Acetaminophen Elimination Half-Life in Humans Is Unaffected by Short-Term Consumption of Sulfur Amino Acid-Free Diet

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ABSTRACT

Sulfation and glutathione (GSH) conjugation are important pathways for elimination of acetaminophen (APAP). Previous studies in rodents show that limitation of dietary sulfur amino acids (SAAs) reduces biosynthesis of 3'-phosphoadenosine-5'-phosphosulfate, the precursor for sulfation, and GSH, the precursor for the mercapturic acid pathway. The amount of SAA needed for the metabolism of two doses of APAP is equivalent to 62% of the recommended dietary allowance (RDA) for SAA in humans. A decrease in the activity of these metabolic pathways could lead to decreased elimination of the reactive metabolite of APAP and possibly affect risk of APAP use. To determine whether intake of a SAA-deficient diet alters APAP metabolism, a pilot clinical study with a double-blind, cross-over design was performed. Subjects received the RDA for SAA for 3 days for equilibration. After admission to the clinical research unit, subjects were given a chemically defined diet with 100 or 0% of the RDA for SAA for 2 days. On day 3, two doses of APAP (15 mg/kg) or placebo were given successively within a 6-h interval. Plasma samples were collected at baseline and hourly for 12 h, and two 6-h urine aliquots were collected during this time course. The data show that SAA limitation 1) did not change the pattern of APAP metabolites in plasma or urine and 2) did not alter APAP pharmacokinetics. Thus, the results show that 2 days of diet completely devoid of SAA have no effect on APAP metabolism or disposition in healthy humans.

Acetaminophen (APAP) is one of the most widely used antipyretic and analgesic drugs in the world. After therapeutic doses, approximately 3% APAP is excreted in the urine unchanged (Brodie and Axelrod, 1948). Approximately 90% of APAP is metabolized by way of conjugation reactions where two-thirds is metabolized through glucuronidation (Johnson et al., 1989). The remaining 5 to 9% is converted by human cytochrome P450 2E1 to its reactive intermediate, N-acetyl-p-benzoquinonimine (Dahlin et al., 1984). In the presence of glutathione (GSH), this metabolite is rapidly detoxified and excreted in the urine as GSH, cysteine (Cys), and mercapturic acid conjugates (Mitchell et al., 1974; Andrews et al., 1976).

Sulfur amino acids (SAAs), methionine (Met) and Cys, are required for drug metabolism through the use of Cys for biosynthesis of sulfate and GSH (Jones et al., 1995; McCarver and Hines, 2002). Previous studies of SAA availability and APAP metabolism showed that prolonged dietary deficiency of Cys and Met significantly lengthened the half-life \([t_{1/2}]\) of a low dose of APAP in rodents (Price and Jollow, 1989). The markedly slower elimination of APAP in SAA-deficient animals was caused primarily by a depression of sulfate conjugation. Short-term dietary deficiency of Cys and sulfate slowed the elimination of APAP from the blood and significantly decreased the urinary excretion of the APAP-sulfate conjugate (Gregus et al., 1994). Moreover, rodents with diet-induced SAA deficiency converted more APAP into its toxic metabolite, as suggested by increased excretion of APAP-Cys and APAP-mercapturate conjugates (Gregus et al., 1994). Taken together, these studies suggest that nutritional deficiency of SAA can affect the individual pathways of APAP metabolism by impairment of sulfate conjugation. This impairment could potentially enhance the susceptibility to APAP-induced liver injury.

Although the mean SAA intake in Americans is considerably higher than the recommended daily allowance (RDA) of 1.9 g/day (Food and Nutrition Board, 2005), individual SAA
intake is variable and can range from <0.3 to >5 g per day (Flagg et al., 1994). Considering that a molar equivalent of approximately 0.2 g of Cys is used for metabolism of a maximal therapeutic dose of 1 g of APAP, four doses per day would consume 0.8 g, or more than half of the RDA. Because of variation of intake in the population, the total equivalents of SAA needed for metabolism of the maximum daily dose of APAP can exceed individual intake of SAA.

The present study was designed to determine whether short-term SAA insufficiency alters APAP metabolism in healthy individuals. The experiment was designed with two study periods in which each individual was equilibrated to either the RDA or 0% of the RDA for SAA before APAP administration in two successive doses of 15 mg/kg, 6 h apart. Young healthy individuals were studied under conditions where there are no known risks for either APAP use or short-term SAA insufficiency. The specific hypothesis was that 2 days of a SAA-free diet would cause changes in the metabolic pattern and pharmacokinetics of APAP in the plasma and urine.

Materials and Methods

Materials. Authentic standards of APAP metabolites (APAP-glucuronide, APAP-sulfate, APAP-Cys, APAP-GSH, and APAP-mercapurate) were a generous gift from Dr. José Manautou (University of Connecticut, Storrs, CT). Methanol and acetic acid were high-performance liquid chromatography (HPLC) grade.

