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HEPATIC GLUTATHIONE AND LIPID PEROXIDATION IN RATS TREATED WITH THEOPHYLLINE

EFFECT OF DOSE AND COMBINATION WITH CAFFEINE AND ACETAMINOPHEN

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Abstract—As theophylline, caffeine and acetaminophen (APAP) are commonly found in combination in prescription and non-prescription drugs, the present study was designed to evaluate changes of hepatic glutathione (GSH) and lipid peroxidation in rats treated concurrently with these widely used drugs. In rats treated with different doses of theophylline, a dose-related depletion of hepatic GSH was observed ($r = -0.88$, $P < 0.001$). After a dose of 100 mg/kg of caffeine given alone and in combination with 50 mg/kg of theophylline, hepatic GSH levels were decreased by 22.5% ($P > 0.05$) and 35.5% ($P < 0.01$) of the control value, respectively. Also, the challenge with 500 mg/kg of APAP significantly depleted hepatic GSH in rats pretreated with either saline or the combination of both theophylline and caffeine (50 and 100 mg/kg, respectively), $P < 0.001$. In the latter group, hepatic GSH levels were approximately 47% of the corresponding values in rats that received APAP ($P < 0.02$). Such severe depletion of hepatic GSH, in rats that received the three drugs, was associated with a significant rise in the extent of lipid peroxidation ($P < 0.05$). The other experimental groups treated with one or two drugs did not exhibit similar changes. These results suggest that the concomitant administration of theophylline, caffeine and APAP may enhance the susceptibility of the liver cells to the toxic effect of APAP by severely depleting hepatic GSH with subsequent induction of lipid peroxidation.

Key words: acetaminophen, caffeine, theophylline, glutathione, lipid peroxidation

Modulation of cellular GSH† levels may greatly affect the susceptibility of various organs to chemically-induced cellular injury [1–3]. For example APAP is known to be metabolized by the hepatic cytochrome P450 enzyme system to an electrophilic metabolite, *N*-acetyl-*p*-benzoquinone imine, which upon overdosage causes hepatic GSH depletion, lipid peroxidation and necrosis [4–7]. GSH conjugation is certainly an important metabolic pathway for detoxifying this reactive intermediate of APAP metabolism and, accordingly, prior depletion of hepatic GSH potentiates APAP hepatotoxicity [8–10].

In view of the wide popularity of methylxanthines and the presence of caffeine in combination with APAP in some analgesic products, the effect of caffeine on APAP hepatotoxicity has received considerable attention over the last years. Several studies in rats have demonstrated enhanced APAP-induced hepatic GSH depletion and toxicity by concomitant administration of caffeine [11–14]. Also, the interaction between APAP and theophylline, a dimethylxanthine commonly used as a bronchodilator, was the topic of a recent study. Kalhorn *et al.* [13] reported that the simultaneous administration of APAP and theophylline potentiates APAP-induced hepatotoxicity in rats. These investigators, however, did not evaluate the effects of theophylline on hepatic GSH content and lipid peroxidation as possible mechanisms of interaction with APAP.

Therefore, the present study examined the effect of both theophylline and the concomitant administration of theophylline and caffeine on APAP-induced GSH depletion and lipid peroxidation. This investigation consists of two parts: first, an assessment of the effects of different doses of theophylline on hepatic GSH content; and second, an evaluation of APAP-induced hepatic GSH depletion and lipid peroxidation in rats pretreated with theophylline at either a large dose alone or a small dose given in combination with caffeine.

MATERIALS AND METHODS

Animals and treatments. Adult male albino rats of a local strain (Medical Research Institute, Alexandria University, Egypt) weighing between 85 and 140 g were used throughout the study. The animals were kept under observation for at least 1 week before use with free access to food and water. At the time of experimentation, the rats were randomly divided into groups with four or five rats per group. All drugs were injected intraperitoneally. Control rats injected with 0.9% NaCl solution were run in parallel.

To study the effects of theophylline on hepatic

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† Abbreviations: GSH, glutathione; APAP, acetaminophen.

Table 1. Dose-dependent effect of theophylline on hepatic GSH content

Dose (mg/kg)	Hepatic GSH	
	μmol/g liver	% control
0	7.74 ± 0.69	—
50	6.82 ± 0.42	88
100	5.21 ± 0.16*	67
150	4.49 ± 0.03†	58

Values shown are means ± SEM with four rats per group. The rats were killed 4 hr after theophylline administration.
*$P < 0.05$, †$P < 0.01$ as compared with control values.

