Characterization of Silver Deposits in Tissue Resulting from Dermal Application of a Silver-Containing Pharmaceutical

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Abstract □ A procedure was developed to characterize the chemical nature of silver salts or complexes deposited in body tissues. The solubility of silver-containing material in tissues from kidneys of rabbits treated topically with silver sulfadiazine cream was determined in various solvents. The presence of silver was detected by a microchemical test and atomic absorption analysis. The toxicological significance of such characterizations is also discussed.

Keyphrases □ Silver deposits in tissue—method of determining chemical nature of salts or complexes, rabbits □ Toxicology—silver deposits in tissue after dermal application of silver-containing pharmaceuticals, method to determine chemical nature of deposits, rabbits □ Dermal toxicity—silver-containing pharmaceuticals, method to determine chemical nature of silver tissue deposits, rabbits

Silver nitrate solution has enjoyed wide utilization in the treatment of severe burns covering large areas of the body (1). Additionally, several creams containing silver salts (acetate, lactate, *etc.*) have been used (2). In particular, the silver salt of sulfadiazine has been recommended as a chemotherapeutic agent in the prevention of infection in burns (3).

When an agent is used topically, there is a possibility that dermal absorption will occur and result in systemic activity. Since the skin is damaged in burns, the likelihood that agents applied in burn therapy will be found systemically is increased. Low levels of silver have, in fact, been found in the urine of patients undergoing extensive topical treatment with silver nitrate solution (4). In addition, silver has been found deposited in several tissues following such treatment (5).

Studies concerned with the systemic presence of silver have been directed to the determination of its concentration but not of its chemical form in the affected tissue. The chemical nature of the complex or salt in which silver is found in the tissue is important, however, since the nature of the silver complex or salt determines the extent of its dissociation and, consequently, its toxicity.

DISCUSSION

Reported studies of silver in biological tissues (6) normally involve a preliminary ashing ("wet" or "dry") to remove interfering material and convert the silver into a form suitable for the analytical scheme. These procedures, however, prevent a characterization of the chemical nature of the silver in tissue. Therefore, a study was designed to examine the nature of the silver in tissues by avoiding a preliminary ashing procedure.

Kidneys from albino rabbits[1] that received daily applications, over 100 days, of 1% silver sulfadiazine cream to abraded skin at dosages of 5.0, 10.0, or 15.0 g./kg./day were used in this study. Corresponding control rabbits received the ointment base at a dosage level of 10.0 g./kg./day. At the termination of the study at 100 days, the kidneys exhibited a discoloration of the renal pyramid which appeared as a green staining. No other structural damage of the kidney or renal impairment was noted.

These affected tissues were brought into direct contact with a number of solvents including nitric acid, hydrochloric acid, acetic acid, ammonium hydroxide, and thiourea. The solutions were then tested qualitatively for the presence of silver by a microchemical test with potassium dichromate. Atomic absorption analysis was also used to detect the presence of silver in the solutions and to confirm the results of the microchemical test.

In this manner, it was possible to determine the solubility of the silver tissue deposits in a number of different solvents. This solubility spectrum was then used to characterize the chemical nature of the silver deposits in the rabbit kidney.

EXPERIMENTAL

Qualitative Test for Sulfadiazine—An initial determination for the presence of sulfadiazine in the affected tissue was necessary to ascertain its role in any silver complex or salt formation in the tissue.

The tissue samples were homogenized, acidified, and extracted with chloroform. The chloroform extracts were reduced in volume and applied to a TLC system designed to detect 1 mcg. of sulfadiazine (7). No sulfadiazine was detected in any tissue extract *via* this procedure.

Microchemical Test for Silver—Four tissue samples were taken from each kidney examined, two from the affected region and two from the unaffected region. The samples were sectioned and placed on a microscope slide. Ten microliters of a test solution (silver nitrate solution equivalent to 0.20 mg. Ag/ml.) were placed on one of the tissues sectioned from the unaffected region of the kidney. This "seeded" unaffected tissue section was used to demonstrate a positive reaction for silver with the test reagent, while the remaining unaffected tissue section served as a blank. All of the tissue sections were allowed to remain uncovered for 2 hr.

