# Sulfate Homeostasis. III. Effect of Chronic Naproxen or Sulindac Treatment on Inorganic Sulfate Disposition in Arthritic Patients with Renal Impairment

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The purpose of the present investigation was to examine the influence of chronic naproxen (500 mg twice daily) or sulindac (200 mg twice daily) therapy on the disposition of inorganic sulfate in arthritic subjects with impaired renal function. Subjects were studied during a control period (after a 7-day NSAID washout) and after 14 days of treatment with either naproxen or sulindac. During the control period subjects in this investigation exhibited higher serum sulfate concentrations and lower sulfate renal clearance values than reported for younger subjects with normal renal function. Treatment with either sulindac or naproxen significantly decreased creatinine clearance. Sulindac therapy also increased the serum sulfate concentration and decreased the clearance of sulfate; a similar trend was observed after naproxen therapy but the average change was smaller and not statistically significant. There were significant correlations between the creatinine and the sulfate clearances or serum concentrations. The glomerular filtration rate of inorganic sulfate was not altered by drug treatment and there was no impairment of reabsorption. The serum concentrations and renal clearance of other electrolytes (sodium, potassium, magnesium, calcium, phosphorus) were largely unaffected. Therefore, chronic treatment with naproxen or sulindac decreases the renal clearance of endogenous sulfate in humans: this appears to be a consequence of the decrement in renal function observed in subjects with preexisting mild renal impairment.

**KEY WORDS:** sulfate; naproxen; sulindac; renal clearance; creatinine; nonsteroidal antiinflammatory drugs.

## INTRODUCTION

Inorganic sulfate is involved in the conjugation of exogenous compounds including acetaminophen, isoproterenol, and α-methyldopa and many endogenous compounds such as glycosaminoglycans, cerebrosides, steroids, and catecholamines (1). Low sulfate availability results in decreased incorporation of sulfate into both xenobiotics (2,3) and endogenous substrates (4–9). In humans, maximal sulfate incorporation into glycosaminoglycans occurs at plasma sulfate concentrations of 0.4 m*M* [approximately the physiologic plasma concentration (10,11)] and decreases at sulfate concentrations below 0.2 m*M* (6). Sulfated glycosaminoglycan synthesis is also suppressed in mice and rats when plasma sulfate concentrations fall to approximately one-half of the normal physiologic level (7–9). Administration of the sulfate-depleting drug salicylamide to pregnant rats results in decreased incorporation of sulfate into fetal skeletal glycosaminoglycans, which may represent the cause of the drug's teratogenicity (12,13).

Recently we have found that acute treatment with salicylic acid or ibuprofen in rats results in decreased serum concentrations of inorganic sulfate due to an increased renal clearance of the anion (14,15). Aspirin, salicylic acid, and ibuprofen, as well as other nonsteroidal antiinflammatory drugs (NSAIDs) including phenylbutazone, flufenamic acid, indomethacin, tolmetin, and fenoprofen, can inhibit sulfated glycosaminoglycan synthesis (16–21). The mechanism of this interaction is unknown. Although salicylates can inhibit various reactions in sulfated glycosaminoglycan synthesis *in vitro* including glucosamine-6-phosphate synthesis and uridine-5′-diphosphoglucose reactions (22,23), sulfate depletion may be an important factor *in vivo* (9). Sodium salicylate and acetaminophen inhibit sulfated glycosaminoglycan synthesis in patellar cartilage *in vivo* and this is due to a drug-induced reduction in endogenous sulfate (8,9). Therefore, although NSAIDs are widely used in the management of arthritis, these agents themselves may compromise the connective tissue metabolism of cartilage.

The objective of the present investigation was to evaluate the effects of chronic treatment with the NSAIDs, naproxen and sulindac, on inorganic sulfate homeostasis in arthritic patients with decreased prestudy creatinine clearance values. The present investigation was part of a larger study designed to evaluate the effect of naproxen of sulindac treatment on the renal function of subjects with preexisting mild to moderate renal dysfunction (24).

