Penetration of Trolamine Salicylate into the Skeletal Muscle of the Pig

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Abstract □ Studies to determine the extent of local tissue penetration of topically applied trolamine [^{14}C]salicylate were conducted in domestic pigs. The preparation was applied onto a 100-cm^2 shaved area of skin overlying the biceps femoris at a concentration of 0.7 mg of salicylate/cm^2 to closely approximate the actual use in humans. At least 82% of the topically applied trolamine salicylate was absorbed over a 2-h period. Based on blood and muscle salicylate levels, a localization of the absorbed drug occurred in muscle underlying the treated area within 120 min. Muscle from the treated area had a concentration of salicylate that was 13 times that of blood and 49 times that of muscle taken from untreated areas. Blood samples taken from the treated area at 10, 20, and 30 min showed that salicylate levels ranged from 15.8 to 5.3 μg/g. Less than 0.5% of the applied drug was excreted in the urine during the 2-h period.

Keyphrases □ Trolamine salicylate—percutaneous absorption, local tissue penetration, domestic pigs □ Percutaneous absorption—trolamine salicylate, domestic pigs, local tissue penetration □ Tissue penetration—local, percutaneous absorption of trolamine salicylate, domestic pigs

Trolamine salicylate is the active ingredient in a number of topical analgesic creams that are used for sore muscles, joint stiffness, and strains. The creams are also used as a topical adjunct for arthritis and rheumatism therapy. Although the percutaneous absorption of salicylate is well known, only one published report on the penetration of salicylate into canine knee joints has shown that preferential uptake and localization of salicylate occurs in tissue and fluids beneath the area of salicylate application (1). This study was carried out to determine whether localization of trolamine salicylate could be demonstrated in the muscle of the domestic pig after topical administration.

The most commonly used laboratory species have loose-fitting skin, a heavy hair coat, and dermal vasculature that does not resemble that found in humans (2). The pig, on the other hand, has a sparse hair coat, close-fitting skin, a thick epidermis with a well-differentiated lower limit, a dermis with a distinct papillary body, a highly elastic tissue content (3, 4), and vascularization similar to that of humans (2, 5). These findings have been confirmed ultrastructurally by Karasek and Oehlert (6, 7). In addition, the thickness of the dermis and epidermis (8), the distributed enzymes (9), the keratinous proteins (10), and the permeability characteristics of pig skin closely resemble those of humans (11, 12).

EXPERIMENTAL SECTION

Materials—The [7-^{14}C]salicylic acid[1] had a specific activity of 0.37 mCi/mg. Scintillation fluid[2], carbon dioxide trapping agent[2], cellulose cups[2], powdered cellulose[3], ^{14}C-recovery standards[3], and scintillation fluid[4] were commercially obtained. All other materials were reagent grade, obtained from commercial sources, and used without further purification.

Animals—Six healthy female Yorkshire swine (weighing 23–40 kg), maintained on a commercial swine diet[5], were used.

Dosage Preparation—A trolamine [^{14}C]salicylate formulation based on a commercial topical cream[6] was prepared. The trolamine [^{14}C]salicylate was incorporated into a white hydrophilic cream base by dissolving [^{14}C]salicylate (1 μCi/mg) and trolamine in stoichiometric proportions in the aqueous phase of the cream. The oil phase of the cream was then added and thoroughly mixed. The final concentration of trolamine [^{14}C]salicylate was 10% (w/w).

Drug Treatment and Sample Collection—Before the experiment, each pig was dosed with acepromazine (1.1 mg/kg im) followed 30 min later by administration of pentobarbital[7] (30 mg/kg ip). Phenobarbital was administered intravenously as required during the experimental period. The animals were quickly washed with water and dried, and their hind quarters were clipped and carefully shaved. Shaving was performed with a safety razor and warm water. The trolamine [^{14}C]salicylate formulation was not applied until 45–60 min after shaving. During the 45–60-min pretreatment, the urinary bladder was catheterized with a polypropylene catheter, and a 10- × 10-cm area was marked over the biceps femoris. The radiolabeled cream was applied to this area with a syringe fitted with a 16-gauge blunt needle and spread evenly over the area by gentle rubbing. The syringe was calibrated to deliver 1.5 g of the preparation, which contained the equivalent of 72 mg of salicylic acid. This application closely approximates the amounts and conditions of application of trolamine salicylate applied in actual practice by humans.

