Influence of Aspirin Formulation and Dose on Concentration of Total Salicylate in Rat Kidney

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Abstract □ Rats received the equivalent of 100, 250, 500, or 700 mg/kg of ^{14}C-aspirin orally as a suspension or as a solution of a buffered, effervescent, aspirin-containing product. Animals in each dose group were sacrificed at time intervals ranging from 0.5 to 24 hr after dosing. The ^{14}C content of whole blood, plasma, and homogenized whole kidney (after perfusion) was determined. The concentration of total salicylate proved to be dose dependent and was lower in tissues from rats receiving the buffered, effervescent product, especially at the higher doses. The results suggest that salicylate-induced renal toxicity should be less likely to occur after administration of the buffered, effervescent formulation.

Keyphrases □ Aspirin—effect of formulation and dose on total salicylate concentration in rat kidney □ Salicylate—concentration in rat kidney, effect of formulation and dose □ Renal excretion—salicylates, effect of formulation and dose, rats

Chronic, oral administration of high (500 mg/kg) single daily doses of aspirin was reported (1) to produce renal papillary necrosis in rats. The incidence of nephropathy was enhanced if the rats were dehydrated for 16 hr each day and reduced by measures that alkalinized the urine.

The latter observation is interesting in view of the demonstration (2) that the renal excretion of salicylates is profoundly influenced by urinary pH and is much more rapid in alkaline than in acid urine. The increased rate of salicylate excretion observed when the urine is alkaline is generally accepted to reflect a reduction in the filtered salicylate reabsorbed from tubular urine (3). Therefore, salicylate levels in renal tissue (defined as renal tubular epithelial cells and the interstitial spaces between these cells and renal blood vessels, excluding renal blood and tubular urine) would arise largely as a consequence of the passive reabsorption processes involved in excretion. Thus, renal tissue salicylate levels would be maximal when tubular urine is acidic and minimal when tubular urine is alkaline. This condition has important toxicological implications since, as Prescott (4) stated, "the pharmacological and toxic effects of most therapeutic agents are thought to be related to the concentration of free drug at the receptors of the target cells."

This report describes experiments in which kidney tissue levels of total radioactive salicylate were determined, after perfusion, in rats receiving various equivalent single doses of aspirin or an effervescent aspirin product[1]. The latter formulation, when dissolved in water as intended, becomes essentially sodium acetylsalicylate in sodium citrate buffer, a medium well known to alkalinize urine (5). The results produced by the two formulations were compared to support the contention that aspirin administered as a buffered formulation should be less likely to produce renal toxicity.

EXPERIMENTAL

^{14}C-Aspirin[2] was added to pure unlabeled aspirin[3] or to powdered, buffered, effervescent aspirin. Each dose contained 12.4 μCi of ^{14}C/kg. Aspirin was administered by gavage as single doses of 0.5, 1.25, 2.5, or 3.5% aqueous suspensions (4 ml/200 g of body weight, providing 100, 250, 500, or 700 mg/kg, respectively). The other formulation was similarly administered as an aqueous solution (doses contained equivalent amounts of aspirin as the sodium salt).

Animals—One hundred and sixty-eight male Charles River COBS-CD rats, 130–182 g, were randomly assigned to eight groups of 21 rats each. The animals were fasted overnight prior to drug administration but were fed thereafter. Drinking water was available *ad libitum*.

Experimental Design—At time zero, rats in four of the eight groups received the aspirin suspension at the doses described and the remaining animals received the buffered, effervescent formulation equivalent in aspirin to the suspension. After dosing, all of the animals were immediately placed in individual metabolism cages.

Three rats from each dose group were killed using chloroform at each of the following intervals: 0.5, 1, 2, 4, 8, 16, and 24 hr. Immediately after death, each rat was opened by ventral midline incision; a sample of whole blood was obtained by cardiac puncture and chilled in an ice bath. Incisions were made in both ventricles, and a 16-gauge perfusion needle then was inserted through the left ventricle into the aorta. Helium was perfused for 5 min at a flow rate of about 2 liters/min. Preliminary experiments indicated that this technique expelled a significant portion of the blood and urine from the kidneys. After perfusion, the kidneys were excised and kept on ice.

Analytical Methods—One kidney from each rat was stripped of fat, weighed to the nearest 0.1 mg, and homogenized[4] in 5 ml of distilled water. A 50-μl sample of each homogenate was transferred to counting vials (low potassium glass) to which were added 1 ml of 2 *M* NaOH, 3.5 ml of a solubilizer[5], and 10 ml of a liquid scintillator[6].