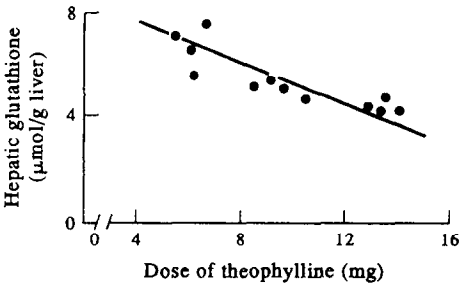

Fig. 1. Correlation between theophylline doses administered to the rats and hepatic GSH levels ($r = -0.88$, $N = 12$, $P < 0.001$).

GSH content, the drug was administered to three groups of rats at doses of 50, 100 and 150 mg/kg. Additional rats treated with theophylline at a dose of 100 mg/kg were injected after 2 hr with APAP at a dose of 500 mg/kg, using a 2.5% supersaturated solution at 40° [4].

To determine the effect of concomitant administration of theophylline and caffeine on hepatic GSH content and lipid peroxidation, groups of rats were injected with theophylline (50 mg/kg), caffeine (100 mg/kg) or simultaneously with both preparations using the same doses. Additional rats treated with both drugs were injected after 2 hr with APAP at a dose of 500 mg/kg. In all experiments, the rats were killed 4 hr after administration of theophylline, caffeine or both compounds.

Analytical methods. Immediately after death, the liver of each rat was removed and assayed for GSH and lipid peroxidation. A part of the liver was homogenized in 5 vol. of ice-cold 5% trichloroacetic acid in 1 mM disodium edetate. The resulting homogenate was used to measure the GSH content as described by Richardson and Murphy [15] and the results were expressed as μmol GSH/g liver. Another part of the liver was homogenized in 9 vol. of ice-cold 1.15% KCl solution. This homogenate was used to measure the extent of hepatic lipid peroxidation by the thiobarbituric acid method as described by Ohkawa *et al.* [16] and the results were expressed by the absorbance at 535 nm [17].

Statistical analysis. The results are presented as means ±SEM. Analysis of variance and Scheffé's multiple comparison procedure were used for statistical comparisons between groups [18]. Values of $P < 0.05$ were considered to represent significant differences between means.

RESULTS

Initially, the effects of increasing doses of theophylline on the GSH content of rat liver were investigated. As shown in Table 1, the treatment of the rats with a dose of 50 mg/kg was without effect. In contrast, the hepatic GSH content fell to 67 and 58% of the control value ($P < 0.05$ and 0.01, respectively) after doses of 100 and 150 mg/kg, respectively. In all rats, when the doses of theophylline were plotted against hepatic GSH contents, a significant correlation was observed ($r = -0.88$, $P < 0.001$, Fig. 1).

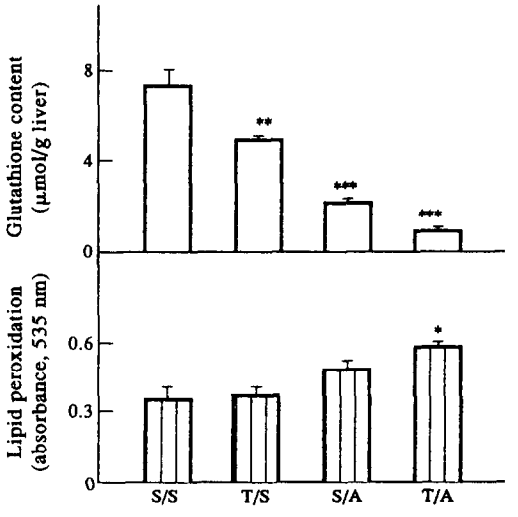

Fig. 2. Hepatic GSH and lipid peroxidation in rats challenged with 500 mg/kg of APAP (A) after the treatment with 100 mg/kg of theophylline (T) or saline (S). Values shown are means ± SEM with five rats per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

As shown in Fig. 2, theophylline-induced depletion of hepatic GSH after a dose of 100 mg/kg was not accompanied by a significant change in the extent of lipid peroxidation. Similarly, APAP (500 mg/kg) alone caused only a significant depletion of hepatic GSH. However, in rats which received both theophylline and APAP, a significant rise in lipid peroxidation and marked depletion of liver GSH were observed ($P < 0.05$ and 0.001, respectively).

Figure 3 demonstrates the effects of theophylline, caffeine and the combination of both compounds with and without the challenge with APAP. In the two groups of rats treated with 50 mg/kg of theophylline or 100 mg/kg of caffeine, hepatic GSH levels were depleted by 11.9 and 22.5% of the

