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Antioxidant effect of acetaminophen in rat liver

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Acetaminophen is a commonly used analgesic drug which is hepatotoxic in high doses [1]. There have been many investigations of its metabolism and toxicity. In the course of studies of vitamin E deficiency we have observed that acetaminophen has antioxidant properties.

Vitamin E is a biological antioxidant. We have recently isolated hepatocytes from vitamin E-deficient rats [2]. Our purpose in developing this hepatocyte model was to employ it in examining mechanisms of liver cell injury by drugs and chemicals. Figure 1A shows that severely vitamin E-deficient hepatocytes maintained in a complete incubation medium lost viability rapidly after 2 hr. Loss of viability was preceded by the appearance of lipid peroxidation products. No such loss of viability or accumulation of TBA-reactive substances was noted in vitamin E-adequate control hepatocytes (Fig. 1B). Thus, the vitamin E-deficient hepatocytes were probably dying of injury caused by spontaneous lipid peroxidation.

The addition of 12.5 mM acetaminophen to the flask containing vitamin E-deficient hepatocytes suppressed lipid peroxidation and increased viability to control levels (Fig. 1A). This concentration of acetaminophen had no effect on control hepatocyte viability or TBA-reactive substances (Fig. 1B). Thus, acetaminophen appears to be able to replace the antioxidant action of vitamin E in this model system. This replacement occurs at concentrations of drug below the toxic range. In this system, 25–50 mM acetaminophen caused cell death during the 4-hr incubation (data not shown).

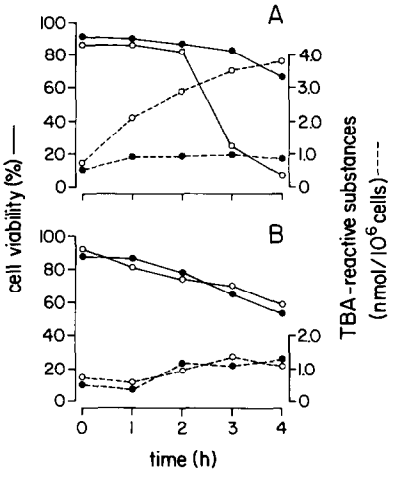

Fig. 1. Effect of acetaminophen on cell viability and TBA-reactive substances in vitamin E-deficient and control hepatocytes. Open circles indicate the absence of acetaminophen and closed circles indicate the presence of 12.5 mM acetaminophen. The solid lines represent cell viabilities and the dashed lines represent TBA-reactive substances. Panel A contains results obtained with hepatocytes isolated from a rat fed a vitamin E-deficient diet for 21 weeks [2]. Panel B contains results obtained with hepatocytes isolated from a rat fed a vitamin E-sufficient diet [2]. These results are from a representative experiment. These experiments have been repeated six to seven times. Hepatocytes were isolated according to Ref. 2. Cell viability was determined by trypan blue exclusion. TBA-reactive substances, which are products of lipid peroxidation and usually are referred to as malonaldehyde, were measured as an index of lipid peroxidation [3]. After isolation, the hepatocytes were allowed to stabilize for 1 hr prior to the start of the experiment.

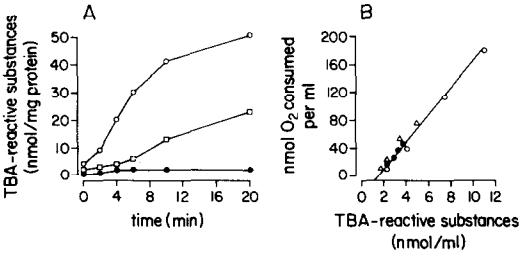

Fig. 2. Effect of acetaminophen on the formation of TBA-reactive substances and on O_2 consumption in the microsomal lipid peroxidation system. Microsomes were isolated and washed once in 0.15 M KCl [3]. They were suspended at a concentration of approximately 10 mg protein/ml. Boiled microsomes were prepared by heating a test tube containing fresh microsomes in a boiling water bath for 1 min. Acetaminophen concentrations used were: (○) none; (□) 5 mM; (△) 12.5 mM; and (●) 25 mM. Panel A: These incubations were carried out at 37° in a shaking water bath in open 25 ml flasks. The buffer was 50 mM Tris–HCl, pH 7.5, containing 0.14 M NaCl. Microsomal protein concentration was approximately 0.9 mg/ml. Other final concentrations were: L-ascorbic acid, 0.5 mM; ADP, 2 mM; and $FeCl_3$, 6 μM. Incubation volume was 5 ml. The ADP and $FeCl_3$ were combined and allowed to stand at room temperature for 1 hr before use. This is a representative experiment. It was carried out three times. Panel B: These incubations were carried out in a Clark-type O_2 electrode and were scaled down in volume but had the same concentrations of constituents as those described for panel A. Also, they were stirred instead of shaken. The oxygen electrode was calibrated with air-saturated buffer and an O_2 depleted solution in which microsomes had been allowed to peroxidize. The oxygen concentration was monitored and samples were taken at various times to measure the content of TBA-reactive substances. Several time points were taken with each concentration of acetaminophen.

