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 was obtained in testis at 6 hr (peak time); in the lung the C\textsubscript{max} was 92 µg 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-benzoquinoniminine (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 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 distilled 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

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

| Organ | C\textsubscript{max} µg/g tissue | Peak time hr |
|-------|-------------------------------|--------------|
| Testis| 114                           | 6            |
| Lung  | 92                            | 8            |

Table 2. APAP concentration as percent of C\textsubscript{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  | 8    | 37   | 28   | 0    |
| Lung  |          | 0    | 4    | 6    | 50   | 84   | 100  | 95   | 0    |

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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 predispone 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 Ca²⁺ 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 Ca²⁺ upon exposure to NAPQI. There are several mechanisms which might account for this effect; NAPQI could have a direct prothonophoric action and/or precipitate Ca²⁺ 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 Ca²⁺ release was initiated. Nevertheless, sustained or secondary mitochondrial Ca²⁺ efflux is probably caused by NAPQI-induced oxidation of pyridine nucleotides.

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