A 100-μl aliquot of whole blood from each rat was placed in counting vials, followed by 1 ml of 2 *M* NaOH and two drops of 30% H_2O_2. The vials were left at room temperature overnight and then were capped and heated at 80° for 30 min. After the solutions returned to room temperature, 3.5 ml of the solubilizer[5] and 10 ml of the liquid scintillator[6] were added to each.

Plasma samples derived from each blood sample (except the 500-mg/kg dose group) were prepared for liquid scintillation counting by transferring 100-μl aliquots to counting vials containing a solution of 8 ml of scintillator[6] and 2 ml of a plasma solubilizer[7].

All samples were counted[8] for a period of time sufficient to guarantee less than 2% counting error. Counting efficiency was deter-

[1] Alka-Seltzer, Miles Laboratories, Elkhart, Ind.

[2] New England Nuclear Corp., Boston, Mass.

[3] Dow Chemical Co., Midland, Mich.

[4] Potter-Elvehjem homogenizer with a Teflon pestle.

[5] Bio-Solv BBS-2, Beckman Instruments, Fullerton, Calif.

[6] A solution containing 4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene/liter of toluene.

[7] Bio-Solv BBS-3, Beckman Instruments, Fullerton, Calif.

[8] Model 3375 liquid scintillation counter, Packard Instrument Co., Downers Grove, Ill.

Table I—Levels of Total Salicylates in Kidney Tissue following the Oral Administration of Aspirin

Dose, mg/kg[a], and Product	Kidney Tissue Total Salicylate Concentration, mg/g[b]						
	0.5 hr	1.0 hr	2.0 hr	4.0 hr	8.0 hr	16.0 hr	24.0 hr
100							
Aspirin suspension	0.071 ±0.042	0.167 ±0.024	0.137 ±0.018	0.200 ±0.035	0.105 ±0.011	0.062 ±0.008	0.020 ±0.006
Buffered, effervescent aspirin	0.170 ±0.003	0.187 ±0.006	0.147 ±0.002	0.126 ±0.011	0.081 ±0.002	0.023 ±0.003	0.016 ±0.006
250							
Aspirin suspension	0.252 ±0.027	0.269 ±0.017	0.289 ±0.017	0.216 ±0.011	0.186 ±0.024	0.062 ±0.031	0.060 ±0.013
Buffered, effervescent aspirin	0.236 ±0.036	0.262 ±0.024	0.225 ±0.020	0.165 ±0.024	0.110 ±0.001	0.043 ±0.015	0.027 ±0.010
500							
Aspirin suspension	0.390 ±0.038	0.529 ±0.069	0.570 ±0.058	0.415 ±0.070	0.363 ±0.041	0.248 ±0.070	0.131 ±0.040
Buffered, effervescent aspirin	0.192 ±0.009	0.293 ±0.004	0.376 ±0.082	0.216 ±0.061	0.205 ±0.069	0.115 ±0.016	0.077 ±0.019
700							
Aspirin suspension	0.277 ±0.015	0.473 ±0.110	0.642 ±0.030	0.435 ±0.030	0.353 ±0.021	0.437 ±0.093	0.225 ±0.029
Buffered, effervescent aspirin	0.216 ±0.043	0.412 ±0.072	0.492 ±0.064	0.367 ±0.067	0.234 ±0.015	0.107 ±0.036	0.140 ±0.090

[a] Aspirin equivalent. [b] Mean ± *SE* (n = 3).

mined by internal standardization, and all count rates were converted first to absolute disintegration rates and then to milligrams of salicylate.

Average kidney total salicylate values were calculated by estimating the area under tissue concentration *versus* time curves using the trapezoidal technique.

RESULTS

The concentration levels of total salicylate in renal tissue of rats after 100-, 250-, 500-, or 700-mg/kg oral doses of aspirin as either the suspension or a buffered, effervescent, aspirin-containing formulation are summarized in Table I. At doses of 100 or 250 mg/kg, no difference in salicylate concentration was observed before 4 hr. However, after the higher doses, the suspension produced higher kidney levels of total salicylate throughout the 24-hr period.

The average concentrations of total salicylate in rat renal tissue following the various doses of each preparation are summarized in Table II. The aspirin suspension produced average concentrations that were 40, 45, 85, or 83% higher than those produced by the buffered, effervescent aspirin when the equivalent doses of aspirin were 100, 250, 500, or 700 mg/kg, respectively. The effect of dose of each product on the average concentration of total salicylate in the kidney is illustrated in Fig. 1.

