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# Comparative Tissue Absorption of Oral $^{14}$C-Aspirin and Topical Triethanolamine $^{14}$C-Salicylate in Human and Canine Knee Joints

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**Abstract:** The local, articular, and systemic absorption of oral and topical salicylates was studied in dogs and humans using radioisotope techniques. Topical triethanolamine $^{14}$C-salicylate was found capable of percutaneous absorption into the knee joint and surrounding tissues. In dogs, topical salicylate application resulted in higher salicylate concentrations than oral aspirin in a number of tissues, despite lower blood levels. In patients with rheumatoid arthritis, intraarticular $^{14}$C-salicylate levels after triethanolamine $^{14}$C-salicylate cream were 60 per cent of those obtained with oral aspirin. Four of six patients reported equal improvement in local discomfort after oral and topical salicylates. A potential role for topical salicylate cream in the treatment of localized rheumatic disorders is suggested

Over 20 million people in the United States suffer from some form of arthritis or rheumatism. Aspirin (acetylsalicylic acid) is the drug of choice for many of these conditions.[1,2] Oral aspirin, however, has a number of well-known adverse effects on the gastric mucosa and has been associated with a significant incidence of gastrointestinal symptoms as well as an increased risk of gastric ulcer and erosion.[3] Tablets may also be difficult for some patients to swallow.

Interest in alternative routes of salicylate administration began in the 1930s when the properties of topical salicylate preparations were first explored.[4-7] In 1952, an ointment of 10% triethanolamine salicylate was found to achieve good cutaneous penetration.[8] After penetration through the skin, the triethanolamine salicylate compound released the salicylate moiety permitting it to exert its analgesic and antiinflammatory properties locally.[8] The penetration of topically applied salicylate into joints, however, has never been investigated.

The purpose of this study was to compare the local, articular, and systemic absorption of oral and topical salicylate preparations using sensitive radioisotope techniques. The levels of $^{14}$C-salicylate in periarticular tissues, synovial fluid, blood, and urine were compared in both dogs and humans after topical administration of triethanolamine $^{14}$C-salicylate and oral $^{14}$C-acetylsalicylic acid administration.

## Methods

### *Preparation of Labeled Salicylates*

$^{14}$C-Aspirin capsules were prepared by grinding nonlabeled aspirin tablets with $^{14}$C-aspirin (New England Nuclear, Boston, Mass.) in an agate mortar to the appropriate specific activity. The mixture was

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then placed in gelatin capsules (Lilly #1, Indianapolis, Ind.).

Labeled triethanolamine salicylate for topical application was prepared by mixing 10% triethanolamine salicylate (Aspercreme, Thompson Medical Co., Inc., New York, N.Y.) with triethanolamine 7-$^{14}$C-salicylate. The triethanolamine 7-$^{14}$C-salicylate was prepared by mixing equal amounts of triethanolamine and 7-$^{14}$C-salicylic acid (New England Nuclear, Boston, Mass.).

The chemical and radioactive purity of all materials was predetermined by thin-layer chromatography, radioassay, and nuclear magnetic resonance (see below).

To ensure equivalent doses of salicylate and radioactivity in each preparation, the following calculations were performed: (a) $^{14}$C-aspirin, 500 mg (molecular weight 180) = 2.77m*M*; (b) triethanolamine $^{14}$C-salicylate, 10,000 mg of 10% triethanolamine salicylate = 1,000 mg triethanolamine salicylate (molecular weight 287) = 3.48m*M* (80% of this = 2.77m*M*) (see under Study Design the reason for 20% correction); (c) 140 disintegrations per min (dpm)/$\mu$g of $^{14}$C-salicylate (chemically assayed) = 140 × 138 (molecular weight of salicylate) = 19320; (d) 19,320 dpm/$\mu M$ salicylate = $19.32 \times 10^6$ dpm/m$M$ salicylate; (e) $53.51 \times 10^6$ dpm/batch of used medication (oral or topical); (f) 24.3 $\mu$Ci present in each medication dose (calculated dose to individual's whole body = 0.65 millirad for $^{14}$C-aspirin = 0.50 millirad for triethanolamine $^{14}$C-salicylate.[9-13]

Triethanolamine $^{14}$C-salicylate was added to the nonlabeled material in small portions until the radioassay of the mixture gave a constant value of 140 dpm/$\mu$g of $^{14}$C-salicylate. In each dose (for both materials), the total dpm given was $53.51 \times 10^6$ dpm, which was about 24.3 $\mu$Ci of a specific activity of 140 dpm/$\mu$g $^{14}$C-salicylate. Each batch of $^{14}$C-aspirin and triethanolamine $^{14}$C-salicylate was assayed for $^{14}$C-specific activity. The $^{14}$C content was determined by radioassay. The chemical content of salicylate was determined by the ACA method.[14] Triethanolamine salicylate was also assayed by nuclear magnetic resonance (NMR) for qualitative and quantitative identification[15] (Fig. 1). When triethanolamine sali-

