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Report

Sulfate Homeostasis. II. Influence of Chronic Aspirin Administration on Inorganic Sulfate in Humans

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The purpose of the present investigation was to determine the effect of chronic aspirin administration on the serum concentration and renal clearance of inorganic sulfate in healthy volunteers. In a randomized crossover study, eight male subjects received either no treatment or 975 mg of enteric-coated aspirin three times daily for 8 days. Blood and urine samples were collected on the eighth day over a 7-hr period. Midpoint salicylic acid concentrations in serum varied between 55 and 182 μg/ml (mean concentration of 109 μg/ml). Serum inorganic sulfate concentrations demonstrated a small but significant decrease on the eighth day of aspirin administration but there was no apparent change in the renal clearance of sulfate. There were significant correlations between the renal clearances, urinary excretion rates, and serum concentrations of creatinine and sulfate, reflecting the dependence of sulfate homeostasis on renal function. The serum concentration and renal clearance of creatinine, sodium, potassium, calcium, magnesium, and phosphorus were unaffected by aspirin treatment.

KEY WORDS: sulfate; aspirin; electrolytes; renal clearance.

INTRODUCTION

Inorganic sulfate is required for the biotransformation of exogenous and endogenous substrates by sulfate conjugation, a physiologic process with important biosynthetic, pharmacologic, as well as detoxification functions. For example, this metabolic pathway is necessary for the formation of sulfated glycosaminoglycans and sulfatides, structural components of membranes (1,2), and for the biologic activity of heparin, heparan sulfate, dermatan sulfate, gastrin, and cholecystokinin (3–5). Additionally, sulfoconjugation represents a detoxification pathway for a wide range of therapeutically important classes of drugs including certain steroids, analgesics, anti-inflammatory agents, adrenergic stimulants, and blockers, as well as endogenous substrates such as catecholamines and bile acids (6).

Homeostasis of inorganic sulfate is maintained predominantly by capacity-limited renal tubular reabsorption (7,8). Acute administration of salicylic acid to rats increases the renal clearance of inorganic sulfate, which results in a decrease in the plasma concentration of this anion (9). Salicylates can decrease the sulfate conjugation of drugs (10,11) and inhibit ^{35}S-sulfate incorporation into sulfated glycosaminoglycans *in vivo* (12,13), an effect which in animal studies is due, at least in part, to the salicylate-induced decrease in plasma inorganic sulfate concentrations (14). This salicylate-induced inhibition of sulfated glycosaminoglycan synthesis may be responsible for the increased perinatal mortality and decreased intrauterine growth associated with aspirin administration during pregnancy (15,16).

In humans, the administration of a single dose of 975 mg of aspirin (approximately 0.08 mmol/kg) (17) or 0.25 mmol/kg of aspirin or salicylic acid (18) has no effect on sulfate homeostasis. However, the influence of chronic high-dose aspirin on inorganic sulfate has not been examined. Therefore the objective of the present investigation was to examine the disposition of inorganic sulfate and other electrolytes in healthy subjects receiving chronic aspirin treatment.

MATERIALS AND METHODS

Protocol. The study group consisted of eight healthy male subjects with a mean age of 25 years (range, 20–36 years) and weight of 80.9 kg (range, 68–118 kg). Any subject with known hypersensitivity or intolerance to aspirin was excluded. Renal function of all subjects was normal based on their untreated values of serum creatinine and creatinine clearance. All subjects were medication-free for 1 week prior to and during the study period and all were studied concurrently. The subjects received either no treatment or 975 mg (3 tablets) of enteric-coated aspirin (Ecotrin, Smith Kline Consumer Products, Lot 37320) three times per day (8 AM, 3 PM, 10 PM) for 8 days in a randomized crossover study design. On the eighth day of treatment, subjects collected urine over a 7-hr period (8 AM–3 PM) and blood samples were drawn at 10 AM, 11:30 AM, and 2 PM. The treated and control study intervals were separated by an 8-day period. Salicylate serum concentrations would be negligible following this 8-day washout period, based on previously reported half-life values (19). The protocol was approved by the Investiga-

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tional Review Board, State University of New York at Buffalo, and written informed consent was obtained from all subjects.

