for the initiation phase of acute renal failure are, besides a decrease in glomerular filtration rate, a decreased reabsorptive and secretory capacity of the nephron. This tubular insufficiency may be seen as a consequence of disturbances in tubular integrity, caused by tubular cell injury and necrosis. These effects are in good accord with a recent investigation of Pfaller [4], who has shown that the initiation phase of $HgCl_2$ induced acute renal failure is accompanied by reduction of cell membrane surface areas of the luminal, the basolateral and the mitochondrial cristae membrane systems in the proximal nephron cell. These structural changes are furthermore substantiated by decreased activities of the corresponding membrane bound marker enzymes. The major finding of the present investigation is the enhanced *in vitro* lipid peroxidation found in homogenates of injured rat kidneys upon an additional load with alkyl-hydroperoxides. The protective capacity against peroxidative processes of $HgCl_2$ and maleate injured kidneys, when exposed to an 'oxidative stress' by addition of cumene hydroperoxide, is grossly diminished (Fig. 3). MDA-formation, indi-

cating lipid peroxidation, can only be kept at a sufficiently low rate by control kidneys, whereas MDA-formation increases by nearly 70% in homogenates of kidneys obtained after 24 hr $HgCl_2$ and 2 hr maleate intoxication, respectively.

A similar finding was reported recently by Fujita and Fujimoto [19], who demonstrated enhanced MDA-formation in kidneys by the Hg containing diuretic drug mersalyl.

The results of this study further substantiate the grossly diminished capacity of $HgCl_2$ and maleate injured renal cells in eliminating highly reactive oxygen intermediates. The complete 'GSH-system', including glutathione, GSH-peroxidase and GSSG-reductase, is affected. The tripeptide glutathione itself is drastically depleted in both models of acute renal failure investigated (Fig. 2). In addition, decreased activities of GSH-utilizing and GSH-regenerating enzyme are found (Fig. 1). The exception is an unaltered GSSG-reductase activity 2 hr after maleate. Several studies [20–22] have shown that isolated cells and perfused organs release glutathione disulphide (GSSG) upon addition of organic hydroperoxides or under conditions of enhanced rates of endogenous H_2O_2 formation. Enhanced GSSG release probably reflects an increased intracellular oxidation of GSH due to the action of GSH-peroxidase.

Intoxication with the analgesic drug paracetamol causes renal and hepatic cell necrosis in rats [23, 24] and markedly depletes the target organs glutathione contents. Wendel *et al.* [25, 26] showed that paracetamol intoxication led to lipid peroxidation due to drug induced glutathione depletion, which is accompanied by microsomal production of activated oxygen.

This implies that the drastical loss in renal cortical glutathione (Fig. 2) and the decrease in glutathione peroxidase (Fig. 1), which is common to both models of acute renal failure studied, will be responsible for the enhanced *in vitro* lipid peroxidation shown in Fig. 3.

Besides the protection of lipids against peroxidation, glutathione plays a crucial role in the maintainance of the cellular thiol status, regulating the thiol : disulphide ratio in integral membrane proteins through interchange reactions [27]. Various membrane functions, including solute transport and oxidative phosphorylation in mitochondria depend upon a suitable thiol : disulphide balance within the proteins involved.

In summary, it can be concluded that the drastic glutathione depletion and the impairment of the cell's natural elimination mechanisms for hydroperoxides in $HgCl_2$ and maleate treated rat kidneys seems most likely to be responsible for a peroxidative damage of renal membrane systems found during the onset phase of these two models of acute renal failure.

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