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# Nonane enhances propranolol hydrochloride penetration in human skin

Joseph L. Melendres, Avinash Nangia, Lena Sedik, Mitsuhiko Hori and Howard I. Maibach

*Department of Dermatology, University of California School of Medicine, San Francisco, CA (USA)*

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## Summary

We compared propranolol hydrochloride penetration through human skin in the presence and absence of a putative enhancer, nonane. During in vivo experiments, 1 mg of propranolol hydrochloride was administered in 1-methyl-2-pyrrolidone, under occlusive conditions to seven human volunteers. Seven others received the same dose and vehicle in the presence of 4% nonane. Propranolol hydrochloride penetration was determined from recovery of tritiated material following urinary excretion; propranolol penetration increased from $0.705 \pm 0.3878$ to $1.402 \pm 0.8358$% (mean ± SE) of the applied dose (1 mg/2.5 $cm^2$) in the presence of nonane ($p < 0.05$). Of clinical interest, all seven subjects receiving the enhancer sustained moderate to severe erythema and slight edema at the application site. Slight eschar formation was observed in two of these seven volunteers after 24 h of exposure. It is postulated that the reactions were a result of stratum corneum disruption due to vehicle, enhancer or both. For comparison, penetration of propranolol HCl from the same formulations was tested during an in vitro experiment using excised human cadaver skin and phosphate buffered saline receptor fluid. Penetration through a 1 $cm^2$ surface area was determined by recovery of tritiated propranolol from the receptor fluid; propranolol bioavailability was increased from $1.03 \pm 0.61$ to $2.28 \pm 0.17$% ($p < 0.05$) of the applied dose (1 mg/1 $cm^2$).

Propranolol hydrochloride is a well characterized, non-selective β-adrenoreceptor antagonist frequently prescribed for treatment of many cardiovascular disorders. Oral dosage forms are often prescribed by physicians for treatment of arrhythmias, angina pectoris, and hypertension. Delivery through the skin could significantly lessen first-pass metabolism of the drug thereby increasing its bioavailability, and possibly offer prolonged, controlled release. Hence, transdermally administered propranolol may offer the advantage of longer sustained bioavailability at therapeutic levels than in oral doses. Although delivering propranolol transdermally during certain situations is theoretically ideal, delivering it near therapeutic levels is a formidable task. Propranolol HCl is hydrophillic (log $P_{o:w} = -0.45$, solu-

*Correspondence to:* J.L. Melendres, University of California School of Medicine, Department of Dermatology, Box 0989, San Francisco, CA 94143-0989, U.S.A.

bility in water = 20 : 1) (Shaefer, personal communication), hence penetration across the stratum corneum is predicted to be low. Delivery of minimally absorbed drugs may be promoted in the presence of pyrrolidone derivatives (Helgard et al., 1988; Sasaki et al., 1988, 1990). Penetration enhancement of propranolol hydrochloride has also been achieved in vitro with various lipids in a rabbit skin model (Ogiso and Shintani, 1990) and *n*-alkanes and *N*-methylpyrrolidone in hairless mouse and rat skin models (Hori et al., 1990). The mouse and rat skin models showed a 6.5- and 8.2-fold increase in propranolol penetration when in the presence of the nine-carbon *n*-alkane analogs, *n*-nonane and *n*-nonanol, respectively. The following investigation examined propranolol hydrochloride penetration in vitro and in vivo in human skin, and determined to what extent propranolol bioavailability can be increased by nonane.

The experiment was designed to compare the penetration of a propranolol hydrochloride (HCl) control solution in 1-methyl-2-pyrrolidone to penetration of the same propranolol HCl solution containing 4% nonane. The control solution consisted of labeled, L-4-[$^3$H]propranolol HCl (NEN, Burbank, CA) combined with unlabeled, crystalline L-propranolol HCl (Sigma Chemicals, St. Louis, MO) in 1-methyl-2-pyrrolidone (Aldrich Chemical Co., Milwaukee, WI), yielding 10 $\mu$Ci mg$^{-1}$ L-propranolol HCl 400 $\mu$l$^{-1}$ dose$^{-1}$. A similar solution containing nonane enhancer was produced by the same method but contained 4% nonane (v : v) (Aldrich Chemical Co., Milwaukee, WI) again yielding 10 $\mu$Ci mg$^{-1}$ L-propranolol HCl 400 $\mu$l$^{-1}$ dose$^{-1}$.