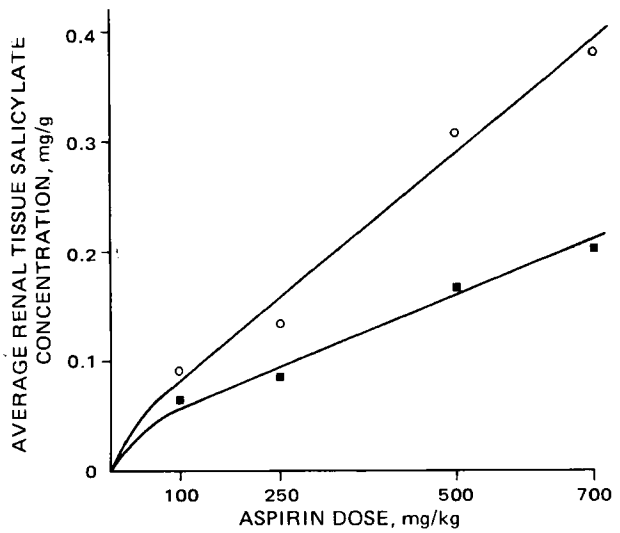

Figure 1—*Average salicylate concentrations in rat kidney after the oral administration of aspirin suspension* (○) *or a buffered, effervescent aspirin-containing formulation* (■).

Total salicylate concentrations in whole blood and plasma are summarized in Tables III and IV, respectively. The estimated average total salicylate concentrations in whole blood and plasma are summarized in Table V. The results of the blood studies agree with the analyses of kidney homogenates and show that higher levels of salicylate are present after these high doses of the aspirin suspension than after similar doses of the buffered, effervescent aspirin.

DISCUSSION

The results of this study show unequivocally that levels of total salicylate in rat kidney tissue are significantly lower when aspirin is administered as the buffered, effervescent formulation than when the aspirin suspension is administered. Since the former preparation produces an alkaline urine and since salicylate excretion is enhanced when the urine is alkaline (2), it is concluded that the lower renal tissue levels are the result of decreased tubular reabsorption of salicylic acid and increased excretion.

At the 700-mg/kg aspirin dose, the buffered, effervescent formulation approached its acute LD_{50} in the rat; indeed, three of the 21 rats died at that dose. Thus, for practical purposes, renal tissue total salicylate levels obtained at 700 mg/kg are maximal for that formulation. Similar aspirin doses as the suspension produced higher renal tissue salicylate levels (Fig. 1). Inasmuch as the acute oral LD_{50} of aspirin in the rat is about 1500 mg/kg (6), much higher kidney salicylate concentrations than those demonstrated are possible after the administration of single doses of the aspirin suspension.

The dose–tissue level relationship indicates that even lethal doses of the buffered, effervescent formulation produce kidney salicylate levels equivalent to about a 350-mg/kg dose of the suspension. This finding, in turn, indicates that it would be impossible to produce, with a single dose of the buffered, effervescent formulation, kidney tissue salicylate levels equivalent to those produced

Table II—Estimated Average Renal Tissue Total Salicylate Concentrations

Dose, mg/kg[a]	Average Renal Tissue Total Salicylate Concentrations, mg/g	
	Aspirin Suspension	Buffered, Effervescent Aspirin
100	0.091	0.065
250	0.136	0.094
500	0.308	0.165
700	0.387	0.212

[a] Aspirin equivalent.

Table III—Levels of Total Salicylates in Whole Blood following the Oral Administration of Aspirin

Dose, mg/kg[a], and Product	Whole Blood Total Salicylate Concentration, mg/ml[b]						
	0.5 hr	1.0 hr	2.0 hr	4.0 hr	8.0 hr	16.0 hr	24.0 hr
100							
Aspirin suspension	0.085 ±0.018	0.105 ±0.028	0.136 ±0.018	0.134 ±0.026	0.047 ±0.003	0.015 ±0.007	0.002 ±0.001
Buffered, effervescent aspirin	0.094 ±0.028	0.108 ±0.013	0.083 ±0.002	0.072 ±0.004	0.039 ±0.002	0.001 ±0.001	0.000 ±0.000
250							
Aspirin suspension	0.239 ±0.017	0.253 ±0.025	0.288 ±0.012	0.244 ±0.011	0.157 ±0.043	0.081 ±0.010	0.040 ±0.009
Buffered, effervescent aspirin	0.198 ±0.012	0.228 ±0.023	0.235 ±0.013	0.187 ±0.003	0.106 ±0.005	0.040 ±0.011	0.003 ±0.001
500							
Aspirin suspension	0.475 ±0.030	0.476 ±0.080	0.521 ±0.095	0.399 ±0.058	0.398 ±0.027	0.282 ±0.075	0.154 ±0.021
Buffered, effervescent aspirin	0.160 ±0.017	0.263 ±0.018	0.362 ±0.067	0.181 ±0.034	0.209 ±0.088	0.129 ±0.014	0.071 ±0.016
700							
Aspirin suspension	0.220 ±0.039	0.365 ±0.040	0.481 ±0.022	0.306 ±0.047	0.374 ±0.037	0.274 ±0.058	0.170 ±0.042
Buffered, effervescent aspirin	0.213 ±0.021	0.277 ±0.042	0.270 ±0.014	0.215 ±0.006	0.185 ±0.046	0.103 ±0.036	0.056 ±0.002