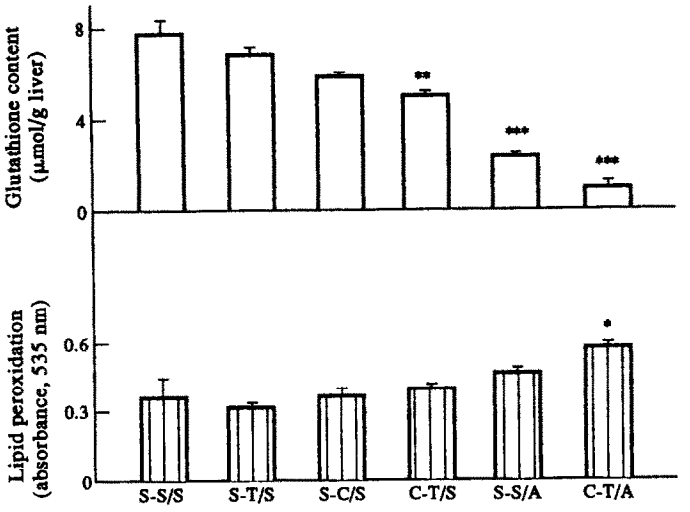

Fig. 3. Hepatic GSH and lipid peroxidation in rats challenged with 500 mg/kg of APAP (A) after the treatment with both 50 mg/kg of theophylline (T) and 100 mg/kg of caffeine (C) or saline (S). Values shown are means ± SEM with four rats per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

control value, respectively ($P > 0.05$). In contrast, the livers of rats treated simultaneously with both compounds using the same doses were depleted of their GSH contents by about 35.5% of the control value ($P < 0.01$). Also, APAP administration resulted in significant depletion of hepatic GSH in rats pretreated with either saline or the combination of both theophylline and caffeine ($P < 0.001$). In the latter group, hepatic GSH level was approximately 47% of that of the saline pretreated group ($P < 0.02$). An enhancement of lipid peroxidation was observed only in the group of rats that received the combination of both theophylline and caffeine before the treatment with APAP ($P < 0.05$).

DISCUSSION

The results of the present study provide evidence for (i) a dose-dependent effect of theophylline on hepatic GSH content, and (ii) marked depletion of hepatic GSH with the induction of lipid peroxidation in rats challenged with APAP after the treatment with either a large dose of theophylline or the combination of a small dose of theophylline with caffeine.

The observation of decreased hepatic GSH levels in rats treated with theophylline is likely due to the utilization of GSH in its metabolism which occurs primarily in the liver. Previous studies in rats and humans have shown that theophylline may be demethylated at N-1 and N-3, and hydroxylated at C-8 by the hepatic microsomal cytochrome P450 enzyme system [19, 20]. The hydroxylation at C-8 appears to be the major route of theophylline metabolism [21]. Following this metabolic step, hepatic GSH may be utilized for the formation of a ring-opened *N*-formyl-diaminouracil metabolite as has been described in studies with the other methylxanthines, theobromine [22] and caffeine [23]. Such an interpretation is supported by two findings. First, in rats treated with different doses of theophylline, hepatic GSH levels correlated significantly with the dose of the drug (Fig. 1). Second, an additive effect on hepatic GSH depletion was observed in rats treated simultaneously with both theophylline and caffeine, compared to the corresponding values in rats treated with the same dose of each drug alone (Fig. 3).

Cellular GSH plays an important role in the detoxification of electrophiles, free radicals and peroxides [24–26]. Thus, hepatic GSH depletion makes the liver cells more susceptible to the development of lipid peroxidation [27–29]. The recent interest in lipid peroxidation stems primarily from the observation that it has been involved in the pathogenesis of liver injury induced by a number of xenobiotics [3–7, 30]. In the present study, the decrease of hepatic GSH levels in rats treated with theophylline, caffeine or APAP was not severe enough to induce lipid peroxidation. On the other hand, when the challenge with APAP was preceded by the treatment with theophylline at either a large dose alone (100 mg/kg) or a smaller dose of 50 mg/kg administered simultaneously with 100 mg/kg of caffeine, hepatic GSH was substantially depleted with a significant rise in the extent of lipid peroxidation (Figs 2 and 3). As GSH depletion and lipid peroxidation are implicated in APAP hepatotoxicity [4–7], the present results suggest that the changes of these parameters following the concomitant administration of theophylline, caffeine and APAP enhance the susceptibility of the liver cells to the toxic effect of the latter agent. Thus, our study extends the available information about the mechanisms of interaction between methylxanthines and APAP which include a stimulatory effect of caffeine on APAP bioactivation by liver microsomes [12, 31].

The importance of the theophylline–APAP interaction in humans is unclear because the high dose of theophylline administered to the animals was 5–10 times greater than the daily dose used in children and adults, respectively, to achieve effective bronchodilatation [32]. However, the results of the combined effect of the smaller dose of theophylline, caffeine and APAP are not without practical interest with regard to the hepatotoxicity of APAP. This drug interaction may be anticipated, in part because of the availability of theophylline, caffeine and APAP in a number of combinations of prescription and non-prescription drugs and in part because of the widespread consumption of caffeine-containing beverages. Thus, it would be worthwhile to consider this type of interaction when the three agents are used concurrently in clinical practice.

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