Human Subjects. This study was reviewed and approved by the Emory Investigational Review Board. The study was designed to determine the effect of 2 days of SAA-deficient diet on APAP metabolism as part of a study that evaluated the effect of APAP on plasma cysteine and glutathione pools. The latter is described along with experimental details in an accompanying article (Mannery et al., 2010). In brief, 12 healthy volunteers (18–40 years old) with a body mass index < 27, no acute and/or chronic illness, and no current smoking history were studied under two different conditions with APAP (two doses; 15 mg/kg) and SAA intake (0 or 100% of RDA) (Table 1). Before each inpatient study period, subjects were given an equilibration diet for 3 days, admitted into the Emory General Clinical Research Center, and given a chemically modified diet containing either 100 or 0% SAA before APAP (two doses; 15 mg/kg) and SAA intake (0 or 100% of RDA) before APAP administration (Table 1). Before each inpatient study period, subjects were given an equilibration diet for 3 days, admitted into the Emory General Clinical Research Center, and given a chemically modified diet containing either 100 or 0% SAA before APAP (two doses; 15 mg/kg) and SAA intake (0 or 100% of RDA) before APAP administration (Table 1).

Experimental design

Plasma samples were collected hourly for 12 h following administration of APAP (15 mg/kg) or placebo at 8:15 AM on day 6. Study periods described above were randomized for order and conducted at least 1 week apart.

TABLE 1

<table>
<thead>
<tr>
<th>Study Period</th>
<th>Days 1–3, Outpatient Equilibration</th>
<th>Days 4–5, Inpatient ± SAA</th>
<th>Day 6, Inpatient ± APAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 Days with 100% RDA for SAA</td>
<td>2 Days in GCRC with 100% RDA for SAA</td>
<td>Two sequential doses of APAP</td>
</tr>
<tr>
<td>2</td>
<td>3 Days with 100% RDA for SAA</td>
<td>2 Days in GCRC with SAA-free diet</td>
<td>Two sequential doses of APAP</td>
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</tbody>
</table>
Limitation of SAA did not alter the pattern of APAP-glucuronide concentration in the plasma (Fig. 1B). Despite a significant increase in APAP-glucuronide at the 7:15 PM time point, AUC data confirm that SAA limitation had no overall effect on plasma APAP-glucuronide concentration (Fig. 2A). Similarly, SAA insufficiency did not alter the pattern of appearance or concentration of plasma APAP-sulfate (Figs. 1C and 2B). The pattern of appearance or concentration of APAP-Cys, APAP-GSH, and APAP-mercapturate were not significantly altered by SAA limitation (Figs. 1D and 2B). However, it should be noted that there was a relatively large variation among individuals for the time course data, so this conclusion is based largely on the AUC data (Fig. 2B).

APAP Pharmacokinetics Were Not Affected by SAA Insufficiency. To determine whether SAA limitation altered APAP pharmacokinetics, the volume of distribution ($V_d$), $t\text{1/2}$, clearance (CL), and rate of elimination were calculated after the first dose of APAP. The results showed that the $t\text{1/2}$ for APAP under conditions of SAA insufficiency was not significantly different from the $t\text{1/2}$ under control conditions (Table 2). The $V_d$ was also similar to control values, indicating that the total aqueous space available for APAP distribution was not affected by SAA insufficiency (Table 2). There was no significant effect of SAA limitation on CL or AUC for APAP, which indicates that SAA insufficiency had no effect on the elimination of APAP (Table 2).

Urinary Excretion of APAP Metabolites Was Not Affected by SAA Limitation. Excretion of APAP in the urine was not affected by SAA limitation (Fig. 3A). Although the concentration of APAP excreted into the urine was higher during the 2:00 PM to 8:00 PM period, this increase was not significant (Fig. 3A). SAA insufficiency did not cause significant changes in the excretion of the glucuronide conjugate (Fig. 3B) or the sulfate conjugate (Fig. 3C) in the urine. SAA limitation appeared to cause an increase in the concentration of APAP-Cys, APAP-GSH, and APAP-mercapturic acid conjugates excreted in the urine during the 2:00 PM to 8:00 PM period; however, the difference was not significant overall (Fig. 3C). Expression of APAP and metabolites as a percentage of total urinary recovery also showed no effect of SAA insufficiency on APAP metabolism (Fig. 4).

Discussion

The present research was a component of a 2 x 2 factorial human study to determine whether APAP alters plasma Cys or GSH pools in healthy individuals with adequate or inadequate SAA intake (Mannery et al., 2010). APAP decreased plasma Cys with both 0 and 100% SAA intake, having the greatest effect after the 2nd dose of APAP with 100% SAA. These results suggest that metabolism adapts to insufficient SAA intake by mobilizing body stores, i.e., by protein and/or GSH breakdown. Consistent with this, plasma GSH was
EhCySS has any health consequences is unknown, but it is activation is responsive to changes in extracellular EhGSSG plasma EhCySS values in the SAA-replete state but not in that APAP administration resulted in the oxidation of processes (Mannery et al., 2010). It should be noted, however, that SAA limitation with SAA insufficiency (Mannery et al., 2010), one may expect that SAA insufficiency would also perturb APAP metabolism. However, the present data show that SAA limitation significantly decreased plasma GSH concentrations in humans (Ookhtens et al., 1985). Moreover, human studies using isotopic tracer methods showed that the fractional turnover of GSH is decreased in humans during SAA insufficiency. Together, the data show that APAP at normal therapeutic doses alter Cys metabolism, but that homeostatic mechanisms largely preserve both Cys and GSH pools.