[a] Aspirin equivalent. [b] Mean ± *SE* (n = 3).

Table IV—Levels of Total Salicylates in Plasma following the Oral Administration of Aspirin

Dose, mg/kg[a], and Product	Plasma Total Salicylate Concentration, mg/ml[b]						
	0.5 hr	1.0 hr	2.0 hr	4.0 hr	8.0 hr	16.0 hr	24.0 hr
100							
Aspirin suspension	0.261 ±0.022	0.242 ±0.031	0.282 ±0.006	0.264 ±0.003	0.159 ±0.006	0.054 ±0.021	0.006 ±0.002
Buffered, effervescent aspirin	0.268 ±0.009	0.258 ±0.013	0.219 ±0.005	0.189 ±0.015	0.129 ±0.009	0.005 ±0.002	0.000 ±0.000
250							
Aspirin suspension	0.325 ±0.055	0.475 ±0.053	0.476 ±0.013	0.422 ±0.015	0.284 ±0.065	0.170 ±0.022	0.068 ±0.009
Buffered, effervescent aspirin	0.373 ±0.019	0.410 ±0.005	0.420 ±0.016	0.342 ±0.006	0.249 ±0.015	0.092 ±0.024	0.009 ±0.001
700							
Aspirin suspension	0.489 ±0.017	0.820 ±0.146	1.008 ±0.037	0.726 ±0.041	0.622 ±0.043	0.615 ±0.091	0.411 ±0.075
Buffered, effervescent aspirin	0.496 ±0.028	0.763 ±0.106	0.732 ±0.084	0.548 ±0.012	0.443 ±0.040	0.113 ±0.075	0.208 ±0.042

[a] Aspirin equivalent. [b] Mean ± *SE* (n = 3).

by a single aspirin dose of 500 mg/kg, *i.e.*, the dose reported to produce renal papillary necrosis in rats (1).

While it is generally difficult to extrapolate the results of acute experiments into a prediction of what might occur on a chronic basis, there is some justification for doing so in this instance. It was reported (1) that chronic coadministration of 500 mg/kg of aspirin and 50 mg/kg of sodium bicarbonate reduces the incidence of analgesic nephropathy by about one-half. The buffered, effervescent formulation, containing about 2.94 g/kg of sodium bicarbonate at a 500-mg/kg aspirin-equivalent dose, would have a more profound effect on urine pH and concomitantly should enhance the urinary excretion of salicylate. This condition should result in lower levels of salicylate in tissues and should, thereby, reduce or eliminate the potential for tissue toxicity.

Table V—Estimated Average Whole Blood and Plasma Total Salicylate Concentrations

Tissue	Dose, mg/kg[a]	Average Total Salicylate Concentration, mg/100 ml	
		Aspirin Suspension	Buffered, Effervescent Aspirin
Whole blood	100	4.7	3.0
	250	13.4	9.0
	500	29.3	13.2[b]
	700	29.0	15.1
Plasma	100	12.2	8.5
	250	24.7	18.4
	500	45.1	25.8[b]
	700	43.0	32.9

[a] Aspirin equivalent. [b] Calculated from values obtained by interpolation.

The results of the blood studies, which showed higher levels of total salicylates after various single doses of the aspirin suspension, substantiate the findings in kidney homogenates. The observation that salicylate concentrations are higher in plasma than in whole blood may be accounted for by the fact that salicylate is bound to plasma protein (7).

