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LOWERING OF PLASMA CONCENTRATIONS OF (+)-6-METHOXY-α-METHYL-2-NAPHTHALENEACETIC ACID (NAPROXEN) BY ASPIRIN IN RATS

MELVIN D. CHAPLIN

Department of Experimental Pharmacology, Syntex Research, Palo Alto, Calif. 94304, U.S.A.

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Abstract—Aspirin is shown to be capable of depressing plasma concentrations of naproxen when the two drugs are given concurrently to rats. However, naproxen, in the dose employed, did not alter plasma concentrations of total salicylate when aspirin was administered simultaneously with naproxen. The effect of aspirin in lowering naproxen plasma levels occurs regardless of the route of administration of the two drugs, but is dose dependent. Binding studies *in vitro* indicate that both aspirin and salicylic acid, at concentrations obtainable *in vivo*, displace naproxen from plasma proteins of the rat. Likewise, 5 min after intravenous administration of aspirin and ^{3}H-naproxen, the per cent of naproxen not bound to plasma proteins is found to be significantly increased over that observed when naproxen is administered alone at the same dose. It is postulated that aspirin lowers plasma concentrations of naproxen by displacing naproxen from plasma proteins, thus making available more naproxen for metabolic transformation or excretion, or both.

NAPROXEN* is a potent anti-inflammatory, analgetic and antipyretic agent.[1] The absorption, distribution and excretion of naproxen in various animal species and man have been reported.[2] In the mature male rat, the plasma half-life of naproxen is approximately 4 hr and about 95 per cent of the drug is excreted in the urine.

Reports in the literature indicate an apparent interaction between aspirin and other nonsteroidal anti-inflammatory agents.[3–5] Likewise, in preliminary studies using the rat carrageen-induced foot edema assay, co-administration of aspirin and naproxen, in some instances, did not cause a greater inhibition of foot edema than that seen with the same dose of naproxen alone.† Since the proposed clinical use of naproxen is for anti-inflammatory and analgesic purposes, aspirin will likely be administered with naproxen in many subjects. Accordingly, as a guide for investigating a possibly clinically important interaction, we have conducted studies in the rat to determine if any biochemical interaction occurs between aspirin and naproxen.

METHODS AND MATERIALS

Male, Sprague–Dawley-derived rats (Simonsen Laboratories, Inc., Calif.), body weight 180–220 g, were employed. Animals were acclimatized to laboratory conditions for at least 4 days before use. Food and water were allowed *ad lib*. After administration

* The correct *Chemical Abstracts* index name for naproxen is (+)-6-methoxy-α-methyl-2-naphthaleneacetic acid, but the compound was identified previously in the chemical and biological literature as *d*-2-(6′-methoxy-2′-napthyl) propionic acid.

† Personal communication from W. H. Rooks, II, Syntex Research.

of radioactive substances, animals were maintained individually in metabolic cages designed for separate collection of urine and feces (Scientific Products, Evanston, Ill.).

Ring-tritiated naproxen* was prepared by the Javorsky and Gorin[6] exchange technique utilizing a boron trifluoride ^{3}H-phosphoric acid complex. Details of the radiochemical synthesis will be published elsewhere,[7] but proof of the structure of the material used in these experiments was accomplished by mass spectrometry and nuclear magnetic resonance (NMR) spectra of the deuterated analog. Radiochemical purity was greater than 99·5 per cent; the specific activity was 610 mCi/m-mole. Aspirin-carboxyl-^{14}C, spec. act. 1·9 mCi/m-mole, was purchased from New England Nuclear Corp., Boston, Mass., and was used without further purification.

Drugs were always administered in solution. Naproxen was dissolved in phosphate buffer at pH 9·0 and aspirin was dissolved in warm water immediately before use.

Blood was collected in heparinized syringes by cardiac puncture from rats that were lightly anesthetized with ether, and plasma was obtained by centrifugation. Urine and fecal samples were collected at designated times and stored frozen.