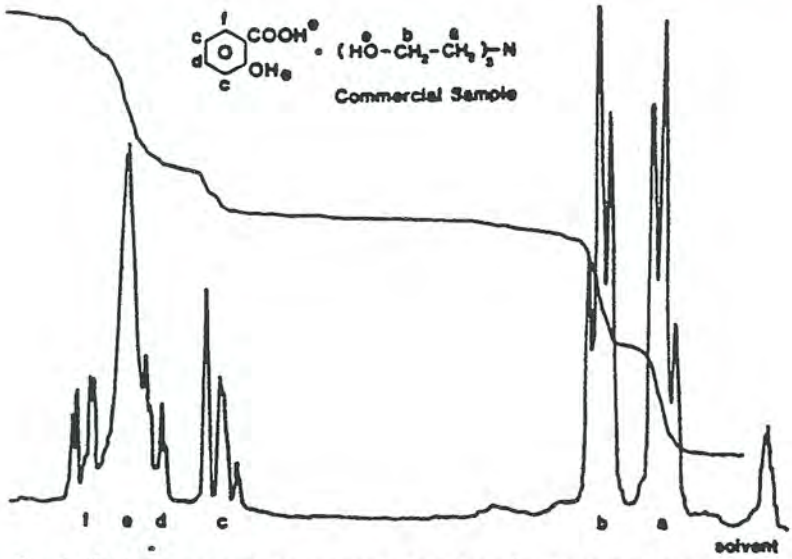


*Fig. 1. Nuclear magnetic resonance of triethanolamine salicylate in Aspercreme. Letters refer to the peaks of the spectrum and relate to the function in the molecular structure. The same spectrum was obtained from synthetic triethanolamine salicylate and the triethanolamine $^{14}$C-salicylate used in this study.*

**TABLE I**

**Canine Study. Average Concentration of $^{14}$C-Salicylate in Various Tissues After Oral and Topical Salicylate Administration**

| Tissue | Average μg $^{14}$C-salicylate·Gm tissue | |
|---|---|---|
| | Oral | Cream |
| Bone | 1.36 ± 0.26* | 1.00 ± 0.10 |
| Bone marrow | 0.90 ± 0.40 | 1.05 ± 0.36 |
| Cartilage | 0.43 ± 0.03 | 1.62 ± 0.49 |
| Fascia | 1.04 ± 0.28 | 16.40 ± 1.96 |
| Fat pad | 1.00 ± 0.10 | 5.60 ± 1.20 |
| Ligament | 0.05 ± 0.16 | 2.00 ± 0.20 |
| Meniscus | 0.62 ± 0.15 | 0.86 ± 0.30 |
| Muscle | 1.76 ± 0.16 | 38.20 ± 5.16 |
| Skin | 0.64 ± 0.09 | 312.20 ± 40.80 |
| Synovium | 0.62 ± 0.10 | 0.74 ± 0.12 |
| Synovial fluid | 1.00 ± 0.10 | 0.80 ± 0.12 |
| Tendon | 0.20 ± 0.03 | 3.00 ± 0.44 |
| Urine | 12.57 ± 5.16 | 0.16 ± 0.09 |
| Whole blood | | |
| 30 minutes | 34.80 ± 2.33 | 2.60 ± 0.02 |
| 60 minutes | 30.60 ± 0.24 | 0.22 ± 0.02 |

* Standard error of the mean. (Data include dogs at various time intervals).

cylate was prepared from authentic materials, its NMR spectrum was found to be identical to that of triethanolamine salicylate in the commercial preparation. The triethanolamine $^{14}$C-salicylate was also assayed and found to have the same NMR spectrum.

*Canine Study*

Each of five male eight-month-old Beagle dogs received by mouth a capsule of 500 mg $^{14}$C-aspirin (specific activity 140 dpm/μg of $^{14}$C-salicylate, 2.77 m$M$ = 24.3 μCi, specific activity 8.77 μCi/m$M$).

Each of a second group of five male, eight-month-old Beagle dogs received 10 Gm labeled triethanolamine salicylate cream containing $^{14}$C-salicylate of the same total equimolecular amount and radioactivity. The right knee of each dog was shaved. The cream was applied and rubbed in thoroughly until it was completely absorbed into the skin.

After 30 or 60 minutes, samples of blood and urine were obtained from the anesthetized dogs. Tissue samples as outlined in Table I were obtained at 1 hour. In the triethanolamine salicylate group, tissue samples were taken at the point of cream application. At the conclusion of the surgery, the dogs were disposed of according to humanitarian rules and the Nuclear Regulatory Commission regulations for $^{14}$C-labeled animal remains.