An antioxidant effect in cells can be mediated through at least two general mechanisms: acetaminophen could be a chemical antioxidant or it could exert its effect metabolically. Some other cytochrome P-450 substrates have antioxidant effects of both types [4–6]. Therefore, we performed experiments to determine if the acetaminophen molecule was a chemical antioxidant which could react directly with oxidizing intermediates.

The ascorbate-iron microsomal lipid peroxidation system [7] was employed using heat-inactivated rat liver microsomes. Figure 2A shows the effect of acetaminophen in this non-enzymatic microsomal lipid peroxidation system. Acetaminophen inhibited lipid peroxidation in a dose-dependent fashion, indicating that it is a chemical antioxidant.

We confirmed the effect of acetaminophen in a system which did not contain ascorbic acid. Ascorbic acid was replaced by 100 μM $FeSO_4$ in the microsomal lipid peroxidation system. Acetaminophen inhibited lipid peroxi-

dation under those conditions indicating that an interaction between acetaminophen and ascorbic acid [8] was not necessary for the acetaminophen effect.

Oxygen consumption is an early event in lipid peroxidation preceded only by free radical formation. TBA-reactive substance formation is a late event. If an antioxidant inhibits TBA-reactive substance formation to a greater extent than O_2 consumption, it is probably acting in the mid-portion of the lipid peroxidation process, perhaps interfering with peroxide breakdown. If both are inhibited to the same extent, the antioxidant is acting early, probably scavenging free radicals. Oxygen electrode experiments shown in Fig. 2B demonstrate that the stoichiometric relationship between TBA-reactive substance production and O_2 consumption was not changed by acetaminophen. This suggests that acetaminophen scavenges free radicals in the system before they can react with O_2.

Several pathways are active in the metabolism of acetaminophen, but a minor one which is dependent on cytochrome P-450 is responsible for the hepatotoxicity of the drug [1]. Recent studies have suggested that *N*-acetylimidoquinone may be the toxic intermediate of the drug [8] and that enzyme systems other than cytochrome P-450 can produce it [8, 9]. The present findings raise the possibility that acetaminophen can be metabolized non-enzymatically, i.e. by lipid peroxidation. Evaluation of this will require further study.

While acetaminophen is an antioxidant under the conditions used in these experiments, it can cause lipid peroxidation as well. Fasted mice given high doses of acetaminophen exhale large amounts of ethane, indicating that lipid peroxidation is occurring *in vivo* [10]. This observation would suggest that the chemical antioxidant effect of acetaminophen may have been overcome by metabolic alterations caused by the compound. Thus, because of its complex metabolic effects, acetaminophen is not a reliable *in vivo* antioxidant. However, consideration of its antioxidant properties would appear to be essential in interpreting the effects of acetaminophen in biological systems.

In summary, acetaminophen, added in subtoxic doses, protected isolated vitamin E-deficient hepatocytes against spontaneous lipid peroxidation and loss of viability. The compound can also inhibit non-enzymatic microsomal lipid peroxidation, indicating that its effect in the cells is due to intrinsic antioxidant properties.

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Actions of certain calcium channel blockers and calmodulin antagonists on inorganic phosphate-induced swelling and inhibition of oxidative phosphorylation of heart mitochondria

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Diltiazem, nifedipine and verapamil, members of the class of drugs known as calcium antagonists or calcium channel blockers [1, 2], were shown previously to mitigate the impairment of mitochondria in the isochemic myocardium [3–5]. Diltiazem and verapamil were also found to prevent inorganic phosphate-induced swelling of heart mitochondria *in vitro* [6]. Recently it was reported that calcium channel blockers bind to purified calmodulin [7, 8]. This observation raised the possibility that the effect of these drugs on mitochondria may be due to the inhibition of a calmodulin-mediated reaction.

This study was carried out to determine whether the effectiveness of calcium channel blocking drugs in preventing inorganic phosphate-induced swelling and inhibition of oxidative phosphorylation follows the rank and order of effectiveness in which they were found to bind to purified calmodulin [7, 8]. The effects of trifluoperazine and R 24 571, two well-known inhibitors of calmodulin mediated reactions [9–11], were also investigated to determine the possible involvement of calmodulin in P_i-induced swelling of mitochondria.

Methods

Mitochondria from rabbit heart [6] and rat liver [12] were isolated as described previously. The initial homogenization medium for rat liver, unlike the reported pro-