## MATERIALS AND METHODS

*Patient Population.* The study population consisted of male and female volunteers over 18 years of age with disease states requiring chronic NSAID therapy and with mild to moderate renal function impairment (defined as a 24-hr creatinine clearance of 35–70 ml/min during the prestudy evaluation) (Table I). The subjects were either not currently taking NSAIDs or could successfully complete a 7-day washout period. Subjects known to be hypersensitive to aspirin or other NSAIDs, as well as individuals who had peptic ulcer disease, gastrointestinal bleeding (within the last 12 months), or unstable diabetes mellitus or had been diagnosed as having cardiovascular, hematologic, hepatic, neurologic, or any other diseases in which the administration of a prostaglandin synthesis inhibitor might be contraindicated were excluded from the study. Additionally, subjects receiving diuretics, beta blockers, angiotensin converting enzyme inhibitors, or corticosteroids were excluded. Subjects who were taking other medications for conditions unrelated to the study could

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Table I. Patient Characteristics[a]

| | Naproxen treatment ($n$ = 7) | Sulindac treatment ($n$ = 8) |
|---|---|---|
| Gender | 2M, 5F | 2M, 6F |
| Age (years) | 67 ± 9 (51–79) | 58 ± 10 (46–69) |
| Weight (kg) | 70.1 ± 16.5 (41.6–90.5) | 75.2 ± 9.1 (62.2–90.0) |
| Body surface area ($m^2$) | 1.73 ± 0.22 (1.35–1.94) | 1.83 ± 0.18 (1.62–2.09) |

[a] Expressed as mean ± SD (range).

continue to administer these medications; none of the other medications taken during study are known to alter sulfate disposition. Diet was not controlled but patients were requested not to change their diet during the study period. All patients remained in the study unit during the 2 days of blood and urine collections and therefore all meals ingested during these periods were similar.

*Study Protocol.* After screening and a 7-day washout period, the subjects were admitted to the study unit for 36 hr. Urine was collected over a 24-hr period and one blood sample was drawn: the results of the analyses of these samples constituted the baseline data. This was followed by 14 days of treatment with either naproxen 500 mg twice daily or sulindac 200 mg twice daily. A randomized single blind study design was utilized, with the investigators blinded to which treatment subjects received. The subjects were readmitted to the study unit for reevaluation after 13 days of therapy and studied on the 14th day of NSAID treatment. The blood sample was obtained at approximately the same time of day (between 1 and 2 PM) in both the pre- and the postdrug evaluations. Compliance was monitored by a tablet count and found to be greater than 95%.

*Electrolyte Analysis.* Inorganic sulfate was determined by single-column anion chromatography (25), using a conductivity detector (Model 213A, Wescan Instruments, Inc., Santa Clara, CA) and an anion-exchange precolumn and analytical column (Wescan Instruments, Inc). Briefly, a mobile phase of 4 m*M* potassium hydrogen phthalate, pH 4.5, at a flow rate of 1.5 ml/min was used. The internal standard was potassium iodide. Serum and urine concentrations of sodium, potassium, calcium, and magnesium were determined by atomic absorption spectroscopy (Model 613, Perkin Elmer, Norwalk, CT). Phosphorus was analyzed by a modification of the colorimetric assay of Fiske and Subbarow (26), using a commercially available kit (Sigma Chemical Co., St. Louis, MO). Creatinine was analyzed by an alkaline picrate assay (Model 8700 Autoanalyzer, Boehringer Mannheim, Indianapolis, IN). Using this assay, the interday coefficient of variation for a serum creatinine quality control sample (1.24 mg/dl) was 12.1% ($n$ = 19). In addition, creatinine clearance values from samples ($n$ = 31) assayed on 2 separate days demonstrated a correlation coefficient of 0.81 and an average percentage mean difference of 12.8%.