Analytical Procedures. Serum salicylate concentrations were determined by reverse-phase HPLC using a C_{18} column (Econosphere 5 μm, Alltech, Deerfield, IL) with a mobile phase of 2% acetic acid and 20% acetonitrile in 3 m*M* monobasic potassium phosphate solution, pH 3.30, at a flow rate of 1.2 ml/min, and UV detection at 280 nm. Serum samples were diluted fivefold and 100 μl of the diluted serum sample was added to 20 μl of distilled water and 50 μl of a 1 *M* perchloric acid solution containing the internal standard phenol (Allied Chemicals, Morristown, NJ). The salicylate standards were prepared in the same manner but 20-μl aliquots of the standard solutions were substituted for the distilled water. All salicylate samples were assayed on the same day and the intraday coefficient of variation for the HPLC assay at concentrations of 13.8 and 27.6 μg/ml (which correspond to the approximate range of salicylate serum concentrations in this study following the fivefold dilution of samples) were 1.1 and 0.8%, respectively. Serum and urine inorganic sulfate concentrations were determined by HPLC (20). Briefly, inorganic sulfate was separated from other ions by means of an anion-exchange column (Wescan Instruments, Inc., Santa Clara, CA), using a mobile phase of 4 m*M* potassium hydrogen phthalate (pH 4.5) at a flow rate of 1.5 ml/min, and was detected with a Wescan conductivity detector (Model 213A). The internal standard was potassium iodide. All serum or urine samples were assayed on the same day and the intraday coefficients of variation of the assay at a serum concentration of 0.35 m*M* or urine concentrations of 1.2 and 2.7 m*M* are 0.07% (n = 3), 2.0% (n = 10), and 1.6% (n = 10), respectively. Creatinine and phosphorus concentrations in serum and urine samples were determined using commercially available kits (Sigma Chemical Co., St. Louis, MO). Serum and urine concentrations of sodium, potassium, calcium, and magnesium were determined by atomic absorption spectroscopy (Model 613, Perkin Elmer, Norwalk, CT).

Data Analysis. The renal clearances of creatinine and electrolytes were calculated by dividing the urinary excretion rate by the midpoint serum concentration. The renal clearances of inorganic sulfate and creatinine were normalized on the basis of body surface area, which was estimated from body weight and height. Clearance ratios were calculated by dividing the renal clearance of inorganic sulfate by the renal clearance of creatinine. Since sulfate in serum is not bound to plasma proteins (7) and therefore is completely ultrafiltrable, the glomerular filtration rate (GFR) of inorganic sulfate was calculated as the product of serum sulfate concentration and creatinine renal clearance. The renal tubular reabsorption rate of sulfate was estimated as the difference between the renal filtration and the urinary excretion rates, assuming negligible renal tubular secretion of sulfate (8). The fraction of the filtered sulfate that was reabsorbed was calculated by dividing the reabsorption rate by the filtration rate.

Statistical Analysis. Statistical analysis of the serum concentrations and renal clearance values was by paired t tests and analysis of variation with a P value of 0.05 or less defined as significant. Linear regression analysis was performed to examine the correlation between sulfate and creatinine serum concentrations, urinary excretion rates, or clearances.

RESULTS

Chronic administration of enteric-coated aspirin in healthy subjects resulted in salicylate serum concentrations that varied over more than a threefold range at any sampling time. The interindividual coefficient of variation of the midpoint salicylate concentrations was 38.9%, with concentrations in subjects ranging from 55 to 182 μg/ml. The incidence of side effects during aspirin treatment was low, with two subjects reporting some tinnitus on the last day of therapy.

Chronic aspirin administration had no effect on renal function as assessed by creatinine clearance (Table I). Serum inorganic sulfate concentrations exhibited a small decrease at the three sampling times following aspirin administration (Fig. 1), but this decrease was statistically significant only at the 11:30 AM time point. There was no change in the urinary excretion rate or renal clearance of sulfate. The individual alterations in sulfate serum concentrations and renal clearance values are presented in Fig. 2, with the mean values given in Table I. The clearance ratio of sulfate to creatinine was 0.24 ± 0.06 (mean ± SD) in the control and 0.23 ± 0.05 in the treated subjects. The fraction of filtered sulfate that was reabsorbed was unchanged during control and treatment periods (0.72 ± 0.08 and 0.74 ± 0.05, respectively). There was no relationship between filtration rate and fraction of filtered sulfate that was reabsorbed.