For determination of radioactivity, plasma and urine samples were mixed with 15 ml of the scintillation fluid previously described[2] and assayed by liquid scintillation spectrometry (model 3320 Tri-Carb scintillation spectrometer, Packard Instrument Co., Downers Grove, Ill.). Feces were dried, weighed and ground to a fine powder and 50- to 100-mg aliquots were combusted to tritiated water by a sample oxidizer (model 300 Tri-Carb automatic sample oxidizer, Packard Instrument Co., Downers Grove, Ill.). All samples were corrected for quenching. The technique used for determination of naproxen by gas–liquid chromatography was briefly described previously[2] and will be described in detail elsewhere.†

Binding of naproxen to plasma proteins was studied by an ultrafiltration technique used extensively by others,[8–12] with minor modifications. Incubation of drugs with undiluted plasma was performed at 37°; drugs were added in the smallest possible volume, usually not more than 25 μl. Dialysis tubing prepared from high purity, regenerated cellulose, with an average pore radius of 24 Å, was obtained from Union Carbide Corp., Chicago, Ill. Tubing of 28 mm diameter was cut into 20-cm segments, soaked in distilled water for 30 min, blotted dry with tissue paper, and securely knotted at one end. Each bag was suspended in a 50-ml polypropylene centrifuge tube with the open end of the bag stretched over the lip of the tube and anchored in place with a plastic stopper. Two-ml plasma samples were added to the dialysis bag and centrifuged for 60 min at 1100 *g* at room temperature (20–22°). This procedure resulted in 0·2 to 0·4 ml of ultrafiltrate. The method of Lowry *et al.*[13] was used to confirm the absence of proteins in the ultrafiltrates.

RESULTS

Ability of orally administered aspirin to lower plasma concentrations of concomitantly administered naproxen. The data in Table 1 and Fig. 1 indicate that simultaneous administration of oral doses of aspirin (25 and 100 mg/kg) with a 10 mg/kg oral dose of naproxen results in lower plasma concentrations of naproxen than those observed when naproxen was administered alone at the same dose. Plasma concentrations of

* The author wishes to thank Dr. W. Hafferl and Mr. A. Hary of Syntex Research, Palo Alto, Calif., for a generous supply of the radioactive compound.

† L. J. Throop and R. J. Leibrand, to be published.

naproxen were estimated from plasma radioactivity and by a specific gas–liquid chromatogry (GLC) assay for naproxen. The two methods yielded similar results (Table 1), which indicates that nearly all of the radioactivity circulating in the plasma is unchanged naproxen. Furthermore, in previous experiments,[2] it was shown by thin-layer chromatography that at least 90 per cent of the radioactivity found in the plasma of adult male rats of the same strain used in the current studies was unchanged

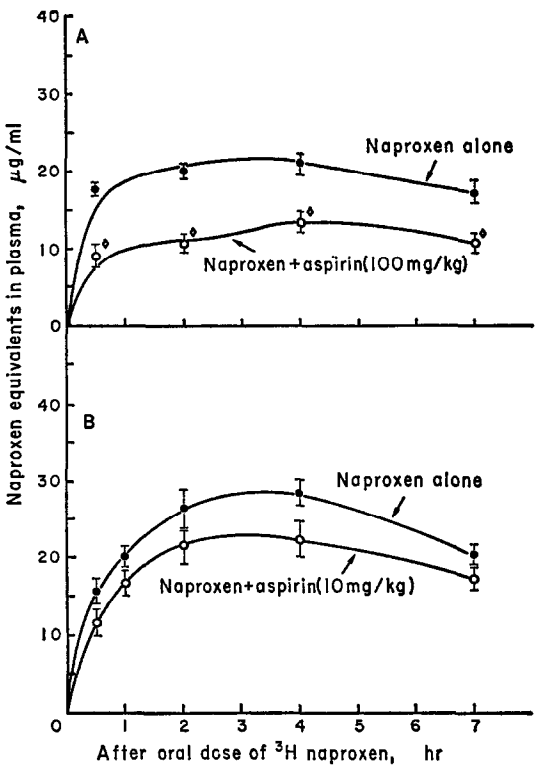

FIG. 1. Effect of aspirin doses of 10 and 100 mg/kg on plasma concentrations of concomitantly administered naproxen. All animals received ^{3}H-naproxen (10 mg/kg) by oral intubation. When both drugs were administered, naproxen was given first, followed immediately by the oral dose of aspirin. Plasma concentrations of naproxen equivalents were estimated from total radioactivity in whole plasma. Each point represents the mean ± S.E. of four (A) or six (B) animals. The symbol (◇) indicates that $P < 0.05$ when compared to animals given naproxen (10 mg/kg) only.

naproxen. The lowering of naproxen plasma levels by aspirin was statistically significant for up to 2 hr post drug administration when the aspirin dose was 25 mg/kg (Table 1) and for 7 hr when the aspirin dose was 100 mg/kg (Fig. 1A); at later time periods the effect of aspirin was negligible. That a dose–response relationship exists for the effect of aspirin on plasma levels of naproxen is further indicated by the data in Fig. 1B, which show that an aspirin dose of 10 mg/kg administered simultaneously with 10 mg/kg of naproxen did not depress naproxen plasma concentrations to a statistically significant degree.