*Data Analysis.* Renal clearance of creatinine and electrolytes was calculated by dividing the urinary excretion rate by the serum concentration. The renal clearance of inorganic sulfate and creatinine was normalized on the basis of body surface area, which was estimated from body weight and height. Clearance ratios are renal clearance of inorganic sulfate divided by renal clearance of creatinine. Since sulfate in serum is not bound to plasma proteins (27) and is therefore completely ultrafiltrable, the glomerular filtration rate (GFR) of inorganic sulfate was calculated as the product of serum sulfate concentration and the creatinine renal clearance. The renal tubular sulfate reabsorption rate was estimated as the difference between the renal filtration and the urinary excretion rates. 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 signed-rank tests, with a $P$ value of 0.05 or less defined as significant. Possible relationships between the serum concentrations or renal clearance values of the electrolytes and creatinine or other parameters were examined using Spearman Rank correlation tests.

## RESULTS

The prestudy creatinine clearance values in our study population initially ranged between 35 and 70 ml/min. Following the 7-day NSAID washout period, control creatinine clearance values in the subjects generally increased from the prestudy values, as has been previously seen (28), and averaged about 70 ml/min (Table II).

Naproxen therapy resulted in a statistically significant decrease in creatinine clearance (Table II). Sulindac therapy also produced a significant decline in renal function as noted by an increase in serum creatinine concentrations and a decrease in creatinine clearance values. Serum inorganic sulfate concentrations were elevated after chronic sulindac treatment and its renal clearance significantly decreased. The urinary excretion rate of sulfate was unchanged. No statistically significant changes in serum inorganic sulfate concentrations or the urinary excretion or the renal clearance of inorganic sulfate occurred following chronic naproxen therapy. The clearance ratio (sulfate/creatinine) in both patient groups was approximately 0.30 during the baseline evaluation and was not affected by naproxen or sulindac treatment (as seen by the slope of the control and treated data in Fig. 1). Furthermore, drug treatment did not significantly influence the fraction of the filtered sulfate that was reabsorbed nor the renal filtration rate of sulfate.

Evaluation of the relationship between creatinine clearance and sulfate clearance for all subjects for both baseline and treated periods demonstrated a significant correlation (Fig. 1) between these parameters. The correlation was also significant for only the subjects in the sulindac study ($r$ = 0.517, $P < 0.05$) or the naproxen study ($r$ = 0.565, $P < 0.05$). There was no correlation between creatinine clearance and serum sulfate concentration, but there was a significant correlation between serum creatinine and serum sulfate concentrations (Fig. 2). This correlation remained statistically significant if only the sulindac-treated subjects were evaluated ($r$ = 0.685, $P < 0.005$). As a consequence of the dependence of serum sulfate concentrations and sulfate renal clearance values on renal function, there was a significant negative

Table II. Effect of Chronic Naproxen or Sulindac Treatment on Renal Function and Inorganic Sulfate Disposition[a]

| | Naproxen ($n$ = 7) | | Sulindac ($n$ = 8) | |
|---|---|---|---|---|
| | Control | Treated | Control | Treated |
| Serum sulfate, m$M$ | 0.49 ± 0.06 | 0.55 ± 0.11 | 0.48 ± 0.06 | 0.64 ± 0.14* |
| Sulfate clearance, ml/min/1.73 m$^2$ | 21.0 ± 7.2 | 16.8 ± 6.2 | 19.2 ± 5.4 | 12.8 ± 2.1** |
| Serum creatinine, mg/dl | 1.0 ± 0.2 | 1.1 ± 0.2 | 1.0 ± 0.4 | 1.5 ± 0.3* |
| Creatinine clearance, ml/min/1.73 m$^2$ | 69.7 ± 14.9 | 54.3 ± 9.8* | 70.1 ± 11.9 | 49.2 ± 6.4** |
| Clearance ratio (sulfate/creatinine) | 0.30 ± 0.07 | 0.31 ± 0.12 | 0.28 ± 0.08 | 0.26 ± 0.05 |
| Fraction of filtered sulfate reabsorbed | 0.70 ± 0.07 | 0.69 ± 0.12 | 0.72 ± 0.08 | 0.74 ± 0.05 |

[a] Results expressed as mean ± SD.
* Different from control, $P < 0.05$.
** Different from control, $P < 0.01$.

correlation between serum sulfate and the normalized sulfate clearance values ($r = -0.513$, $P < 0.005$).