In preliminary experiments (four measurements), the specific activity of large amounts of unabsorbed labeled triethanolamine salicylate placed on the skin of dogs was assayed as a function of time. It was observed that the specific activity ($^{14}$C content per milligram weight of cream recovered) increased 10 to 20 per cent per hour. These results suggested that the lipid contents of the cream preparation were absorbed at different rates from the active ingredient, the lipid being perferentially absorbed. For this reason, the preweighted cream was applied only in small batches and rubbed gently until fully absorbed.

### *Human Study*

Six male subjects (55 to 62 years of age) with seropositive, adult-onset rheumatoid arthritis were studied. All patients met American Rheumatism Association criteria for classical or definite rheumatoid arthritis.[16]

Each had active knee synovitis with recurrent effusions which required synovial fluid aspiration at frequent intervals. Synovial fluid leukocyte counts ranged between 7,500 and 12,000 WBC/mm$^3$. All patients had been on a stable dose of oral aspirin for at least six months. One patient was also taking ibuprofen, and two were on chronic myochrysine therapy.

Informed written consent was obtained from each patient prior to his participation. All studies conformed to the standards set forth by the Declaration of Helsinki and the regulations of the Human Research Subcommittee and Nuclear Medicine Committee of the Philadelphia Veterans Administration Medical Center.

Patients abstained from salicylates for at least 6 hours prior to each study period. Each patient first received a capsule of $^{14}$C-aspirin. Two to six weeks later, each was given 10 Gm of the triethanolamine $^{14}$C-salicylate cream, which was gently massaged into the skin over one knee. Care was taken to continue application of the cream until all of the material was absorbed. In each case the skin surface area to which the topical salicylate was applied was 25 to 30 cm$^2$. In six preliminary experiments in both dogs and humans, it was determined, by reweighing the surgical gloves after use with the cream, that approximately 20 per cent of the weight of the cream remained on the gloves. For this reason, 20 per cent more cream was initially applied in each experiment.

Blood and urine samples were obtained just before the administration of either the oral or the topical medication and again at 60 or 120 minutes, at which time a synovial fluid aspiration was performed under sterile conditions. The synovial aspirations were performed after careful removal of the topical preparation from the skin. The zero-time samples of blood were used to determine salicylate and $^{14}$C background content; if either was above background, the patient was not given the medication.

Each patient was asked to rate his subjective pain relief from both oral and topical salicylate as improved, worse, or unchanged and to report any adverse effects.

### *Measurement of Salicylate Activity*

All tissue samples were weighed. The material was then homogenized with 20 volumes of 0.1*M* sulfuric acid. Aliquots of the acidified homogenates of each of the tissues (20 to 40 ml) were then extracted three times each with 10 volumes of absolute ether. The ether extracts were combined and mixed with 10 to 15 Gm anhydrous magnesium sulfate with shaking. After 3 hours, the magnesium sulfate was filtered and the anhydrous ether solution concentrated to a small volume. The salicylates from the ether extracts were isolated by thin-layer chromatography. Thin-layer plates (Silica Gel HF, Analabs, North Haven, Conn.) were spotted with the concentrated ether solution containing the salicylates. The plates were developed in methanol-acetic acid-ether-benzene 1-18-60-120, v/v/v/v. For acetylsalicylic acid, the *Rf* was determined to equal 0.76, and for salicylic acid, the *Rf* was 0.85.[17] The radioactive spots were scraped from the plate and reextracted in ether. This ether solution was evaporated and the residue was mixed with 13 ml liquid scintillation phosphor solution (Econofluor, Packard) and assayed for radioactivity in a Packard spectrometer #3375 with pulse height discrimination for $^{14}$C. Counting was for a period sufficiently long to ensure a maximum error of ±2 per cent.

## Results

### *Canine Study*

Table I summarizes the results obtained in the dog study. The blood level of $^{14}$C-

**TABLE II**

**Human Study. Average $^{14}$C-Salicylate Concentrations in Synovial Fluid, Blood and Urine 1 and 2 Hours After Oral and Topical Salicylate Administration**

| | μg $^{14}$C-salicylate/ml | | | |
|---|---|---|---|---|
| | 1 Hour (six patients) | | 2 Hours (four patients) | |
| | Oral | Cream | Oral | Cream |
| Synovial fluid | 0.29 ± 0.03* | 0.16 ± 0.02 | 0.40 ± 0.08 | 0.25 ± 0.04 |
| Blood | 10.27 ± 1.04 | 0.03 ± 0.00 | 10.33 ± 1.06 | 0.08 ± 0.01 |
| Urine | 0.64 ± 0.13 | 0.02 ± 0.01 | 1.45 ± 0.27 | 0.18 ± 0.06 |

