Relationship between the Amount of Propranolol Permeating through the Stratum Corneum of Guinea Pig Skin after Application of Propranolol Adhesive Patches and Skin Irritation

Ichiro Kobayashi,* Kyoko Hosaka,* Takashi Ueno,a Hiroki Maruo,a Masashi Kamiyama,a Chohachi Konno,a and Munekazu Gemba

Toxicological Research Center, Nitto Denko Corporation,* 1-2, Shimohozumi 1-chome, Ibaraki, Osaka 567, Japan and Osaka University of Pharmaceutical Sciences,a Kawai, Matsubara, Osaka 580, Japan.

Received November 20, 1995; accepted January 25, 1996

In the present study we evaluated the relationship between the cumulative amount of propranolol permeating through the stratum corneum and the formation of erythema, a skin irritation reaction, after transdermal application of adhesive patches containing propranolol to the skin of guinea pigs. The intensity of erythema was expressed in terms of a* values measured with a chromameter. The a* values increased in guinea pigs after application of the adhesive patches containing 0.4 mg/cm² of propranolol to the skin. Since the adhesive patches showed good adhesion to the skin (propranolol content is less than the saturated concentration in the adhesive base) and the cumulative amount of propranolol permeating through the stratum corneum is small, the development of erythema was considered to be mainly due to physical factors such as peeling. Even in adhesive patches containing 0.8 mg/cm² or 1.2 mg/cm² of propranolol, a* values increased, although adhesion to the skin is low because of crystallization of propranolol in the adhesive base. On the other hand, in these two adhesive patches, the cumulative amount of propranolol permeating through the stratum corneum increased up to 24h after application. These findings suggest that the skin irritation reaction is due to propranolol mainly absorbed transdermally, because there is a high correlation between the cumulative amount of propranolol permeating through the stratum corneum and the a* values (r = 0.928).

Key words propranolol; erythema; skin irritation; chromameter

In recent years, external preparations with systemic effects have been used as transdermal therapeutic drugs. β-Blockers are one group of drugs for transdermal therapeutic delivery.

β-Blockers are widely used for the treatment of hypertension due to various cardiovascular system disorders. Percutaneous absorption of these β-blockers has been reported in many in vitro studies and with diffusion cells such as the Franz type. In in vivo studies, Krishna and Pandit have reported that the bioavailability of propranolol hydrochloride after transdermal application is superior to that after oral administration.

On the other hand, skin irritation by β-blockers has been investigated in a skin irritation study with a triglyceride oil solution of timolol in rats by Cargill et al. They reported that the development of a skin irritation reaction was concentration-dependent. Krishna and Pandit also reported that a skin irritation reaction was observed when a hydroxypropyl methylcellulose patch containing propranolol hydrochloride was applied to the skin. Hirvonen et al. have reported that there was a correlation between the permeability coefficient and the skin irritation of propranolol hydrochloride and timolol tartrate in rabbit pinna skin. Furthermore, Kubota et al. have reported that there was correlation between plasma concentrations and the parameters reflecting the intensity of erythema occurring in volunteers in a study of skin irritation produced by acrylic adhesive patches containing timolol. Mey et al. also reported that a skin irritation reaction was observed after transdermal administration of patches containing propranolol.

These findings suggest that the amount of drug absorbed transdermally is a major factor for the development of a skin irritation reaction after transdermal application of a drug. However, there are a limited number of reports which describe the relationship between the amount of drug absorbed from the skin and the skin irritation reaction.

In the present study we used propranolol as a model drug. We investigated the relationship between the amount of propranolol absorbed from the skin and the a* value, the redness of the skin (measured with a chromameter) by applying acryl adhesive patches containing propranolol to guinea pigs.

MATERIALS AND METHODS

Materials Propranolol (PRL) was prepared by the conventional method from dl-propranolol hydrochloride purchased from Sigma Chemical Co., (St. Louis, MO, U.S.A.). PRL ethanol solutions were prepared as 1, 2.5, 5, 7.5 and 10% concentrations. As for the PRL patch (PRL/P), 0.1 ml of 10% ethanol solution was applied to the lint cloth of an adhesive plaster for the patch test (diameter: 16 mm, Torii & Co., Ltd., Tokyo, Japan) and evaporated by air drying for 30 min (content of PRL: 5 mg/cm²). As for the PRL adhesive patches (PRL/AP), PRL was mixed with acrylic adhesive A-I and laminated (thickness: 40 μm) on polyester film (thickness: 9 μm) by a conventional method to prepare PRL/AP containing 0.4, 0.8 and 1.2 mg/cm² PRL (hereinafter referred to as 0.4-PRL/AP, 0.8-PRL/AP and 1.2-PRL/AP). As controls, adhesive patches without PRL (0-PRL/AP) were prepared.

Animals Male Std/Hartley strain guinea pigs (clean) aged 4—5 weeks were purchased from Japan SLC Inc., (Hamamatsu, Japan). After acclimatization for 11—15 d, animals weighing 270—420 g were used.

Correlation between a* Values Measured with a Chromameter

* To whom correspondence should be addressed.

