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# SHORT COMMUNICATIONS

## Loss of kidney microsomal glucose-6-phosphatase activity following acute administration of *p*-aminophenol

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*p*-Aminophenol induced renal tubular necrosis is being studied as an experimental model for the acute effects of analgesics on the kidney. This compound produces acute proximal tubular necrosis in rats following a single intravenous injection, but no histological changes are observed in other tissues [1]. Current work is designed to elucidate which biochemical changes at the subcellular level are responsible for the necrosis.

We have reported ultrastructural and functional changes in mitochondria isolated from the kidneys of *p*-aminophenol treated rats, which were detectable at 1 hr but became more severe at 4 hr [2]. The results of this study indicated that *p*-aminophenol may initiate changes in the mitochondrial membranes with consequent loss of function. It then became of interest to ascertain whether other intracellular membranes were similarly affected and if the time course of change paralleled that of the mitochondria. For this purpose the activity of microsomal glucose-6-phosphatase was determined. This enzyme is located in the endoplasmic reticulum of certain tissues [3], and loss of activity after toxic treatments is used as an index of damage to that cell compartment [4]. Damage to cellular membranes may be mediated by peroxidation of the lipid moieties of the membranes initiated by a reactive metabolite of the toxic agent [5]. In this communication we also report on the result of attempts to detect endogenous lipid peroxides as well as spontaneous *in vitro* peroxidation in preparations from kidneys of treated rats. Lipofuscin, an end product of lipid peroxidation [6], has been reported to accumulate in the tissues of chronic abusers of phenacetin [7, 8] and in dogs fed phenacetin for several months [7, 9], and the structural relationship between *p*-aminophenol and phenacetin provides further justification for this investigation.

### MATERIALS AND METHODS

Male Sprague–Dawley rats (200–280 g) were lightly anaesthetized with ether and injected in the tail vein with 300 mg/kg *p*-aminophenol hydrochloride dissolved in distilled water. Control animals were injected with distilled water. The animals were killed by bleeding under ether anaesthesia, the kidneys removed, decapsulated, and rinsed in ice-cold homogenizing medium. All subsequent operations were carried out at 0–4°. For measurement of glucose-6-phosphatase activity, the kidneys were homogenized in 9 vol. of 0.25 M sucrose–1.0 mM Tris–0.1 mM EDTA, pH 7.4 in a glass Potter–Elvehjem homogenizer with a teflon pestle, using 4 up and down passages at 1150 rpm. Samples were centrifuged for 10 min at 7,500 $g_{av}$ (Sorvall RC2-B refrigerated centrifuge), the pellet washed once, and the combined supernatants centrifuged for 90 min at 100,000 $g_{av}$ (Beckman model L2-65B ultracentrifuge). The resulting microsomal pellet was resuspended in 1.5 vol. of homogenizing medium, giving a protein content of 10–12 mg/ml. Liver microsomes were prepared by the method used for kidney 1 hr after injection. For lipid peroxidation studies the kidneys were homogenized in 9 vol. of 0.15 M KCl–0.01 M potassium phosphate, pH 7.4. For *in vitro* studies samples prepared from untreated animals were used and *p*-aminophenol was added to the incubation medium.

Microsomal glucose-6-phosphatase activity was measured by a modification of the method of Swanson [10]. 250 μl of 0.1 M sodium malate, pH 6.25 and 50 μl of 0.1 M glucose-6-phosphate (Sigma disodium salt) were preincubated at 37°. The reaction was started by the addition of 10 μl of microsomal suspension and stopped after 15 min with 0.5 ml of 10% trichloroacetic acid. The samples were diluted to 1 ml with distilled water and the amount of inorganic phosphate released determined on an aliquot of the protein free supernatant [11]. Malonaldehyde content was measured by the thiobarbituric acid reaction described by Klaassen and Plaa [12], using 1,1,3,3-tetraethoxypropane (May & Baker) in 40% ethanol as standard. When the effect of *p*-aminophenol *in vitro* was studied, 1–100 μl of a 0.025 M solution of *p*-aminophenol HCl in 0.15 M KCl was added to the incubation flask. For estimation of malonaldehyde production *in vitro* the endogenous level of malonaldehyde (zero time) was subtracted from the level present after 45 min. Diene conjugation was measured on chloroform–methanol extracts of homogenates [12]. Protein was estimated by the Lowry method as described by Munro and Fleck [13]. Statistical significance was determined using a 2-tailed Student's *t*-test on the difference between the means of the control and treated groups.