TABLE 1. EFFECT OF ASPIRIN ON PLASMA LEVELS OF NAPROXEN*

Time (hr)†	Oral dose (mg/kg)		Naproxen plasma concn (μg/ml)	
	Naproxen	Aspirin	Radiochemical assay	GLC assay
0·5	10	0	20·6 ± 3·5	19 ± 3
0·5	10	25	10·7 ± 1·0‡	10 ± 1‡
1·0	10	0	25·2 ± 1·7	21 ± 2
1·0	10	25	17·2 ± 1·8‡	14 ± 1‡
2·0	10	0	26·0 ± 1·1	24 ± 2
2·0	10	25	19·0 ± 1·8‡	16 ± 2‡
4·0	10	0	26·6 ± 1·3	21 ± 1
4·0	10	25	22·0 ± 1·9	19 ± 1
7·0	10	0	15·1 ± 1·0	No assay
7·0	10	25	14·2 ± 1·0	No assay

* Each value represents the mean ± S.E. of six animals. All rats received tritiated naproxen by oral intubation. In those animals receiving both drugs, naproxen was given first, followed immediately by the dose of aspirin.

† Time interval between dosing with tritiated naproxen and collection of blood sample.

‡ $P < 0.05$ when compared to the concentration found at the same time interval without aspirin.

TABLE 2. INFLUENCE OF ROUTE OF ADMINISTRATION ON THE ABILITY OF ASPIRIN TO LOWER PLASMA LEVELS OF NAPROXEN*

Route of administration and dose (mg/kg)		Plasma concn of naproxen equivalents (μg/ml)
Aspirin	Naproxen	
None	p.o.; 10	28·7 ± 1·3 (12)
p.o.; 25	p.o.; 10	18·0 ± 1·4 (12)†
None	i.v.; 10	33·8 ± 2·2 (6)
i.v.; 25	i.v.; 10	24·6 ± 1·3 (6)†
None	i.p.; 10	29·8 ± 0·7 (5)
i.p.; 25	i.p.; 10	22·9 ± 1·1 (5)†

* All blood samples were taken by cardiac puncture precisely 1 hr after dosing with ^{3}H-naproxen. When both drugs were administered, the dose of naproxen was given immediately before the aspirin dose. Plasma concentrations of naproxen were calculated from total radioactivity in plasma; each value represents the mean ± S.E. Numbers in parentheses indicate number of animals used.

† $P < 0.05$ when compared to the concentration after administration of naproxen by the same route without aspirin.

Ability of aspirin administered parenterally to lower plasma concentrations of concomitantly administered naproxen. When aspirin (25 mg/kg) was administered intravenously or intraperitoneally concomitantly with ^{3}H-naproxen (10 mg/kg), the plasma levels of naproxen equivalents obtained 1 hr after naproxen administration were significantly lower than those obtained when naproxen was given alone at the same dose (Table 2). These results suggest that aspirin lowers plasma concentrations of naproxen by a mechanism other than by only inhibiting the absorption of naproxen. Direct evidence that aspirin does not significantly affect the completeness of absorption of naproxen is found in Table 3, which shows that the percentage of an oral dose of naproxen recovered in the feces after concomitant administration of aspirin and naproxen was not significantly different from the percentage of the dose found in the feces following oral administration of the same dose of naproxen alone.

TABLE 3. RECOVERY OF RADIOACTIVITY IN URINE AND FECES FOLLOWING CONCOMITANT ADMINISTRATION OF ^{3}H-NAPROXEN AND ASPIRIN*

Group	Collection period (hr after p.o. dose)	Per cent orally administered radioactivity recovered in 24 hr	
		Urine	Feces
Naproxen only	0–7	33·2 ± 3·4	0·03
	7–24	45·1 ± 5·3	3·77 ± 0·14
	Total	78·3 ± 3·5	3·80 ± 0·14
Naproxen + aspirin	0–7	34·6 ± 3·3	0·03
	7–24	44·3 ± 2·9	4·42 ± 0·94
	Total	78·9 ± 1·5	4·45 ± 0·94

* All animals received ^{3}H-naproxen orally (10 mg/kg). In those animals which received both drugs, naproxen was administered first, followed immediately by the oral dose of aspirin (25 mg/kg). The values are mean ± S.E. of three animals.