Accumulating evidence suggests that the redox balance of the plasma Cys and GSH pools can be important in disease mechanisms even without major changes in concentrations of Cys and GSH per se (Moriarty-Craige and Jones, 2004; Jones and Liang, 2009). Cell surface proteins in endothelial cells contain Cys residues that undergo redox changes in response to a more oxidized EhCys/GSSG (Go and Jones, 2005), and platelet activation is responsive to changes in extracellular EhCys/GSSG (Essex and Li, 2003). The SAA insufficiency caused a more oxidized plasma Cys/CySS redox potential value (Eh Cys) but had no effect on plasma GSH/GSSG redox potential value (Eh GSSG) (Mannery et al., 2010). Whether the effect on EhCys has any health consequences is unknown, but it is worthy of study because the magnitude of effect is similar to changes in EhCys that have been associated with disease processes (Mannery et al., 2010). It should be noted, however, that APAP administration resulted in the oxidation of plasma EhCys values in the SAA-replete state but not in the SAA-insufficient state, suggesting that adaptive responses to SAA insufficiency probably function to protect against adverse effects.

Because the therapeutic, nontoxic doses of APAP significantly decreased plasma GSH concentrations in humans with SAA insufficiency (Mannery et al., 2010), one may expect that SAA insufficiency would also perturb APAP metabolism. However, the present data show that SAA limitation had no effect on the pattern of APAP metabolite concentrations in the plasma (Fig. 1) or urine (Fig. 3). The results are consistent with the interpretation that short-term SAA insufficiency does not compromise APAP metabolism. Pharmacokinetic parameters including half-life, volume of distribution, elimination rate, and CL rate were unaffected by consumption of a SAA-insufficient diet for 2 days (Table 2). Thus, even though APAP affects plasma GSH under SAA-insufficient conditions, the data provide no evidence that APAP metabolism is altered or that risk of adverse effects would be increased by 2 days of SAA insufficiency.

These results are consistent with previous findings concerning APAP metabolism. Glucuronidation is the major pathway for APAP metabolism and is regulated independently of the other pathways. Glucuronidation can be limited by a relative insufficiency of glucose, hypoxia, and alcohol consumption, but the pathway is not known to be influenced by SAA intake. Sulfation is the second most important pathway and, in the short term, is resistant to SAA insufficiency because of the large sulfate pool in the body. In the present study, no measurements of sulfate were obtained, so it is not known whether the SAA insufficiency caused a significant decrease in sulfate availability. However, the effects on Cys and GSH were modest so that one can anticipate that adaptive changes protecting these pools also protected the sulfate pools. The lack of effect on the mercapturate pathway for APAP metabolism is also consistent with known characteristics that are largely determined by the extent of bioactivation of APAP (Mitchell et al., 1973).

The results of the present study in humans with 2 days of SAA-free food and APAP at 15 mg/kg are different from those obtained in rats given 75 mg/kg APAP after 3 days with Met but lacking inorganic sulfate or Cys (Gregus et al., 1994). The latter showed that APAP metabolism and pharmacokinetic properties were altered because of the reduced capacity of rats to sulfate APAP. The decrease in sulfation was not...
compensated by an increase in glucuronidation (Gregus et al., 1994). Instead, the decrease in sulfation resulted in increased conversion of APAP into its toxic metabolite, as suggested by increased excretion of APAP-Cys, APAP-GSH, and APAP-mercapturic acid in the urine. These findings indicate that an SAA-deficient diet causes a decrease in detoxification by sulfation and an increase in toxicity by APAP, which could be more severe after repeated doses of APAP. The current study shows that two 15 mg/kg doses of APAP after 2 days of SAA-free food in humans do not provide evidence for a corresponding shift in metabolism. It must be pointed out, however, that the present study was designed with a modest period of SAA insufficiency and only two doses of APAP to minimize risk of toxicity.

The present study does not completely exclude the effects of SAA insufficiency on APAP toxicity in select subpopulations. The participants in the present study were young, had no known acute or chronic disease, did not smoke, and did not
abuse alcohol. Consequently, extrapolation of these present findings to at-risk populations is inappropriate. Similarly, individuals with malabsorption or prolonged periods of inadequate SAA intake may exhibit different responses.

In conclusion, the present research shows that APAP pharmacokinetics and distribution of metabolites are not affected by SAA deficiency in young, healthy adults. Although there are many unexplained cases of human toxicity after administration of therapeutic doses of APAP, results of the current study indicate that variation in short-term SAA availability is not likely to be a major factor.

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References


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