Effect of Acetaminophen on Glutathione Levels in Rat Testis and Lung

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Glutathione (GSH) levels in rat testis and lung after oral administration (3 g/kg) of acetaminophen (APAP) were studied. At the administered dose APAP is present in each organ and influences the GSH levels. APAP value of 114 μg/g was obtained in testis at 6 hr (peak time); in the lung the C_{max} was 92 μ/g at 8 hr and this value lasted several hours longer than that in testis. GSH levels are also affected differently in the organs studied after APAP administration; the lungs seem to be the primary organ undergoing the depleting action of APAP. This process could not only cause toxicity, but also predispose those organs to the action of toxic compounds responsible for specific pathologies.—Environ Health Perspect Vol 102(Suppl 9):63–64 (1994)

Key words: acetaminophen, glutathione, testis, lung

Introduction

The drug acetaminophen (APAP) has become widely studied because of the specificity in causing serious liver injury after overdose in humans; it remains one of the most frequently chosen drugs in suicide and in suicide attempts (*1*). Occasionally, injury is also noted in people taking only recommended therapeutic doses of the drug. Following therapeutic doses, APAP is primarily eliminated as a nontoxic conjugate of glucuronic acid and sulfate. In a minor pathway, APAP is converted in a purported *N*-acetyliminoquinone (*2–3*), which is normally detoxified by conjugation (*4,6*) with hepatic glutathione (GSH). Following an overdose of APAP, the capacity for its removal by hepatic conjugation with sulfate or glucuronide is exceeded and the extent of formation of the toxic metabolite is increased (*6*). Mechanistic studies largely in animals show that P450 cytochromes convert APAP into a reactive metabolite, *N*-acetyl-*p*-benzoquinoneimine (NAPQI) that indiscriminately arylates macromolecules throughout the cell. This irreversible binding of APAP metabolite closely parallels the degree of the subsequent tissue damage and thus has come to be viewed as the initiating event in a complex process that can culminate in cell death (*7*). NAPQI is a highly reactive metabolite that combines directly with GSH and appears to be responsible for initiating liver damage, although research continues to establish the exact nature and formation mechanism of the toxic species (*8*). Very little is known about APAP distribution in other organs and its relationship with GSH contained there. Having this goal, we measured GSH content and APAP concentration in testis and lungs of rats after oral administration. It must be pointed out that none of the 94 rats, which were observed for 2 weeks, died after the administration of the above dose.

Materials and Methods

One hundred thirty-four male albino rats (Wistar strain), weighing 170 to 200 g, maintained for 2 weeks on balanced diet (Mil, Morini, S. Polo d'Enza, Italy) with free access to water and kept under a standardized temperature of about 2°C and divided in six groups each with its control group, were used. The treated animals received orally APAP suspended in arabic gum (2% in distillated water) at the dose of 3 g/kg; the control group received vehicle only. At 4, 6, 8, 12, 192, and 336 hr after the drug administration, but at the same hour each day (16:00), the rats were killed by decapitation and exsanguinated (*9*). Immediately lung and testis were removed, weighed, washed in saline, and homogenized properly in phosphate-EDTA buffer, pH 7.4. One ml of 25% trichloroacetic acid was added to 1 ml of the homogenate and then centrifuged at 2000g for 10 min at 0° C.

In the supernatants GSH levels were determined by the method of Tietze and expressed as nmole/mg of protein (*10*). The protein concentration was determined by the method of Lowry (*11*). All the procedures were performed at 0°C. The differences between GSH means were evaluated by the Student's two-tailed *t*-test. No statistical differences were observed among the control groups; thus, they were collected in a single organ pool.

In the homogenate of testis and lungs, APAP was determined by HPLC method (*12*).

Results and Discussion

In Table 1 the maximum APAP concentration (μg/g tissue) in lungs and testis and

Table 1. Maximum concentration and peak time in rat testis and lung after APAP administration (3 g/kg os).

Organ	C_{max}, μg/g tissue	Peak time hr
Testis	114	6
Lung	92	8

Table 2. APAP concentration as percent of C_{max} (100%) in rat testis and lung after APAP oral administration (3 g/kg).