### RESULTS AND DISCUSSION

Microsomal glucose-6-phosphatase activity was reduced at 1 and 4 hr after injection of *p*-aminophenol to 50 per cent of control value (Table 1). However when added *in vitro* *p*-aminophenol had no effect on the activity of the enzyme in either microsomes or homogenates of kidney from untreated animals (Fig. 1). Liver microsomal glucose-6-phosphatase activity 1 hr after injection did not differ significantly from the control value, being 51.8 ± 5.8 μg Pi released/mg protein/15 min compared with 55.9 ± 9.6 for the corresponding control. Endogenous lipid peroxides in homogenates from treated animals were unchanged when measured by diene conjugation, but when measured in terms of malonaldehyde (thiobarbituric acid reaction) were significantly reduced at 10, 30 and 60 min. In kidney homogenates incubated at 37° for 45 min the extent of malonaldehyde formation was reduced in samples from treated animals at all times (Table 1). The *in vitro* production of malonaldehyde in homogenates from untreated animals was also reduced if *p*-aminophenol was included in the incubation mixture (Fig. 2).

Previous studies with *p*-aminophenol have shown that mitochondrial respiration and oxidative phosphorylation are impaired 1 hr after treatment, and are further reduced at 4 hr [2]. These results indicate that *p*-aminophenol may damage mitochondrial membranes, and so measurement of microsomal glucose-6-phosphatase activity was undertaken to see whether membrane damage was a general

Table 1. Effect of *p*-aminophenol treatment on glucose-6-phosphatase activity and on lipid peroxidation

| Time after injection | Glucose-6-phosphatase (μg Pi/mg protein/15 min) | Diene conjugation (OD at 243 nm/mg protein) | Malonaldehyde (nmoles/mg protein) Zero time | Malonaldehyde (nmoles/mg protein) 45 min–Zero time |
|---|---|---|---|---|
| 10 min C | 96.9 ± 5.4 | 0.005 ± 0.001 | 0.17 ± 0.01 | 0.72 ± 0.01 |
| T | 96.9 ± 13.9 | 0.004 ± 0.001 | 0.12 ± 0.01* | 0.37 ± 0.08* |
| 30 min C | 84.6 ± 8.4 | 0.005 ± 0.001 | 0.21 ± 0.03 | 0.82 ± 0.11 |
| T | 76.9 ± 5.2 | 0.005 ± 0.001 | 0.09 ± 0.02* | 0.17 ± 0.04* |
| 60 min C | 53.7 ± 7.6 | 0.008 ± 0.001 | 0.25 ± 0.04 | 0.98 ± 0.15 |
| T | 25.5 ± 3.6* | 0.008 ± 0.001 | 0.11 ± 0.01* | 0.26 ± 0.04* |
| 240 min C | 51.4 ± 3.5 | 0.004 ± 0.001 | 0.15 ± 0.01 | 0.79 ± 0.05 |
| T | 25.8 ± 1.5* | 0.006 ± 0.004 | 0.13 ± 0.03 | 0.15 ± 0.07* |

Glucose-6-phosphate activity was measured on kidney microsomal preparations. Diene conjugation was measured on chloroform–methanol extracts of kidney homogenates. Malonaldehyde content of homogenates was determined by the thiobarbituric acid reaction at zero time and after 45 min aerobic incubation at 37°. Details of the assay procedures are given in Materials and Methods. All values are the means ± S.E.M. of at least four animals. (C = control, T = *p*-aminophenol treated. * indicates $P < 0.01$).

phenomenon of *p*-aminophenol induced renal injury. The significant decline in glucose-6-phosphatase activity after *p*-aminophenol treatment indicates that damage to the endoplasmic reticulum membranes does occur. This, in conjunction with the mitochondrial studies [2], suggests that generalized membrane damage is a consequence of the treatment. In contrast with the changes in mitochondrial function, loss of microsomal glucose-6-phosphatase activity was maximal at 1 hr. Variation in levels of kidney glucose-6-phosphatase in control animals (Table 1) may be due to the ether anaethesia given for intravenous injection. Similar variations in liver microsomal enzymes, including glucose-6-phosphatase, have been noted after short periods of ether anaesthesia [4, 14].