In pigs 1 through 4, 30 min after the application of the dose, one-half of the 100-cm^2 area was washed with a damp paper towel until the cream was no longer visible. An incision of ~4 cm was then made in the center of the washed area with a scalpel. The scalpel blade was changed often during the cutting of the skin. The subcutaneous fat (when present) was then removed with scissors to expose the underlying muscle. Three samples of the exposed muscle (0.4–0.6 g) were then removed with scissors and pressed firmly between gauze to remove blood. Muscle was also taken from the contralateral side (control) by the same procedure. At the same time, blood samples were collected from the orbital sinus. The procedures were repeated at 120 min postdose. Skin wipes taken at 120 min were retained for analysis of carbon-14. Skin removed from the total area of application (10 × 10 cm) was assayed for carbon-14 in pigs 1 and 4.

The concentration of salicylate in blood at the site of application was determined in pigs 5 and 6. The area of drug application was cleaned as described above, and blood samples were taken from shallow incisions (1–1.5 mm) at 10, 20, and 30 min postdose.

All samples were placed in cellulose cups and allowed to air-dry for 48 h before combustion in an oxidizer[8]. The amount of radioactivity on the paper towel wipes was determined by placing the wipes into a 500-mL beaker containing a magnetic stirring bar and by adding 300 mL of toluene. The beaker was covered and placed on a magnetic stirrer to stir overnight. Duplicate 1-mL samples were removed the next morning and added to 10 mL of scintillation fluid[4]. The carbon-14 was quantitated by liquid scintillation counting.

RESULTS AND DISCUSSION

Skin wipes taken at 120 min after application of the dose indicated 7.9 ± 0.5% (mean ± *SEM*) of the dose remaining on the skin, whereas assays of skin tissue showed 9.3 ± 0.7% remaining in the skin. These figures were calculated from data obtained at the 120-min sampling time and multiplied by a factor of two, since one-half of the area remained at this time. Thus, at least 82% of the dose was found to be absorbed within 120 min after application. The excellent absorption of trolamine salicylate shown by the data was not a result of incomplete extraction of the paper towels. Extraction of paper towels containing a known amount of cream indicated that 49.1, 78.1, 90.7, 93.7, 97.7, 101.0, and 100% of the applied carbon-14 could be recovered at 30, 60, and 90 min and 2, 3, 4, and 7 h, respectively. The literature indicates that the absorption of salicylate after topical administration to humans varies considerably. Feldmann and Maibach have found that only 13% of the applied salicylate was absorbed in a 24-h period (13), whereas Taylor and Halprin have

[1] New England Nuclear Corp., Boston, Mass.
[2] Permafluor V, Carbosorb, Combusto Cones; Packard Instrument Co., Inc., Downers Grove, Ill.
[3] Packard Instrument Co., Downers Grove, Ill.
[4] Thrift-Solve; Kew Scientific, Inc., Columbus, Ohio.
[5] Yoeman, Plain City, Ohio.
[6] Myoflex creme (trolamine salicylate); Adria Laboratories, Inc., Dublin, Ohio.
[7] Nembutal.
[8] Model 306 Sample Oxidizer, Packard Instrument Co.