Chronic naproxen treatment did not produce changes in the serum concentrations or renal clearance of phosphorus, potassium, magnesium, or calcium (Table III). There was a small but statistically significant decrease in the serum concentration of sodium. Sulindac treatment resulted in a significant increase in serum phosphorus concentrations but all other electrolytes were unchanged. Serum phosphorus concentrations and phosphorus renal clearance values were not significantly related to creatinine clearance, although the phosphorus renal clearance was correlated with sulfate renal clearance ($r = 0.507$, $p < 0.005$).

## DISCUSSION

Naproxen and sulindac treatment produced significant decreases in renal function in the study subjects, as evidenced by alterations in serum creatinine and creatinine clearance values. The control creatinine clearance values in our study groups were low (about 70 ml/min) compared with average population values (29–31); the decreased creatinine clearance could be due to age-related decrements in renal function (29–31) or renal impairment due to other causes. Serum creatinine values in the control period were within the normal population range; the finding of no elevation in serum creatinine values in an elderly population, even in the presence of renal impairment, is not unexpected due to the decreased production of creatinine in elderly subjects (29–31).

Sulindac treatment caused a significant decrease in the renal clearance of inorganic sulfate which resulted in elevated serum sulfate concentrations; naproxen treatment resulted in an elevated mean serum sulfate concentration and a decreased mean sulfate clearance but the changes were not statistically significant. Alterations in sulfate disposition following sulindac treatment appear to be the consequence of the altered renal function observed following sulindac therapy. The lack of statistically significant changes in sulfate

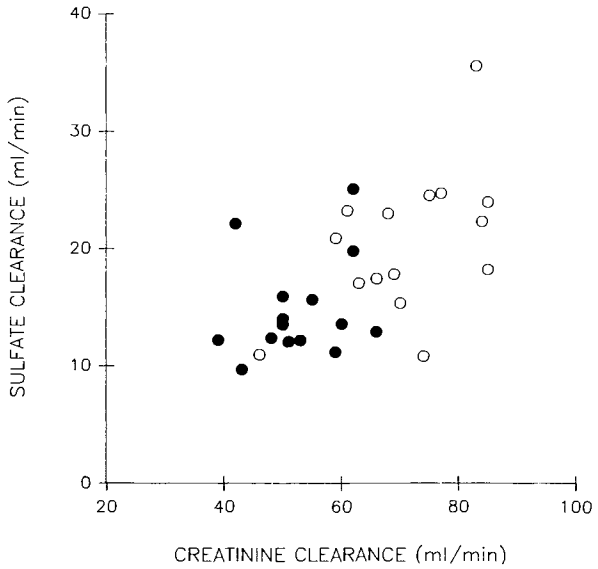


Fig. 1. Relationship between creatinine clearance and sulfate clearance for subjects during the control (○) and treated (●) periods ($r$ = 0.579, $P < 0.002$).

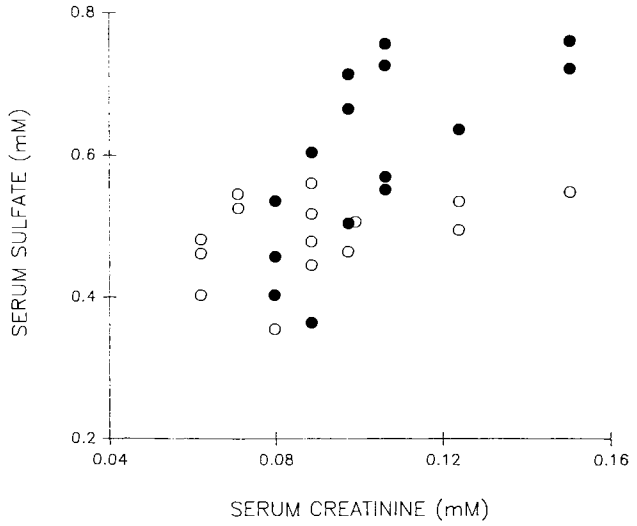


Fig. 2. Relationship between serum creatinine and serum sulfate concentrations for subjects during the control (○) and treated (●) periods ($r$ = 0.627, $P < 0.001$).