Ten microliters of the solvent to be tested (16 *M* nitric acid, 30% ammonium hydroxide, 50% acetic acid, 37.5% hydrochloric acid, or 9.0% thiourea) was placed directly on each tissue section. The tissue sections were then microscopically observed under low magnification with reflected light. Contact of the solvents with the tissue sections was assured by manipulation with a fine glass rod. After 10 min., the solutions from the tissue sections were removed and placed on microscope slides. A small crystal of potassium dichromate was added to the solutions and each was again observed microscopically under low magnification. A positive reaction for silver (8) was the formation of characteristic yellow, triclinic crystals of silver dichromate.

In this test, positive reactions for the presence of silver were observed only in those affected tissues in contact with 16 *M* nitric acid. Four ambiguous results were noted in the 50 tissue sections: two unaffected tissue sections and one affected tissue section in contact with ammonium hydroxide and one unaffected tissue section in contact with hydrochloric acid.

Atomic Absorption Analysis for Silver—Accurately weighed sections of tissue (0.40–0.75 g.) were taken for analysis, five from an affected and five from an unaffected region of the kidney. These tissue sections were ground to a fine paste with 4.0 ml. of distilled water and transferred to a separator. After extraction with ether to remove fatty material, 10 ml. of the solvents used in the microchemical test was added to the aqueous extracts of the tissue sections and mild heat was applied to facilitate dissolution.

The mixture was filtered and washed with the solvent used, and the filtrate was adjusted to pH 4.5 with 16 *M* nitric acid. The filtrate was then extracted several times with dithizone (13 p.p.m.) in carbon tetrachloride (9). The combined carbon tetrachloride extracts were

[1] Donated by Marion Laboratories, Inc., Kansas City, Mo.

Table I—Atomic Absorption Analysis of Silver in Various Solvents

Test Tissue	Solvent	Tissue Weight, g.	Volume, ml.	Solution[a] Concentration, p.p.m.	Tissue Concentration, mg. Ag/g. Tissue
Female No. 6					
Affected	HNO_3	0.32	43	1.84	0.2470
Normal	HNO_3	0.75	46	(−)	Under 0.0122
Affected	NH_4OH	0.38	30	(−)	Under 0.0151
Normal	NH_4OH	0.66	41	(−)	Under 0.0124
Affected	CH_3COOH	0.47	39	(−)	Under 0.0165
Normal	CH_3COOH	0.95	43	(−)	Under 0.0091
Affected	H_2NCSNH_2	0.45	43	(−)	Under 0.0191
Normal	H_2NCSNH_2	0.85	41	(−)	Under 0.0096
Affected	HCl	0.53	48	(−)	Under 0.0181
Normal	HCl	0.90	41	(−)	Under 0.0091
Female No. 7					
Affected	HNO_3	0.38	47	1.75	0.2160
Normal	HNO_3	0.81	39	(−)	Under 0.0096
Affected	NH_4OH	0.48	35	(−)	Under 0.0145
Normal	NH_4OH	0.92	38	(−)	Under 0.0082
Affected	CH_3COOH	0.55	42	(−)	Under 0.0152
Normal	CH_3COOH	0.82	41	(−)	Under 0.0100
Affected	H_2NCSNH_2	0.30	38	(−)	Under 0.0253
Normal	H_2NCSNH_2	0.96	35	(−)	Under 0.0729
Affected	HCl	0.43	41	(−)	Under 0.0094
Normal	HCl	0.84	37	(−)	Under 0.0088
Male No. 8					
Affected	HNO_3	0.51	47	1.87	0.1723
Normal	HNO_3	0.86	38	(−)	Under 0.0088
Affected	NH_4OH	0.45	43	(−)	Under 0.0191
Normal	NH_4OH	0.72	38	(−)	Under 0.0105
Affected	CH_3COOH	0.54	35	(−)	Under 0.0129
Normal	CH_3COOH	0.85	50	(−)	Under 0.0117
Affected	H_2NCSNH_2	0.64	34	(−)	Under 0.0106
Normal	H_2NCSNH_2	0.86	35	(−)	Under 0.0081
Affected	HCl	0.50	41	(−)	Under 0.0164
Normal	HCl	0.89	40	(−)	Under 0.0089
Male No. 9					
Affected	HNO_3	0.41	41	2.00	0.2000
Normal	HNO_3	0.75	37	(−)	Under 0.0098
Affected	NH_4OH	0.52	47	(−)	Under 0.0180
Normal	NH_4OH	0.86	39	(−)	Under 0.0091
Affected	CH_3COOH	0.59	46	(−)	Under 0.0155
Normal	CH_3COOH	0.87	44	(−)	Under 0.0101
Affected	H_2NCSNH_2	0.41	35	(−)	Under 0.0170
Normal	H_2NCSNH_2	0.88	41	(−)	Under 0.0093
Affected	HCl	0.37	48	(−)	Under 0.0259
Normal	HCl	0.86	40	(−)	Under 0.0093