There was no significant correlation between the salicylate serum concentrations and the serum concentrations or clearance values of inorganic sulfate. The normalized urinary excretion rate of sulfate was significantly related to that of creatinine and this correlation remained even if the excre-

Table I. Effect of Chronic Aspirin Treatment on the Serum Concentration and Renal Clearance of Creatinine and Several Electrolytes[a]

	Control	Treated
Creatinine		
Serum conc., mg/dl	1.05 ± 0.19	0.94 ± 0.16
Clearance, ml/min	124 ± 40	129 ± 62
Inorganic sulfate		
Serum conc., m*M*	0.37 ± 0.05	0.33 ± 0.04*
Clearance, ml/min	29.1 ± 10.6	27.9 ± 10.7
Sodium		
Serum conc., m*M*	178 ± 6	178 ± 4
Clearance, ml/min	0.83 ± 0.53	0.95 ± 0.53
Potassium		
Serum conc., m*M*	4.54 ± 0.62	4.32 ± 0.40
Clearance, ml/min	8.83 ± 3.92	12.2 ± 4.85
Magnesium		
Serum conc., m*M*	0.86 ± 0.05	0.84 ± 0.05
Clearance, ml/min	3.03 ± 0.93	2.65 ± 1.53
Calcium		
Serum conc., m*M*	2.62 ± 0.09	2.57 ± 0.15
Clearance, ml/min	0.60 ± 0.23	0.53 ± 0.28
Phosphorus		
Serum conc., mg/dl	4.10 ± 0.47	4.24 ± 0.35
Clearance, ml/min	11.3 ± 3.9	13.0 ± 5.5

[a] Results expressed as mean ± SD; n = 8.
* Significantly different from control, P = 0.05.

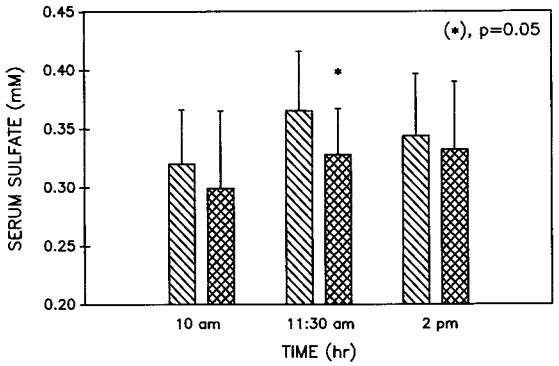

Fig. 1. Serum inorganic sulfate concentrations in eight healthy subjects determined at three times (10 AM, 11:30 AM, and 2 PM) on a control day (▨) and on the eighth day of chronic aspirin treatment (▩).

tion rates during the control ($r = 0.723$, $P < 0.05$) or treatment ($r = 0.758$, $P < 0.005$) periods alone were examined. Statistically significant correlations were also found between normalized sulfate and creatinine clearances ($r = 0.707$, $P < 0.05$, for control period; $r = 0.771$, $P < 0.05$, for treatment period; $r = 0.731$, $P < 0.002$, for data from both periods) (Fig. 3). Serum sulfate was significantly related to serum creatinine concentrations ($r = 0.574$, $P < 0.02$) for the combined data from control and treatment periods.

Aspirin administration had no significant effect on the serum concentrations and renal clearance of sodium, potassium, magnesium, calcium, or phosphorus (Table I).

DISCUSSION

Inorganic sulfate is formed in the body by the oxidation of cysteine and methionine (21,22); it can be acquired, as such, from dietary sources as well (23,24). The anion is eliminated mainly in unchanged form by urinary excretion (25,26). The renal clearance of inorganic sulfate in man is approximately 30% of the GFR under normal physiological conditions (8,17,27) and increases to a rate approximately equal to GFR when serum sulfate concentrations are increased (8), suggesting saturable reabsorption and little, if any, tubular secretion. This capacity-limited reabsorption is of primary importance in sulfate homeostasis.

The chronic administration of enteric-coated aspirin, 2925 mg per day, resulted in large intersubject variability of salicylate serum concentrations (midpoint concentrations of

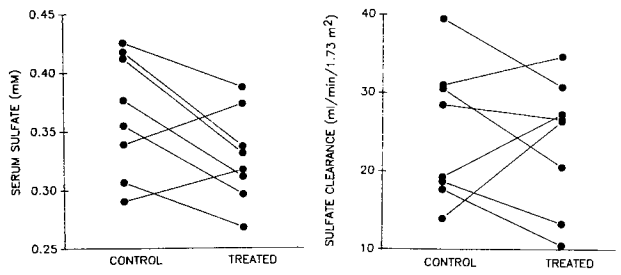

Fig. 2. Serum sulfate concentrations (11:30 AM sample) and sulfate renal clearance in eight healthy subjects during a control period and during chronic aspirin treatment.