Organ	Time, hr								
	0	2	4	6	8	12	192	336	
Testis	0	4	6	100	37	28	0	0	
Lung			0	50	84	100	95	0	0

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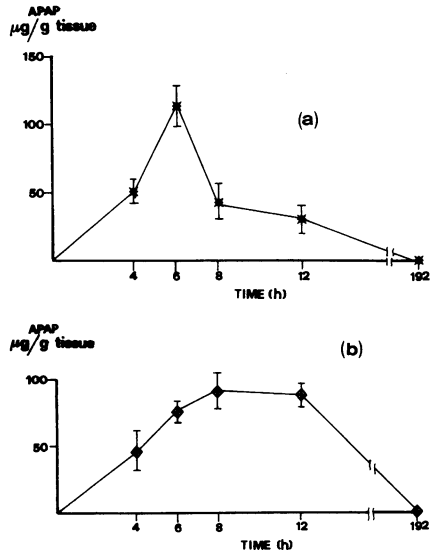

Figure 1. Kinetics of APAP in rat testis (*a*) and lung (*b*) after oral administration (3 g/kg). Each point represents the mean value of five animals with standard deviation.

the peak time are reported. Table 2 shows the APAP concentration as percent C_{max} (100%). Figure 1 shows the time-course of APAP. Figure 2 reports the GSH levels variations in two studied organs of rats after oral APAP administration.

It is evident that, at the administered dose, APAP is present in each organ in different amounts and with various peak times. From the obtained results one can say that APAP administration on GSH levels influences these levels according to the organ considered. The lung appears to be the organ that primarily evidences the depleting action of APAP. Our data demonstrate that APAP at the studied dose exerts not only a specific toxicity, but can also predispose those organs to the action of toxic agents and thus indirectly can be the cause of specific pathologies.

The experiments presented here suggest that APAP (and NAPQI) can be added to the list of compounds which can disrupt Ca^{2+} homeostasis and that this may be a common pathway of cell toxicity for agents which affect the redox status of cells, as seen previously (*13*).

It is obvious that depletion of GSH by NAPQI would allow a greater proportion of the metabolite to interact with protein thiol groups. Jollow et al. (*5*) were the first to observe the association between the extent of covalent binding of APAP metabolites to protein and the degree of liver necrosis. The binding of NAPQI to cysteinyl groups of proteins leads to loss of available protein thiols, a feature of NAPQI toxicity observed by Moore et al (*14*). NAPQI can directly or indirectly oxidize NADPH and, concomitantly, deplete GSH by conjugation and/or oxidation. Mitochondria immediately release their sequestered Ca^{2+} upon exposure to NAPQI. There are several mechanisms which might account for this effect; NAPQI could have a direct protonophoric action and/or precipitate Ca^{2+} release by affecting the redox status of mitochondrial thiols. The oxidation of pyridine nucleotides could also account for this release, although significant NADPH oxidation occurred after Ca^{2+} release was initiated. Nevertheless, sustained or secondary mitochondrial Ca^{2+} efflux is probably caused by NAPQI-induced oxidation of pyridine nucleotides.

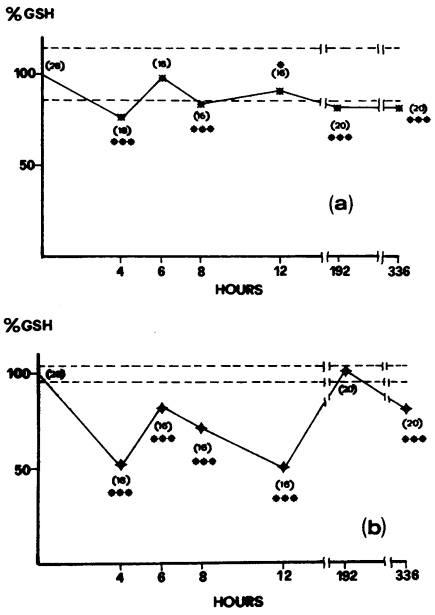

Figure 2. Time course of GSH levels after APAP oral administration (3 g/kg) in (*a*) rat testis and (*b*) lung. The control value is equal to 100. The dotted lines represent in every picture the upper and the lower limits (standard deviation of the control). The number of animals is reported in parentheses. ***p<0.001.

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