[a] The lower limit of detection was determined to be 0.2 p.p.m.

shaken with a solution of 3.0 ml. each of 20% sodium chloride solution and 0.03 *M* hydrochloric acid. This aqueous solution was then diluted for analysis[2] according to the method of Galetrous and Willis (10).

Calculations for the tissue concentration of silver were based on the volume of aqueous filtrate prior to dithizone extraction. Calibration curves used in the analysis were obtained by adding a volume of the standard solution (silver nitrate solution equivalent to 0.20 mg. Ag/ml.) to an aqueous extract of an unaffected tissue section. A blank solution was represented by an aqueous extract prepared from an unaffected tissue section.

Representative results from four different kidneys are summarized in Table I.

CONCLUSIONS

The silver involved in the tissue discoloration of rabbit kidneys is in a form that is soluble only in an oxidizing acid such as nitric acid. The insolubility of the silver form in the solvents used in this study eliminates the chloride, oxide, carbonate, sulfide, phosphate, or albuminate salts of silver from consideration, since each of these salts is soluble in one or more of the solvents tested (11). Other forms of silver can also be eliminated as possibilities.

Since the silver exists in a form that can be solubilized only by an oxidizing acid, it would appear that its dissociation under normal physiological conditions would be minimal. Hence, the tissue deposits of silver would not seem to constitute a significant physiological threat, other than possible mechanical interference with kidney function.

The general procedure of solubility determinations developed here could be useful in determining the form and toxicological significance of other heavy metal tissue deposits.

[2] A Perkin-Elmer model 303 double-beam atomic absorption spectrophotometer, equipped with a "high solids" burner head and a slit width of 10.16 × 0.064 cm. (4 × 0.025 in.), was used. Acetylene was used as the fuel and air was used as the oxidant. The silver line at 3392.9 Å was used with a lamp current of 9 mamp. and a bandwidth of 4 Å.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 9, 1971, from the *School of Pharmacy, University of Missouri–Kansas City, Kansas City, MO 64110*
Accepted for publication May 17, 1972.
The authors thank Marion Laboratories for their financial support of this study.
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Effect of Halofenate on Binding of Various Drugs to Human Plasma Proteins and on the Plasma Half-Life of Antipyrine in Monkeys

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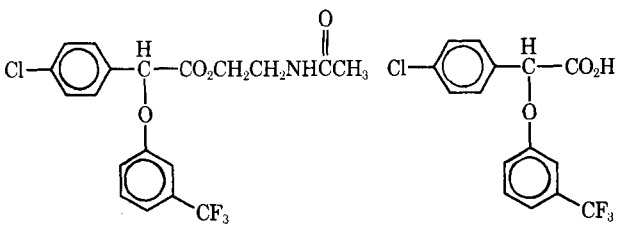
Abstract □ Halofenate free acid was shown to reduce markedly the binding of salicylic acid and aspirin in human plasma. Similar, but much smaller, effects were observed on binding of chlorothiazide, tolbutamide, digitoxin, and phenylbutazone. No effect was seen on binding of warfarin, dicumarol, or indomethacin. Halofenate administration did not alter the plasma half-life of antipyrine in rhesus monkeys.