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Table I—Concentration of [^{14}C]Salicylate in Pig Muscle [a]

Sample	Pig	Drug Concentration, ng/g[b]		T/B[c]	
		30 min	120 min	30 min	120 min
Blood	1	56.4	38.7	—	—
	2	47.7	84.5	—	—
	3	35.4	—	—	—
	4	31.6	41.6	—	—
Control muscle	1	28.7	55.2	0.5	1.4
	2	0.0	8.6	0.0	0.1
	3	8.7	—	0.2	—
	4	6.8	23.8	0.2	0.6
Treated muscle	1	635.0	313.5	11.3	8.1
	2	150.9	582.3	3.2	6.9
	3	724.5	—	20.5	—
	4	236.8	560.1	7.5	13.5

[a] [^{14}C]Salicylate trolamine applied topically (0.6–0.7 mg of salicylic acid equivalents/cm^2) over biceps femoris (treated muscle). Biopsy from treated muscle and contralateral (control) muscle assayed for total carbon-14. Blood was taken from the orbital sinus. [b] Concentration of drug is expressed as free salicylic acid equivalents. [c] Ratio of tissue to blood.

Table II—Penetration of [^{14}C]Salicylate into Pig Muscle [a]

Parameter	Pig				Mean ± *SEM*
	1	2	3	4	
Concentration in muscle, ng/g[b]	635 (30 min)[c]	582 (120 min)	725 (30 min)[d]	560 (120 min)	625 ± 37
Carbon-14 Ratios					
Treated muscle/blood	11.3	6.9	20.5	13.5	13.1 ± 2.8
Control muscle/blood	0.5	0.1	0.2	0.6	0.4 ± 0.1
Treated muscle/control muscle	22.1	67.7	83.2	23.5	49.1 ± 15.5

[a] [^{14}C]Salicylate trolamine was applied topically (0.6–0.7 mg/cm^2) over biceps femoris (treated muscle). Biopsy from treated muscle and contralateral (control) muscle assayed for total carbon-14. Blood was taken from orbital sinus. [b] Concentration of drug is expressed as free salicylic acid equivalents. [c] Number in parentheses represents the time of biopsy (30 or 120 min) when concentration was higher. [d] Only data at 30 min were obtained. Animal died from anesthesia before 120-min sampling time.

shown that 60% of the dose was absorbed within a 24-h period (14). In the latter study, the peak plasma level occurred at 5 h. The extreme differences shown in the percentage of the dose absorbed between these two studies are probably due to the vehicle and the treatment of the dose after application. Feldmann and Maibach applied salicylate to the skin dissolved in a small volume of acetone, whereas Taylor and Halprin used 60% propylene glycol and 19.4% alcohol as a vehicle and occluded the areas for 10 h. However, not only the vehicle, but also the salt form of salicylate affect the rate of its percutaneous absorption. Gaudin has demonstrated that the percutaneous absorption of the trolamine salt of salicylate after 24 h is 1.7 times greater than that of the sodium salt form (15). The fact that the dose of salicylate applied to the skin of the pig in the present study was the trolamine salt in a hydrophilic cream base most likely accounts for the rapid absorption of the dose.

Although the dose was well absorbed, urinary excretion of carbon-14 was found to be minimal. Urine collected during the first 30 min postdose contained 0.13 ± 0.12% of the dose, and 0.45 ± 0.07% (mean ± *SEM*) of the dose was excreted after 2 h.

Data obtained from the blood and tissue samples taken at 30 and 120 min are shown in Table I. Greater concentrations of carbon-14 were observed in the treated area at 30 min of pigs 1 and 3, whereas pigs 2 and 4 were found to have a greater concentration of carbon-14 at 120 min. Pig 3 died from the anesthesia after the 30-min muscle biopsy, but the data obtained at that time were similar to those in pigs 1, 2, and 4, which had greater concentrations of carbon-14.

A summary of those data representing the greater concentration of salicylate in muscle is presented in Table II. Whether these data represent the highest concentrations of carbon-14 achieved during the experimental period is not known since samples were only obtained at 30 and 120 min to minimize the possible effects of local trauma.