* Standard error of the mean.

salicylate at 30 and 60 minutes was 10 to 100 times lower after topical triethanolamine salicylate than after an equimolecular quantity of oral aspirin. Despite this, triethanolamine salicylate application resulted in higher local salicylate concentrations than did oral aspirin. These results clearly suggest that topical triethanolamine salicylate was primarily absorbed locally by direct penetration. As expected, the skin showed the highest salicylate level after topical application. In addition, superior salicylate concentrations were also seen in a number of other tissues including ligament, tendon, cartilage, fascia, and fat pad. The adjacent muscle showed an average of 20 times greater salicylate concentrations from topical triethanolamine salicylate than from oral aspirin. Approximately equal salicylate concentrations were noted in bone, synovial fluid, and synovium.

At distances greater than 10 cm in all directions from the point of cream application, tissue samples showed only trace salicylate levels.

### *Human Study*

Table II summarizes the results obtained in the patient study. Oral $^{14}$C-aspirin was well absorbed into blood and synovial fluid and was excreted in the urine. After triethanolamine $^{14}$C-salicylate application, $^{14}$C-salicylate was found in synovial fluid, indicating that it was absorbed into the joint through the skin. This absorption increased after 2 hours as compared with 1 hour.

The $^{14}$C-salicylate concentrations in synovial fluid after the application of the triethanolamine $^{14}$C-salicylate were found to be approximately 60 per cent of the concentrations found after the oral ingestion of $^{14}$C-aspirin at 1 and 2 hours, respectively. Blood $^{14}$C-salicylate levels remained low after topical triethanolamine salicylate application at both time intervals. The $^{14}$C-salicylate blood levels at each time interval tested were hundreds of times less after topical application than after oral aspirin administration.

Four of six patients reported equal subjective improvement at 1 and 2 hours in the treated knee after both topical salicylate and oral aspirin. There were no cases of gastrointestinal symptoms, skin irritation, or other adverse effects.

## Discussion

Previous animal studies have shown that triethanolamine salicylate is rapidly absorbed through the skin.[8] Higher levels of salicylate were found in the underlying muscle with the topical preparation than with oral aspirin, despite lower blood levels. In humans, significant blood and urinary

salicylate levels were obtained within 3 hours of topical application of triethanolamine salicylate to the skin of the lower extremities.[18] Clinical studies of the effectiveness of triethanolamine salicylate cream have demonstrated favorable results in a variety of localized rheumatic disorders.[7,18] In a double-blind study of patients with rheumatic pain, topical salicylate was found to provide prompt subjective relief with fewer adverse effects than oral aspirin.[18]

The present study was designed to obtain accurate quantitative bioavailability data on the comparative absorption of oral and topical salicylates in specific tissues. For this purpose, $^{14}$C-radioactive tracer methods were used. The use of trace amounts of radioactive materials facilitated the accurate measurement of salicylate levels in the tissues assayed, without significant human exposure. Specifically, information on the ability of salicylates to penetrate the joint space and periarticular tissues was obtained. Our results demonstrate that topical salicylate can be absorbed effectively through the skin achieving significant salicylate levels in the knee joint and surrounding tissues.

In dogs, topical salicylate was clearly superior to oral aspirin in terms of the local tissue concentrations achieved, despite lower blood levels. In canine tendons, for example, the salicylate level obtained after triethanolamine salicylate application was ten times greater than that from oral aspirin. The highest salicylate concentration was found in muscle as observed in previous studies. In addition, intraarticular penetration of salicylate after topical application compared favorably with that after oral aspirin.

In patients with rheumatoid arthritis, the synovial fluid salicylate levels after topical salicylate application were approximately 60 per cent of those found after oral administration. Again, these levels were achieved despite low blood salicylate levels. This further supports direct penetration as the route of absorption. Data obtained after 2 hours suggest that a longer duration of contact of the cream with the skin may increase absorption. After topical triethanolamine salicylate, four of six patients reported definite improvement in local discomfort equal to that after oral aspirin.

The nuclear magnetic resonance spectra data demonstrated that the triethanolamine salicylate preparation is a salt. This salt is lipid soluble and as such could be transported intact through the lipid layers of the skin. This property may explain the high local tissue levels observed despite low blood levels.

We have shown that significant tissue and intraarticular salicylate levels can be achieved with topical triethanolamine salicylate. The lipid solubility of this material permits it to remain near the site of application, from which it appears to be slowly absorbed into the blood stream and excreted. This is a highly desirable feature which permits relief of local discomfort without systemic side effects.

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