Effect of naproxen on plasma concentration of total salicylates. To determine if naproxen affects the plasma levels of total salicylate when the two anti-inflammatory agents are simultaneously administered orally, rats were dosed with naproxen (10 mg/kg) and aspirin (25 mg/kg). The latter drug was labeled with ^{14}C in the carboxyl group. The data in Table 4 demonstrate that the plasma levels of total salicylate obtained when the two drugs are given concomitantly are not significantly different from those obtained when the same dose of aspirin was administered alone.

Displacement of naproxen from plasma proteins by aspirin and salicylic acid in vitro. Since aspirin does not depress plasma levels of naproxen by interfering with absorption, other possible mechanisms must be considered. One possibility would be for aspirin to compete with naproxen for plasma protein binding sites. In all species examined, naproxen is extensively bound to plasma proteins over a wide concentration range.[14,15] The drug is bound primarily to albumin.[14] In the rat, at concentrations in the range of 20–70 μg/ml, naproxen is 98–99 per cent bound to plasma proteins. We reasoned that should aspirin be capable of competing with, or displacing, naproxen from plasma proteins, the percentage of naproxen not bound to plasma proteins would increase,

TABLE 4. EFFECT OF NAPROXEN ON PLASMA CONCENTRATION OF TOTAL SALICYLATE*

Time (hr)†	Plasma concn of total salicylate (μg/ml)	
	Aspirin only	Aspirin + Naproxen
0·5	85·7 ± 8·8	87·8 ± 4·6
1	91·9 ± 6·8	82·9 ± 1·7
2	70·8 ± 12·2	79·9 ± 8·5
4	79·9 ± 1·4	71·9 ± 3·3

* Naproxen (10 mg/kg) was given orally as a solution. Immediately after the naproxen dose, ^{14}C-aspirin (25 mg/kg) was given orally as a solution. Plasma concentrations of total salicylate were calculated from total radioactivity in plasma. Values presented in the table are the mean ± S.E. of five animals.

† Indicates time elapsed between dosing with ^{14}C-aspirin and collection of blood sample by cardiac puncture.

TABLE 5. CAPABILITY OF ASPIRIN AND SALICYLIC ACID TO DISPLACE NAPROXEN FROM PLASMA PROTEINS *in vitro**

Salicylate studied and concn (mM)	Per cent free naproxen
None	1·44 ± 0·02
Aspirin, 0·4	1·84 ± 0·10†
Aspirin, 0·8	2·55 ± 0·15†
Aspirin, 4·0	6·75 ± 0·52†
None	2·79 ± 0·13
Salicylic acid, 0·4	3·98 ± 0·27‡
Salicylic acid, 0·8	5·16 ± 0·31‡
Salicylic acid, 4·0	12·32 ± 0·43‡

* Tritiated naproxen was added to 22 ml of rat plasma to a final concentration of 21·5 μg/ml (0·094 mM) in the aspirin study or 70·0 μg/ml (0·304 mM) in the salicylic acid study. After incubating at 37° for 15 min in a Dubnoff metabolic shaker (60 oscillations/min), 5-ml samples were withdrawn, aspirin or salicylic acid was added to its specified concentration, and the samples were incubated at 37° for an additional 30 min. After incubation, aliquots of each sample were removed and assayed for radioactivity (concentration of naproxen in total plasma); 2-ml aliquots of each sample were then placed into dialysis bags and centrifuged for 60 min at 1100 *g* at room temperature (20–22°) and the resulting ultrafiltrates were assayed for radioactivity. The values represent the mean ± S.E. of three separate determinations, using different plasma samples.

† $P < 0·05$ when compared to per cent free naproxen with no aspirin added.

‡ $P < 0·05$ when compared to per cent free naproxen with no salicylic acid added.

which, in turn, would render naproxen more susceptible to metabolic degradation or excretion with subsequent lowering of plasma naproxen concentrations.

The data in Table 5 demonstrate that aspirin and salicylic acid can effectively displace naproxen from plasma proteins of the rat *in vitro*. Salicylate concentrations of 4·0 and 0·8 mM displaced naproxen from plasma proteins to a high degree, while a salicylate concentration of 0·4 mM displaced naproxen to a lesser, but statistically significant, extent. In Table 4 it was shown that 30 and 60 min after intravenous administration of 25 mg/kg of aspirin to rats orally, the plasma concentration of total salicylate was 85–90 μg/ml. This calculates to be about 0·5 mM, which indicates that even 1 hr after intravenous administration, the concentration of salicylate in the plasma is high enough to effectively displace naproxen from plasma proteins of the rat.