Table II shows that [^{14}C]salicylate was detectable in muscle underlying the site of application in greater concentrations (49 times) than that taken from the contralateral (control) muscle. Gross differences in skin thickness were observed not only from pig to pig, but also from areas as close as 3.5 cm within the same pig. This may have influenced the differences in the time of detection of the greater concentration from pig to pig. Table II also shows the ratios of [^{14}C]salicylate levels in muscle to [^{14}C]salicylate levels in blood taken from the orbital sinus. The data show that the carbon-14 concentration was ~13 times greater in the muscle underlying the site of drug application than in the blood. The concentration of salicylate in pig muscle was low compared with that obtained in the dog (1). This difference may be attributed to the application of a much lower dose in the pig (1.5 *versus* 10 g) without massaging the preparation into the skin, as was done in the dog. However, despite methodological differences, preferential uptake and localization of salicylate in tissues underlying the area of drug application were found in both studies.

Analysis of blood taken from the applied area also indicated that the penetration of salicylate through the dermis to underlying muscle occurs fairly rapidly. Whether partitioning of the salicylate between the local vasculature and the muscle tissue accounts for the high concentration of salicylate in the muscle beneath the area treated with trolamine salicylate is not known. However, blood collected from shallow incisions in the dermis of pigs 5 and 6 had carbon-14 concentrations equivalent to 15.8 ± 4.6, 6.2 ± 3.3, and 5.3 ± 1.8 μg of salicylate/g (mean ± *SEM*) at 10, 20, and 30 min, respectively. Data obtained from control muscle demonstrate that salicylate has a muscle-blood partition coefficient of 0.1. If this coefficient is applicable in the tissues underlying the point of trolamine salicylate application, then the local blood salicylate concentrations of 5–16 μg/g would be expected to result in salicylate concentrations of 500–1600 ng/g in the treated muscle. This agrees with the mean value of 625 ng/g that was actually found.

Several studies have demonstrated the percutaneous absorption of salicylic acid and its ultimate excretion from the body. However, the significance of this absorption with regard to a local pharmacological effect on muscles or other tissues beneath the area of application has not been fully investigated. The present studies, as well as an earlier study (1), have indicated that salicylic acid does, indeed, penetrate the skin and is taken up by muscle tissue beneath the treated area. Although these studies shed little light on the pharmacological significance of the concentration of salicylic acid present in the muscle of the treated area, the fact that significant amounts of salicylate were transported through the local area and permeated the musculature provides a basis for the possible efficacy of topically applied trolamine salicylate.

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Simultaneous Determination of Nitrate and Nitrite in Toothpastes by High-Performance Liquid Chromatography

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Abstract □ A stability-indicating analytical method is described for the simultaneous determination of nitrate, and if present, its reductive degradation product, nitrite, in toothpastes. Nitrate and nitrite were extracted from the toothpaste using distilled water and separated from other water-soluble excipients by two RP-8 columns (250 mm × 4 mm i.d.) using a mobile phase containing 0.2% (w/v) sodium acetate and 2.5% (v/v) glacial acetic acid in distilled water. A UV detector set at 313 nm was used for quantitation. The method was applied to commercial toothpastes containing 5% potassium nitrate and yielded an average recovery of 100.1% with a relative standard deviation of 1.43%. Average recovery of nitrate and nitrite from spiked samples were 100.6% and 96.4%, respectively. The minimum detectable concentration for nitrite was 50 μg/g of toothpaste.

Keyphrases □ Nitrate—simultaneous determination with nitrite, toothpastes, HPLC □ Nitrite—simultaneous determination with nitrate, toothpastes, HPLC □ Dentifrices—simultaneous determination of nitrate and nitrite, HPLC

Potassium nitrate, reportedly a tooth desensitizing agent (1-3), is the active ingredient in commercial dentrifrices for the treatment of dental hypersensitivity. As nitrate ion can be converted to nitrite by reducing agents or bacteria, a simple, specific, and sensitive method for simultaneous determination of intact nitrate and its reductive degradation product is desirable for routine quality control and stability evaluation.