As a preliminary experiment, penetration of propranolol HCl was measured in vitro using 0.5 mm cadaver skin, dermatomed from the upper thigh of a 69-year-old white female, within 24 h of autopsy. Prior to experiment the skin was stored in phosphate-buffered saline at 5–10°C, cut into eight 4 cm$^2$ pieces, and placed inside one of eight flow-through diffusion cells (Laboratory Glass, Inc., Berkeley, CA); the diameter of the application site was 1 cm$^2$, and phosphate-buffered saline was used as receptor fluid, maintained at 37°C by a water bath in the exterior cell jacket (Bronaugh and Stewart, 1985). Four cells received 400 $\mu$l of the control solution and the other four received 400 $\mu$l of the solution containing nonane. The skin was exposed to the test material for a total of 24 h, and 5 ml receptor fluid fractions were collected every 4 h for 120 h by a fraction collector (ISCO, Inc., Lincoln, NE). The receptor fluid samples were combined with 10 ml of liquid scintillation cocktail (Universol ES, ICN Biomedicals, Costa Mesa, CA). At the end of 24 h, the skin surface was washed three times with alternating soap-water (50% v : v, ivory soap) and water washes. After 120 h the skin was removed from the diffusion cell apparatus and digested in 3 ml of soluene cocktail (Packard Technologies, Arlington Heights, IL) for 48 h or until a homogeneous solution was achieved. 300 $\mu$l of 80% acetic acid was added followed by 10 ml of liquid scintillation cocktail (Ultima Gold, Packard Technologies) in preparation for scintillation counting. Receptor fluid samples, surface washes, and skin digests were counted in duplicate in a liquid scintillation counter (Packard 1500, Arlington Heights, IL). The amount of propranolol HCl that penetrated was determined from the cumulative amount of tritiated material detected in the receptor fluid after 120 h of sampling. The data that follows was calculated from the percent of the applied dose recovered in the receptor fluid based on penetration through a 1 cm$^2$ area. Since the skin was used from the same anatomical site and source, the control vs nonane penetration data were compared by employing a Student's *t*-test for paired samples.

14 healthy volunteers (ages 21–50 years) were dosed with one of either test solution after informed consent was obtained. Seven volunteers received the control solution, and seven received the test solution containing nonane. 400 $\mu$l of solution was applied to the absorbent pad inside an occlusive polypropylene chamber (Hilltop Research, Inc., Miamiville, OH) and applied to a pre-marked 2.5 cm$^2$ site on the ventral forearm, approx. 4 inches from the anticubital fossa. The chamber was secured to the skin by application of an adhesive dressing (Tegaderm, 3M Medical Surgical Division, St. Paul, MN) on top of the chamber.

TABLE 1

*In vitro propranolol HCl recovery ($\mu g / cm^2$ of skin determined from recovery of L-4-[$^3$H]propranolol HCl)*

| Cell | 1 | 2 | 3 | 4 | Mean | ±SD |
|---|---|---|---|---|---|---|
| Control group | | | | | | |
| Washes | 918.78 | 861.56 | 911.65 | 896.88 | 897.22 | 25.46 |
| Skin | 1.60 | 0.12 | 0.18 | 10.61 | 3.13 | 5.04 |
| Rec. Fluid | 15.13 | 14.34 | 9.71 | 1.84 | 10.26 | 6.10 |
| Nonane group | | | | | | |
| Washes | 892.46 | 846.42 | 985.67 | 893.20 | 926.94 | 68.69 |
| Skin | 5.16 | 2.37 | 1.01 | 2.08 | 2.66 | 1.77 |
| Rec. Fluid | 24.01 | 22.91 | 24.08 | 20.36 | 22.84 | 1.74 |

All volunteers were exposed to the material for 24 h after which the chamber was removed and the site washed with two 50% (v:v) ivory soap-water washes and three water washes. The skin was air exposed for the remaining 6 days in order to minimize potential irritancy that may result from long-term occlusion. Urine samples were collected from each volunteer every 24 h for 7 days. Volunteers were provided collection containers (Fisher Scientific; Santa Clara, CA), and were instructed to void each day's urine into designated containers for 7 days. The volume voided by each volunteer was measured in a graduated cylinder. Duplicate 5 ml aliquots were collected and combined with 10 ml of liquid scintillation cocktail. Tritium content was determined by liquid scintillation counting. Absorption of L-propranolol HCl was determined from recovery of tritiated material following urinary excretion (Wester and Maibach, 1989). The percent dose calculated from the data was corrected for excretion of radiolabeled propranolol through other routes by inclusion of excretion data following intravenous administration of L-[$^3$H]propranolol HCl in rhesus monkeys. Specifically, the percent of the applied dose detected in urine following topical administration to volunteers was divided by the percent recovered following parenteral administration to rhesus monkeys (mean % recovered in urine ± SD = 89.1 ± 7.7%). The mass absorbed was calculated from the percent of applied propranolol HCl that penetrated 2.5 $cm^2$ area of skin. The absorption data were compared by a Wilcoxon signed-rank test.