Table III. Effect of Chronic Naproxen or Sulindac Treatment on Electrolyte Disposition[a]

| | Naproxen ($n$ = 7) | | Sulindac ($n$ = 8) | |
|---|---|---|---|---|
| | Control | Treated | Control | Treated |
| Phorphorus | | | | |
| Serum conc., mg/L | 34.9 ± 4.1 | 36.4 ± 5.7 | 31.0 ± 3.6 | 35.8 ± 4.0* |
| Clearance, ml/min | 13.5 ± 5.2 | 14.0 ± 4.2 | 14.9 ± 5.4 | 14.2 ± 3.5 |
| Sodium | | | | |
| Serum conc., m$M$ | 138 ± 8 | 126 ± 9* | 138 ± 4 | 136 ± 7 |
| Clearance, ml/min | 0.89 ± 0.44 | 0.91 ± 0.52 | 1.22 ± 0.38 | 1.03 ± 0.24 |
| Potassium | | | | |
| Serum conc., m$M$ | 4.47 ± 0.41 | 4.50 ± 0.42 | 4.38 ± 0.21 | 4.43 ± 0.22 |
| Clearance, ml/min | 22.9 ± 8.9 | 21.5 ± 8.3 | 25.3 ± 6.6 | 22.1 ± 4.4 |
| Magnesium | | | | |
| Serum conc., m$M$ | 0.82 ± 0.06 | 0.81 ± 0.07 | 0.82 ± 0.04 | 0.81 ± 0.04 |
| Clearance, ml/min | 3.77 ± 1.34 | 3.75 ± 0.97 | 2.80 ± 1.08 | 3.09 ± 1.03 |
| Calcium | | | | |
| Serum conc., m$M$ | 2.37 ± 0.06 | 2.35 ± 0.13 | 2.32 ± 0.11 | 2.39 ± 0.05 |
| Clearance, ml/min | 0.78 ± 0.31 | 0.66 ± 0.17 | 0.50 ± 0.14 | 0.56 ± 0.14 |

[a] Results expressed as mean ± SD.
* Different from control, $P < 0.05$.

disposition after naproxen therapy may be explained by the smaller alterations in renal function observed after naproxen treatment, compared with that seen following sulindac treatment. Endogenous inorganic sulfate is eliminated almost entirely by renal excretion (32,33); its elimination involves glomerular filtration and capacity-limited tubular reabsorption (10,32). Sulfate retention in the presence of renal failure has been known for many years (34–36). Freeman and Richards (37) have reported a significant correlation between serum creatinine and serum sulfate concentrations in adults; however, they were studying patients with end-stage renal disease. In addition, Hänze (36) observed a progressive increase in serum sulfate concentrations in subjects with creatinine clearance values below 70 ml/min. In our patient population, hypersulfatemia was present during our baseline evaluation of these subjects [normal serum sulfate is about 0.3 to 0.4 m$M$ (10,11)], although our subjects' creatinine clearance values averaged 70 ml/min. Additionally, the baseline sulfate renal clearance values were lower than seen in a population with normal renal function (average sulfate clearance of 31.7 ml/min/1.73 $m^2$) (11). These changes may reflect the mild renal dysfunction present in our study population due to age-related or other reasons; in addition, it is possible that these changes in sulfate serum concentrations may reflect, at least in part, an influence of age, which may be unrelated to renal function.