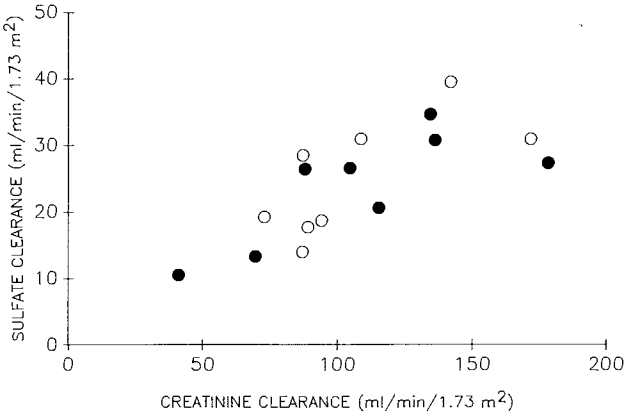

Fig. 3. Relationship between the renal clearance of inorganic sulfate and creatinine in healthy subjects during control (○) and aspirin treatment (●) periods ($r = 0.731$, $P < 0.002$). Renal clearance values were normalized to a body surface area of 1.73 m^2.

55 to 182 μg/ml) in healthy volunteers, as has been previously observed (28). Following aspirin administration there was a small but significant decrease in serum sulfate concentrations but no change in sulfate renal clearance. Sulfate renal clearance would normally be decreased in the presence of lowered serum sulfate concentrations, in order to maintain sulfate homeostasis (27,29). Since this does not occur, this may reflect a relative increase in the renal clearance of sulfate above expected values, possibly because of aspirin treatment. However, considering the small decrease in serum sulfate concentrations observed in this investigation and the variability of sulfate urinary excretion rates in humans (24), the lack of a significant alteration in sulfate renal clearance is not unexpected.

The serum concentrations and renal clearance values of inorganic sulfate determined in the present study were similar to those previously reported in humans (17,27). The mean serum sulfate concentrations varied over the 7-hr control period, possibly reflecting a circadian rhythm. A circadian variation of inorganic sulfate in humans has been observed by Meier and Schmidt–Kessen (30) but they reported higher serum concentrations in the late afternoon and evening. The significant correlations between the serum concentrations, urinary excretion rates, and renal clearance estimates for endogenous sulfate and creatinine indicate that sulfate homeostasis is highly dependent on renal function. Such behavior suggests that saturation of the reabsorption of sulfate occurs at normal physiologic serum concentrations of sulfate (8,29). Relationships between sulfate and creatinine urinary excretion rates in healthy subjects (31) and between serum concentrations and renal clearance values of creatinine and inorganic sulfate in patients with renal impairment (32; unpublished results[3]) have been previously observed. Although high-dose aspirin can alter renal function (reviewed in Ref. 33), creatinine clearance was not significantly altered following chronic aspirin treatment in the present study.

[3] Morris, M. E., Freer, J. P., and Watson, W. A. Effect of chronic naproxen or sulindac treatment on inorganic sulfate disposition in arthritic patients with renal impairment.

The findings of the present investigation differ from the results of animal studies. In rats, salicylic acid, at steady-state concentrations of 250 μg/ml, produces a 50% decrease in sulfate serum concentrations as a consequence of the pronounced increase (about 100%) in its renal clearance (9). Similar alterations in serum sulfate concentrations have been observed following the administration of a 200-mg/kg dose of sodium salicylate to mice (12). Previous studies have examined the influence of a single dose of aspirin or salicylic acid on serum sulfate concentrations in healthy human subjects and showed no alterations (17,18). Based on the results of the present study, chronic aspirin treatment also has little effect on sulfate homeostasis. However, the salicylate concentrations obtained in this study were, on the average, low compared with the therapeutic concentrations of aspirin achieved in the treatment of rheumatoid arthritis and the effects of higher concentrations, such as that obtained in the animal study (9), are unknown. In addition, aspirin was used in the clinical studies, while salicylic acid was directly administered in the animal studies. Although aspirin is rapidly hydrolyzed to salicylate in the body, with a half-life of 15 to 20 min (34), it is possible that aspirin may differ from salicylate in its effect on sulfate homeostasis. For example, salicylate but not aspirin treatment produces a diuresis and natriuresis in dogs and swine, an effect which may be related to the differing magnitude of inhibition of renal prostaglandin synthesis seen with salicylate and aspirin (35,36).

In summary, the chronic administration of aspirin caused a small decrease in serum sulfate but no change in its renal clearance. The elimination of inorganic sulfate is highly correlated with that of creatinine in healthy subjects, reflecting the dependence of sulfate homeostasis on renal function.

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