Table 1 compares the recovery of propranolol HCl in $\mu g/cm^2$ of skin from each formulation. The amount of drug absorbed was calculated from the cumulative recovery of radiolabeled material in the receptor fluid. The data indicate that more propranolol HCl was recovered in the presence of nonane (mean ± SD = 22.84 ± 1.74 $\mu g/cm^2$) than without enhancer (10.26 ± 6.10 $\mu g/cm^2$) ($p < 0.05$). There was no difference in the amount of propranolol removed by washing following 24 h of exposure or the amount that remained in the skin after 120 h ($p > 0.05$). Mass balance for the control and nonane groups was 91.06 ± 2.54 and 95.24 ± 7.02% of the applied dose, respectively.

Table 2 compares propranolol HCl penetration based on urinary excretion. The data show

TABLE 2

*Cumulative in vivo penetration of propranolol HCl ($\mu g$ per $cm^2$ of skin determined from urinary excretion of L-4-[$^3$H]propranolol HCl)*

| Subject | Propranolol HCl | Propranolol HCl + nonane |
|---|---|---|
| 1 | 1.84 | 4.41 |
| 2 | 3.58 | 3.41 |
| 3 | 2.34 | 6.97 |
| 4 | 1.29 | 5.38 |
| 5 | 2.35 | 4.67 |
| 6 | 4.29 | 10.76 |
| 7 | 4.04 | 3.63 |
| Mean | 2.82 | 5.61 |
| ±SD | 1.15 | 2.57 |

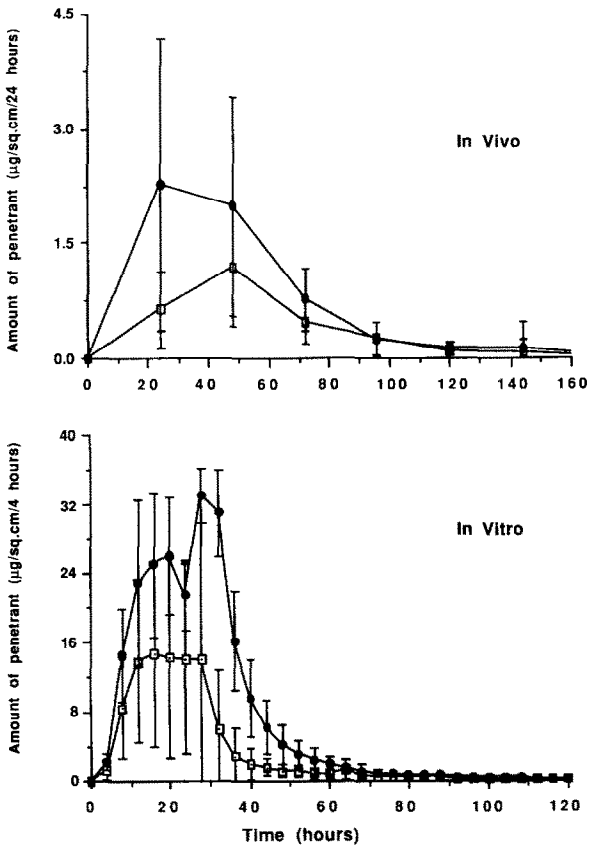


Fig. 1. Time course of propranolol HCl penetration through human skin. (□) Control formulation without nonane enhancer; (●) formulation containing nonane.

significantly more propranolol HCl penetrated the skin of volunteers from the enhancer group ($5.6 \pm 2.6$ $\mu g/cm^2$) than in the control group ($2.8 \pm 1.2$ $\mu g/cm^2$) ($p < 0.05$). Fig. 1 summarizes the amount of radiolabeled propranolol HCl per $cm^2$ that penetrated over time during in vitro and in vivo experiments.