The mechanism of the change in the microsomes remains obscure. Enhanced breakdown of lipid moieties of subcellular membranes sometimes occurs via lipoperoxidation [5]. Measurement of sample diene conjugation and malonaldehyde production are frequently used to monitor the extent of this process. In neither parameter did the level found after *p*-aminophenol administration exceed the control values. With diene conjugation levels there was no change whereas with malonaldehyde production the values in the treated samples were lower than controls. Addition of *p*-aminophenol *in vitro* also reduced malonaldehyde production. It is possible that *p*-aminophenol acted as an antioxidant to inhibit lipoperoxidation. Another explanation would depend on the fact that some degree of lipoperoxidation involves an active microsomal enzyme complex [15]. Damage to the endoplasmic reticulum by *p*-aminophenol treatment could in fact interfere with this process and decrease the endogenous lipid peroxide levels. To clarify this point studies on the effect of *p*-aminophenol on various microsomal processes are in progress.

The failure of *p*-aminophenol addition *in vitro* to alter glucose-6-phosphatase activity parallels the findings obtained with the *in vitro* studies on mitochondrial function [2]. These results indicate that a direct attack on the membrane does not occur, but rather metabolic activation is first required. The site of activation is uncertain. It would be expected that major metabolic transformation would occur in the liver, and that the liver would then be a target for toxic damage. We have shown that glucose-6-phosphatase activity in the liver is unaffected by *p*-aminophenol treatment. This supports the findings that no histological evidence of cell injury is found in the liver [1].

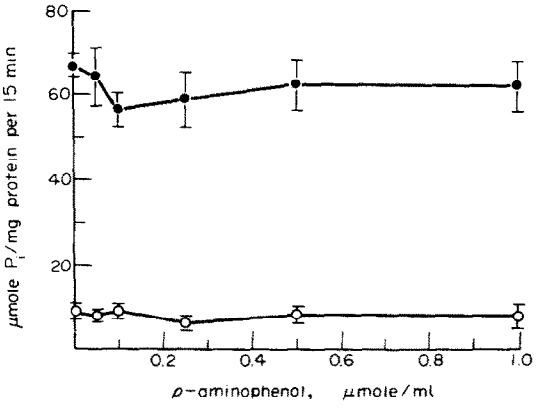


Fig. 1. Effect of *p*-aminophenol *in vitro* on glucose-6-phosphatase activity. Glucose-6-phosphatase activity was measured in kidney microsomes (●) and homogenates (○) as described in Materials and Methods. Results are given as the mean ± S.E.M. of determinations on preparations from four animals.

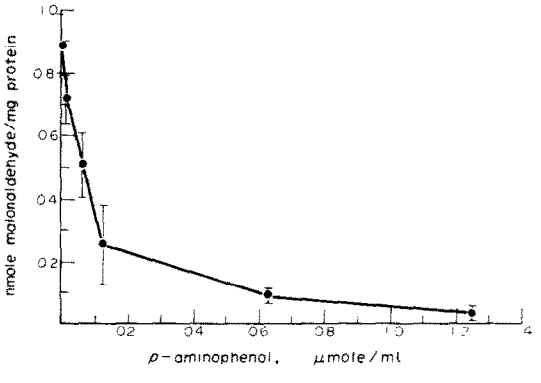


Fig. 2. Effect of *p*-aminophenol *in vitro* on malonaldehyde production in rat kidney homogenates. Homogenates were incubated for 45 min at 37° as described in Materials and Methods. Results are given as the mean ± S.E.M. of determinations on preparations from four animals.

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