TABLE 6. ABILITY OF ASPIRIN TO DISPLACE NAPROXEN FROM PLASMA PROTEINS OF THE RAT *in vivo**

Group	Naproxen concn (μg/ml)		Per cent free
	Total plasma	Ultrafiltrate	
Naproxen only	72·1 ± 2·4†	0·720 ± 0·032‡	1·00 ± 0·02§
Naproxen + aspirin	62·5 ± 1·9†	1·422 ± 0·084‡	2·29 ± 0·15§

* Rats were given either ^{3}H-naproxen (10 mg/kg) alone or ^{3}H-naproxen (10 mg/kg) followed immediately by aspirin (25 mg/kg). Both drugs were given intravenously. Five min after injection of ^{3}H-naproxen, blood was withdrawn by cardiac puncture and plasma was obtained. Aliquots of the plasma fraction were assayed for total radioactivity in order to ascertain the concentration of naproxen in undiluted plasma (values presented in "Total plasma" column). Two-ml aliquots of the remaining plasma were transferred to dialysis bags and centrifuged as described in Methods. Aliquots of the resulting ultrafiltrates were assayed for radioactivity. Naproxen concentrations were calculated from radiochemical data, assuming that all of the radioactivity represented naproxen. Plasma protein concentrations were the same in both groups of animals. The values presented represent the mean ± S.E. of six individual animals.

† Significantly different, $P < 0.05$.

‡ Significantly different, $P < 0.001$.

§ Significantly different, $P < 0.001$.

Ability of aspirin to displace naproxen from plasma proteins in vivo. Evidence that aspirin displaces naproxen from plasma proteins of rats *in vivo* is presented in Table 6. Rats were given ^{3}H-naproxen intravenously (10 mg/kg) followed immediately by the intravenous dose of aspirin (25 mg/kg). Five min later, blood was obtained by cardiac puncture, plasma was obtained, and the amount of naproxen not bound to plasma proteins was determined. The data indicate quite clearly that not only is the concentration of naproxen in total plasma significantly lower in the rats which received both aspirin and naproxen compared to the plasma levels of naproxen obtained in those animals which received only naproxen but, in addition, the concentration of naproxen found to be not bound to plasma proteins was about twice as high in those animals receiving both drugs as compared to those which received only naproxen.

DISCUSSION

Several reports have appeared in recent literature indicating an interaction between nonsteroidal anti-inflammatory agents. Mielens *et al.*[3] demonstrated that aspirin and

indomethacin had no additive effect in inhibiting carrageenin-induced rat paw edema. Swingle *et al.*[4] confirmed these findings and also demonstrated the lack of additive effects when rats were given phenylbutazone with either aspirin or indomethacin. In studies utilizing adjuvant-induced arthritis in the rat, Van Arman and Nuss[16] showed that aspirin and indomethacin actually antagonized each other. The doses of aspirin employed were in the range of 1 to 3 mg/kg. The antagonistic effect of aspirin on the anti-inflammatory action of indomethacin in Van Arman's studies apparently could not be related to any alteration of the pharmacokinetics of indomethacin, since Duggan and Duncan[17] showed that pretreatment with salicylate at doses antagonizing the anti-inflammatory properties of indomethacin had no significant effect on the metabolism of indomethacin *in vivo*. However, the results of Mielens *et al.*[3] and Swingle *et al.*[4] might be explained, at least in part, by the finding of Yesair *et al.*[5] that administration of high doses of salicylic acid 1 or 3 hr after indomethacin decreased plasma concentrations of ^{14}C-indomethacin equivalents in plasma by 30–60 per cent, enhanced biliary and fecal excretion of indomethacin and its metabolites, and modified tissue concentrations.

Very few reports have been generated on the possibility of interactions in humans when two nonsteroidal agents are administered concurrently. The question of an interaction between aspirin and indomethacin was raised by the report of the Cooperating Clinics Committee of the American Rheumatism Association,[18] which demonstrated that indomethacin was not more effective than placebo when *ad lib.* use of aspirin was permitted. Subsequently, Jeremy and Towson[19] reported that aspirin decreased serum levels of ^{14}C-indomethacin equivalents in man. This effect was attributed to impaired gastrointestinal absorption of orally administered indomethacin, since urinary excretion of ^{14}C-indomethacin metabolites was reduced while fecal excretion was increased. However, in a later study, Champion *et al.*[20] could not demonstrate an effect of buffered aspirin on serum indomethacin concentrations.