As expected, penetration of propranolol HCl in the absence of the nonane enhancer was low during in vivo and in vitro experiments ($2.82 \pm 1.15$ and $10.26 \pm 6.10$ $\mu g/cm^2$, respectively). During in vitro assays, the average amount of propranolol HCl absorbed per $cm^2$ doubled in the presence of nonane ($22.84 \pm 1.74$ $\mu g/cm^2$). Although the sample size was small ($n = 4$), we view the results of this experiment with interest. This in vitro study suggests that the penetration of propranolol HCl through the skin is enhanced by nonane and this is furhter validated by our observations in vivo. Note that in vivo, the average amount of propranolol HCl absorbed per $cm^2$ also doubled ($5.61 \pm 2.57$ $\mu g/cm^2$) ($p < 0.05$) in the presence of nonane.

Thus, in vitro and in vivo assays indicate that nonane can increase propranolol HCl bioavailability and enhance penetration through human skin. Note, however, that comparison of these two models must be approached with caution. During in vitro assays, the application of drug and vehicle (10 $\mu Ci\ mg^{-1}$ 400 $\mu l^{-1}$ $dose^{-1}$) was the same as in vivo; however, the surface area was 1 $cm^2$ of excised, unoccluded cadaver skin. The difference in concentration of propranolol HCl per surface area of skin (in vitro, 2.5 $\mu g\ \mu l^{-1}\ cm^{-2}$; in vivo, 0.1 $\mu g\ \mu l^{-1}\ cm^{-2}$) possibly accounts for the higher cumulative penetration of drug in vitro from both control and nonane vehicles relative to in vivo.

Exhaustive pharmacokinetic comparisons between the models were not intended during the design of the study nor should they be attempted. The in vitro model serves merely as an efficient means for comparison. These in vitro results from human skin generally agree with previously reported in vitro mouse skin models that employed the same vehicle and *n*-alkane enhancer (Hori et al., 1990).

Of clinical interest, during the first 4 h of exposure, three volunteers reported feeling some discomfort at the application site. By the fifth hour this subsided, and the volunteers continued to participate in the study. These three volunteers received propranolol HCl with nonane. Inspection of each volunteer's application site after 24 h exposure by a dermatologist revealed no evidence of irritancy in any of the volunteers receiving the control formulation other than very slight erythema attributable to occlusion of the skin. However, all volunteers receiving propranolol HCl with nonane sustained moderate to severe erythema and slight edema at the application site. Two volunteers sustained slight eschar formation; the volunteers were two of three that complained of a stinging sensation localized at

the application site. The third complaint came from a volunteer who sustained slight edema at the application site, with no eschar formation.

The irritancy observed in volunteers receiving the propranolol + nonane in NMP solutions is possibly explained by any of the following theories:

(i) Nonane enhanced penetration by extensive barrier alteration of the stratum corneum and epidermis, and the irritancy sustained by the volunteers was solely a result of the enhancer's physicochemical properties. The enhancer acted as an irritant and decreased the barrier properties of the skin without destroying it.

(ii) Nonane enhanced penetration of propranolol HCl, and the increased bioavailability was sufficient to induce irritancy in the skin. Previous incidence of skin irritancy attributable to propranolol and other $\beta$-adrenoreceptor antagonists (i.e., timolol, mepindolol) have been reported (De Mey et al., 1989a,b; Kubota et al., 1991). However, these experiments employed the free base form of the drug at higher concentrations, which greatly differ physicochemically from the salt form employed in this study (i.e., log $P_{o:w}$, $pK_b$). The exact mechanism of skin irritancy attributable to $\beta$-adrenoreceptor antagonists remains unresolved.

(iii) It is possible that the enhancer increased the penetration of *N*-methylpyrrolidone and contributed to irritating the skin. Recently, penetration enhancement of vehicles during hairless mouse skin assays in vitro has been reported (Hori et al., 1992). Since 1-methyl-2-pyrrolidone is a known irritant it seems likely that the irritancy we observed was an attribute of the vehicle.

(iv) Finally, the remote possibility of synergy between all components in the formulation to induce irritancy cannot be ruled out.

Our observations indicate that propranolol HCl penetration through human skin can be enhanced by delivering it in a 1-methyl-2-pyrrolidone vehicle containing 4% nonane. Unfortunately, increasing its transdermal delivery to near or beyond therapeutic levels in this manner would be impractical because of irritancy. We postulate that the irritancy sustained by the volunteers receiving enhancer was more likely an attribute of the enhancer and/or vehicle than the drug itself since the amount of propranolol applied was merely 400 $\mu g/cm^2$ (1 mg/2.5 $cm^2$). Nevertheless, irritancy attributable to all components of the formulation remains possible. Our hypotheses would be more conclusively tested in assays designed specifically to examine irritancy. Consequently, even the enhanced penetration noted here would not yield therapeutic plasma levels.

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