Keyphrases □ Halofenate—effect on binding of various drugs to human plasma proteins and on the plasma half-life of antipyrine in monkeys □ 2-Acetamidoethyl (*p*-chlorophenyl)(*m*-trifluoromethylphenoxy)acetate (halofenate)—effect on binding of various drugs to human plasma proteins and on the plasma half-life of antipyrine in monkeys □ Plasma protein binding—effect of halofenate on various drugs □ Half-life, antipyrine—effect of halofenate administration, monkeys □ Antipyrine half-life—effect of halofenate administration

Halofenate[1] is a new drug which reduces concentrations of several lipid parameters in the rat (1) and is currently in clinical trial for treatment of patients with hypercholesterolemia and/or hypertriglyceridemia (2–6). The new agent also has been shown to have hypouricemic activity in man (2). Halofenate is completely metabolized in man by hydrolysis to the halofenate free acid, which is extensively bound to plasma proteins (7).



halofenate halofenate free acid

[1] 2-Acetamidoethyl (*p*-chlorophenyl)(*m*-trifluoromethylphenoxy)acetate.

The pharmacological activity of many drugs is altered by coadministration of drugs that compete for binding sites on plasma proteins and inhibit or induce drug metabolism or excretion (8). We have studied two aspects of possible drug interactions with halofenate, namely, its effects on the binding of other drugs to plasma proteins and its effects on the plasma half-life of antipyrine in rhesus monkeys. The latter technique was recommended as a means to detect induction of drug metabolism (9); for this reason the present report also includes studies with clofibrate and phenobarbital.

EXPERIMENTAL

Materials—The following drugs were used in the study: aspirin-^{14}C (carboxyl)[2], 10.4 μc./mg.; salicylic-7-^{14}C acid[2], 34.8 μc./mg.; digitoxin-^{3}H (generally labeled)[2], 5.8 mc./mg.; dicumarol-^{14}C (methylene)[2], 83 μc./mg.; warfarin-^{14}C[3], 23 μc./mg.; and tolbutamide-^{35}S[3], 21 μc./mg. Chlorothiazide-^{3}H (12 μc./mg.), indomethacin (8.4 μc./mg.), and phenylbutazone-^{3}H (36 μc./mg.) were synthesized[4]. All compounds were found to be radiochemically pure by paper or thin-layer chromatography.

Binding—Fresh blood from human donors was citrated on collection and centrifuged, and the plasma was separated. Solutions of the various drugs were prepared as follows: indomethacin in 0.1 *M* phosphate buffer, pH 8.0; halofenate free acid, salicylic acid, chlorothiazide, warfarin, dicumarol, and phenylbutazone in 0.01 *N* NaOH; aspirin in water; digitoxin in ethanol; and tolbutamide in 5% aqueous K_2CO_3. All drugs were added in the smallest possible volume to 70 ml. of plasma, the pH of which was not affected by addition of the drug.

Binding was measured by ultrafiltration as described by Borga *et al.* (10). Six samples were prepared for each concentration used. After final centrifugation, 0.2 ml. of the ultrafiltrate and 1 ml. of the plasma inside the dialysis tubing were pipeted directly into polyethylene counting vials containing 20 ml. of counting medium. The counting medium consisted of 7 g. diphenyloxazole, 0.23 g. of 1,4-

[2] New England Nuclear Corp.
[3] Amersham-Searle.
[4] By Dr. Mertel, Dr. Ellsworth, and Dr. Meriwether of the Merck Sharp & Dohme Research Laboratories.