In our study, concurrent administration of aspirin (25 or 100 mg/kg) and naproxen (10 mg/kg) to rats resulted in lower plasma concentrations of naproxen than those obtained when naproxen was given alone at the same dose. The ability of aspirin to depress plasma levels of simultaneously administered naproxen was significant for up to 7 hr post oral administration when the aspirin dose was 100 mg/kg. This effect of aspirin appears to be dose related, since an aspirin dose of 10 mg/kg did not significantly affect the plasma concentration of naproxen.

That the interaction of aspirin with naproxen is not solely related to an effect on gastrointestinal absorption is clearly demonstrated by the observation that aspirin lowers plasma concentrations of naproxen when both drugs are given parenterally. Furthermore, when aspirin and tritiated naproxen were given orally, no more radioactivity was recovered in the feces within 24 hr than was found when the same dose of labeled naproxen was given orally.

A likely explanation for the interaction of aspirin with naproxen in the rat is that aspirin (or salicylic acid) displaces naproxen from plasma proteins. Both salicylates, at concentrations obtainable *in vivo* in the rat, were shown to be capable of displacing naproxen from plasma proteins in experiments *in vitro*. Furthermore, 5 min after concomitant intravenous administration of aspirin and ^{3}H-naproxen, the concentration of naproxen not bound to plasma proteins was more than double that found to be not bound after intravenous administration of naproxen alone at the same dose.

Thus a greater concentration of naproxen becomes susceptible to metabolic transformation or excretion, or to both. This sequence of events would be expected to lead to reduced plasma concentrations of naproxen.

It is generally accepted that a variety of acidic drugs are attached to one or two sites on albumin and that this protein has a limited carrying capacity for these drugs.[21] Thus, if two drugs are presented in sufficiently high concentration, competition for the available binding sites occurs. Among the drugs shown to inhibit competitively the binding of phenylbutazone to human albumin are tolbutamide, indomethacin, sulfamethoxypyridazine and chlorophenoxyisobutyric acid.[22] Similarly, chlorophenoxyisobutyric acid,[22] phenylbutazone[23] and trichloroacetic acid[24] have been shown to displace warfarin from plasma proteins. Since aspirin and naproxen are both organic acids, the finding that the two drugs compete for binding sites on plasma albumin is not surprising.

There are several precedents in the literature, pertaining to interactions between two acidic drugs, which substantiate the notion that increasing the concentration of naproxen not bound to plasma proteins would lead to a reduction in the plasma levels of naproxen. Aggeler *et al.*[23] found that phenylbutazone displaces warfarin from human plasma albumin and postulated that this led to both the observed increased hypoprothrombinemic response of warfarin and the increased rate of disappearance of the anticoagulant from the plasma. A similar mechanism was considered for the observation that, in the rat, aspirin caused decreased plasma concentrations of bishydroxycoumarin.[25] Furthermore, the primary metabolite of chloral hydrate, trichloroacetic acid, has been shown to displace warfarin from plasma albumin with a 25 per cent reduction of the plasma half-life of the anticoagulant.[24]

It is recognized that other factors may be involved in this apparent interaction between aspirin and naproxen. It appears unlikely that aspirin causes a significant redistribution of naproxen into tissues, since the apparent volumes of distribution are approximately the same in rats receiving both drugs and in animals receiving only naproxen. However, it is possible that aspirin inhibits the tubular reabsorption of naproxen. This possibility has not yet been studied.

On the basis of our experiments it appears that aspirin lowers plasma levels of naproxen in the rat by a mechanism different from that proposed for the ability of salicylic acid to lower plasma concentrations of indomethacin. Yesair *et al.*[5] proposed that salicylic acid inhibits the renal excretion of both indomethocin and desmethylindomethacin in rats and that secretion of indomethacin in the bile was increased. Displacement of indomethacin from plasma proteins by salicylic acid was considered to contribute only a minor amount to the interaction between these two agents.

In our experiments, the dose of aspirin required to demonstrate a significant lowering of plasma concentrations of naproxen is within the dose used in some clinical situations. The question arises as to whether this interaction between aspirin and naproxen observed in the rat might occur in man. This problem is under current investigation and will be reported in detail in a separate communication, but preliminary data indicate that aspirin, in some volunteers, depresses plasma concentrations of naproxen.

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