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Measurement of Sulfamethizole Clearance Rate by Nonthrombogenic Constant Blood-Withdrawal System

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Abstract □ A method for the measurement of the total body clearance rate (*CR*) of drugs is described. It involves a single intravenous injection of a known quantity of the drug (*D*) and automatic integration of the plasma concentration curve, using a portable, nonthrombogenic, constant blood-withdrawal system. When blood withdrawal is carried out until the concentration of the drug in the plasma approaches zero, the concentration of the drug in the collected pool, the integrated concentration (IC_T) multiplied by the time of collection (*T*) yields the integral of the concentration curve: $\int_0^\infty X'\,dt = IC_T \times T$ and $CR = (D/\int_0^\infty X'\,dt)$. The method was tested by measuring the clearance rate of sulfamethizole in five dogs by the established constant infusion method. At three plasma levels (25, 75, and 200 mg/liter), the plasma concentration had no significant effect on the clearance rate. The clearance rate of sulfamethizole was subsequently measured in the same dogs by the new single-injection constant withdrawal method. Multiple blood samples were collected at 15-min intervals simultaneously with the constant withdrawal of blood. There was no significant difference between the clearance rate of sulfamethizole measured by the two methods. The initial peak mean concentration of the drug from the time of injection ($t = 0$) to the time of the first blood sampling ($t = 15$ min) was calculated from the difference between $\int_0^\infty X'\,dt$ obtained by the constant withdrawal method and that obtained from the results of the multiple blood withdrawals by the trapezoidal rule. The integrated concentration IC_{15} was significantly higher than its estimation by the semilogarithmic linear regression method.

Keyphrases □ Sulfamethizole—measurement of total body clearance rate, nonthrombogenic constant blood-withdrawal system, dogs □ Clearance rate, total body—sulfamethizole, measured using nonthrombogenic constant blood-withdrawal system, dogs □ Blood-withdrawal system—nonthrombogenic, use in measurement of total body clearance rate of sulfamethizole, dogs

The total body clearance rate of drugs (*CR*) is defined as the volume of blood containing the amount of the drug under study that is irreversibly removed in a unit of time. Two established methods for measuring the clearance rate are currently in use (1–5): (*a*) a steady-state constant infusion method, in which the clearance rate is derived from the infusion rate at a situation where both reach equilibrium; and (*b*) a single-pulse injection method, in which the value $\int_0^\infty X'\,dt$ of the disappearance curve is calculated from multiple determinations of X'.

A new method for the measurement of $\int_0^\infty X'\,dt$ is proposed. The new method became possible by the development of a nonthrombogenic, constant blood-withdrawal system (6). A single determination of the integrated concentration of the drug in the pool obtained by constant withdrawal yields the $\int_0^\infty X'\,dt$. This method is not dependent on an assumed knowledge of the equation of the disappearance curve. The purposes of this work were to demonstrate the use of this new method and to test its accuracy in comparison with the established method.

EXPERIMENTAL

Apparatus—A constant infusion pump[1] was used for the constant infusion method. Nonthrombogenic, presterilized, disposable Kowarski sets and withdrawal pump[2] were used for the withdrawal method.

The method of Bratton and Marshall (7) was used to assay sulfamethizole[3] spectrophotometrically[4] in plasma and in saline solutions.

Clearance Rate Measurement by Constant Infusion Method—An intravenous catheter was placed in a jugular vein and another in a hindleg cephalic vein. The jugular catheter was then connected to a 100-ml syringe containing a sterile solution of sulfamethizole in saline. Three experiments were carried out on each dog. In each experiment, the concentration of sulfamethizole was maintained at 15, 35, or 100 mg/ml.

The syringe was then placed in a constant infusion pump. The pump was set to infuse at a rate of 4 ml/hr and continued without interruption for at least 5.5 hr. While the sulfamethizole solution was infused through the jugular vein, blood samples were drawn every 15 min from the cephalic vein into a heparinized syringe. Each blood sample was centrifuged and the plasma was stored at 4°.

At the end of 5.5 hr of infusion, the syringe was disconnected from the catheter in the jugular vein, without stopping the pump. The pump effluent was delivered into a measuring cylinder for an additional hour, and the rate of delivery was noted. The concentration of sulfamethizole in both the plasma sample and in the infusion solution was measured.

Since there was no detectable fluctuation in the plasma level of sulfamethizole during the final 2 hr of infusion, the mean of these results was considered the concentration at equilibrium (X^c). The infusion rate (*IR*) was calculated from the measured concentration in the infusion solution and the volume actually delivered into the measuring cylinder during the final hour. The total body clearance rate was then calculated:

$$CR = \frac{IR}{X^c} \qquad \text{(Eq. 1)}$$

[1] Model 352, Sage Co., Oriane Research, Cambridge, Mass.
[2] Sigmamotor Inc., Middleport, N.Y.
[3] Ayerst Laboratories, New York, N.Y.
[4] Beckman DU.