The widely used colorimetric procedures for nitrate (4, 5) and nitrite (6-12) are subject to interference by other ions and generally require tedious sample treatment. In addition, those methods involving the cadmium reduction of nitrate to nitrite (6, 7) prior to color development often suffer from nonstoichiometric conversion.

Methods involving derivatization followed by HPLC (13) or GC (14, 15) are lengthy. The potentiometric method with a nitrate ion-selective electrode (16-18), differential pulse polarography (19), and enthalpimetric analysis for nitrate (20) are specific, but they measure only one ion (nitrate or nitrite). The newly developed ion chromatography (21) and its variations (22-27) are selective, sensitive, and suitable for one-step differentiation and determination of nitrate and nitrite. However, the need for additional expensive instrumentation (an ion chromatography system with conductivity or electrochemical detector and ion-exchange columns) hinders the use of this technique.

Recently, Skelly (28) reported the use of an eluant containing the octylamine salt of a mineral acid to separate inorganic anions on a conventional reverse-phase column, with detection at 205 nm. Leuenberger *et al.* (29) and Cortes (30), using phosphate buffer as an eluant, have described the resolution of nitrate from nitrite on an amino normal-phase column. The separation mechanism of the former is dynamic ion-exchange, while the second is weak base ion-exchange. Both methods, which directly determined nitrate and nitrite by conventional HPLC with low-wavelength detection, are simple and sensitive, but they have not been applied to the toothpaste matrix.

The purpose of this paper is to report the separation and simultaneous determination of nitrate and nitrite by high-performance liquid chromatography (HPLC) using two RP-8 columns with acetate buffer as mobile phase. The separated nitrate and nitrite were monitored by a UV detector set at 313 nm. No sample treatment or cleanup was required, and the method was found to be simple, rapid, precise, and selective for the measurement of nitrate and nitrite in a complex toothpaste matrix.

EXPERIMENTAL SECTION

Chemicals and Reagents—Potassium nitrate[1] and sodium acetate[2] were used without further purification. Glacial acetic acid[3] and 0.1 M sodium nitrite solution[4] were used as received.

Apparatus—The liquid chromatograph[5] was fitted with a manual septumless injector[6], a fixed-wavelength UV detector (313 nm)[7], and a strip-chart recorder[8]. The recorder was connected to a laboratory data system[9] through an A/D converter[10]. Two 250 mm × 4.0-mm i.d. columns containing 10-μm Lichrosorb RP-8 packing[11] and one precolumn containing 37-40-μm octadecylsilane packing[12] were used.

Chromatographic Conditions—The chromatographic solvent was 0.2% (w/v) sodium acetate in distilled water containing 2.5% (v/v) glacial acetic acid, adjusted to pH 3.0 ± 0.1 with glacial acetic acid or sodium hydroxide. This solvent was vacuum-filtered[13] through a 0.45-μm filter[13] and deaerated

[1] Baker Analyzed Reagent, J. T. Baker Chemical Co., Phillipsburg, N.J.
[2] MCB Manufacturing Chemical Inc., Cincinnati, Ohio.
[3] VWR Scientific Inc., South Plainfield, N.J.
[4] Orion Research Inc., Cambridge, Mass.
[5] Model 6000 A Pump; Waters Associates, Milford, Mass.
[6] Valco Model CV6-UHpa-N6o, 7000 psi sample injection valve equipped with a 100-μL sampling loop.
[7] Model 440; Waters Associates, Milford, Mass.
[8] Houston Instrument, Austin, Tex.
[9] Model 21MX Computer with model 2645A terminal; Hewlett-Packard, Avondale, Pa., and model DP 8000 printer; Anadex, Chatsworth, Calif.
[10] Model 18652A; Hewlett-Packard, Avondale, Pa.
[11] Cat. #9318; Merck, Elmsford, N.Y.
[12] Waters Associates, Milford, Mass.
[13] Millipore Corp., Bedford, Mass.

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