Inorganic sulfate clearance is about 30% of creatinine clearance in normal adult subjects (10,11,37). This is consistent with our results, which demonstrated a sulfate-to-creatinine clearance ratio of about 0.3 which was not changed following NSAID therapy. Although creatinine clearance decreased following sulindac treatment, serum sulfate concentrations increased so that the sulfate filtration rate was unchanged. At a similar sulfate filtration rate, the fraction reabsorbed was unchanged, suggesting no impairment in the capacity-limited reabsorption of sulfate with the decreasing creatinine clearance values in our study population.

Other studies have demonstrated alterations in renal function following NSAID therapy in patients with preexisting renal impairment (reviewed in Ref. 38). Although creatinine clearance is extensively used clinically to estimate GFR, creatinine clearance tends to overestimate the true GFR or inulin clearance in patients with renal dysfunction (39,40), although not in subjects with age-related renal impairment (29). A portion of the creatinine cleared renally undergoes tubular secretion; with a decline in GFR due to pathological reasons, there is an increase in the proportion of the creatinine clearance that results from secretory mechanisms (39,40). This leads to a greater disparity between the creatinine clearance value and the true GFR in patients with renal dysfunction. Although it is possible that naproxen and sulindac therapy could decrease creatinine clearance by inhibiting the active secretion of creatinine, sulindac has been shown to have no acute effect on creatinine clearance in patients with chronic renal failure (41). In addition, Toto *et al.* (42) have demonstrated that ketoprofen, which is structurally similar to naproxen, causes a significant decrease in inulin clearance in patients with renal insufficiency, suggesting that ketoprofen therapy alters renal function in these patients. Inasmuch as inorganic sulfate is not cleared renally by tubular secretion, naproxen and sulindac would not decrease the clearance of sulfate by inhibition of the renal tubular secretion of this anion. Therefore, a direct effect of these drugs to decrease sulfate renal transport does not appear likely.

The results of this clinical study involving the chronic administration of naproxen and sulindac contrast with the findings in animal studies where sulfate homeostasis was examined following the acute administration of two other NSAIDs, salicylic acid and ibuprofen. Salicylic acid at a mean steady-state serum concentration of 250 μg/ml caused a 50% decrease in the serum concentrations of inorganic sulfate as a result of a doubling of its renal clearance (14). Ibuprofen, at an average serum concentration of 58 μg/ml, also increased the renal clearance of inorganic sulfate, al-

though its effect was less pronounced than that seen with salicylic acid (15). However, neither drug had any effect on renal function following their acute administration in these animal studies. Therefore, either chronic therapy of naproxen and sulindac does not increase the renal clearance of endogenous sulfate in humans in a manner similar to that of salicylic acid and ibuprofen in animals or such an effect was masked by the opposing changes produced by the decrement in renal function observed in this study. Thus, the conclusions of this investigation should currently be restricted to patients with mild renal impairment for whom decreases in renal function occur following chronic naproxen, sulindac, or other NSAID therapy.

Little change was seen in the other electrolytes examined in this investigation. Although renal prostaglandin synthesis inhibition can result in reductions in the urinary excretion of sodium, magnesium, and calcium (43–45), no changes in the renal clearance of these electrolytes were found following naproxen or sulindac therapy. A small but statistically significant elevation in serum inorganic phosphorus occurred following sulindac treatment. A significant correlation between serum sulfate and serum phosphorus concentrations in subjects with renal disease has previously been noted (36), but no such relationship was found in this investigation. The renal clearances of these two electrolytes, though, were significantly correlated in this study; since the phosphorus clearance was not significantly related to that of creatinine, this correlation may not be a consequence of the alterations in renal function observed in this investigation.

In summary, endogenous serum sulfate concentrations were significantly elevated, and sulfate renal clearance was significantly decreased following sulindac therapy. These changes in inorganic sulfate were related to the altered creatinine clearance values seen after sulindac treatment and were much more pronounced than any alterations observed with other electrolytes. The clinical consequences of hypersulfatemia are largely unknown, although the retention of inorganic sulfate does not appear to produce any acute toxicity (10,37).

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