© 1996 Pharmaceutical Society of Japan
Table 1. Evaluation of Skin Reactions

<table>
<thead>
<tr>
<th>Skin reaction</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Erythema and eschar formation</td>
<td></td>
</tr>
<tr>
<td>No erythema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight erythema</td>
<td>1</td>
</tr>
<tr>
<td>Well defined erythema</td>
<td>2</td>
</tr>
<tr>
<td>Moderate to severe erythema</td>
<td>3</td>
</tr>
<tr>
<td>Severe erythema (beet redness) to slight eschar formation (injuries in depth)</td>
<td>4</td>
</tr>
<tr>
<td>B. Edema</td>
<td></td>
</tr>
<tr>
<td>No edema</td>
<td>0</td>
</tr>
<tr>
<td>Very slight edema (barely perceptible)</td>
<td>1</td>
</tr>
<tr>
<td>Slight edema</td>
<td>2</td>
</tr>
<tr>
<td>(edges of area well defined by definite raising)</td>
<td></td>
</tr>
<tr>
<td>Moderate edema (edema approximately 1 mm)</td>
<td>3</td>
</tr>
<tr>
<td>Severe edema (raised more than 1 mm and extending beyond area of exposure)</td>
<td>4</td>
</tr>
</tbody>
</table>

Draize J. H. et al. \(^{18}\)

**mameter and Visual Scores** Plasters for the patch test having different concentrations of PRL in ethanol solutions were applied tightly to previously shaved bilateral flanks of guinea pigs for 1, 2, 8 or 16 h. The redness of the skin following application of the plaster, the \(a^*\) value, was measured with a Minolta chromameter CR-200 (Tokyo, Japan) and was also evaluated by a visual score according to the criteria of Draize et al. \(^{18}\) shown in Table 1. The \(a^*\) values measured before application of plasters for the patch test were used as the initial values. These measurements and evaluations were made with respect to time after the end of the application and the correlation between the measurements and evaluations was examined. The \(a^*\) values show changes in colors from red to green of the \(L^*a^*b^*\) color space parameters described in JIS Z 8729 recommended by the Commission Internationale d’Eclairage (CIE) in 1976.

**Adhesion of PRL/AP to Guinea Pig Skin** A piece of PRL/AP (10 mm × 50 mm) was applied to the previously shaved flank in the dorsal region and the peeling force was measured 30 min after application using a digital gauge (Model 7502B, Aiko Engineering, Tokyo, Japan) under pentobarbital sodium anesthesia (Nembutal\(^{18}\), Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) and at a peeling rate of 300 mm/min and an angle of 180°.

**Percutaneous Absorption and Skin Irritation Following Transdermal Application to Guinea Pig Skin** Two pieces (right and left) of PRL/AP or PRL/P (diameter: 16 mm) were applied tightly to previously shaved bilateral flanks of guinea pigs for 1, 2, 4, 8, 16 and 24 h. At the end of each application time, PRL/AP and PRL/P were removed and the \(a^*\) values of the skin at the application site were measured with a Minolta chromameter CR-200, and a visual evaluation was made according to the criteria of Draize et al. \(^{18}\). The initial value of \(a^*\) was measured before application of PRL/AP or PRL/P. After the end of the measurement of the \(a^*\) values and visual evaluation, guinea pigs were killed by CO\(_2\) gas, and the corneum at the application site was peeled 10 times with cellophane tape. A skin sample (diameter: 20 mm) after removal of the corneum was taken from the stripped skin.

The extraction of PRL from the collected PRL/AP, PRL/P and corneum collected with cellophane tape was as follows: Add 20 ml, 25 ml and 10 ml ethanol, respectively, to the samples, shake at 37 °C for 60 min, repeat 3 times, and recover ethanol solutions. The extraction of PRL from the stripped skin was as follows: Slice the stripped skin, homogenize the slices in 6 ml 0.1 n-sodium hydroxide solution with an ultradisperser (LK-21 type, Yamato Scientific Co., Ltd., Tokyo, Japan), mix with 40 ml ethyl acetate, centriﬁuge at 3000 rpm for 5 min and recover ethyl acetate solution. For the precipitate, add 40 ml ethyl acetate again and repeat the process, recover ethyl acetate solution and mix with the previously obtained PRL. After evaporating the recovered ethanol and ethyl acetate under reduced pressure at 40 °C, dissolve PRL in 10 ml ethanol again and quantitate PRL by HPLC (column, Cosmosil 10C18, Nacalai Tesque Inc., Kyoto, Japan; UV wavelength, 290 nm; ﬂow rate, 1.5 ml/min; mobile phase, acetonitrile:distilled water: phosphoric acid = 30:70:0.2; injection volume, 10 µl).

The cumulative amount released was obtained by subtracting the PRL content of PRL/AP or PRL/P collected after application from the PRL content of the unused PRL/AP or PRL/P; the PRL content in the peeled corneum was regarded as the PRL content of the stratum corneum and the PRL content of the stripped skin was regarded as the PRL content of the skin from which the stratum corneum was removed. The PRL amount obtained by subtracting the PRL content of the stratum corneum from the cumulative amount released was regarded as the cumulative amount of PRL permeating through the stratum corneum.

**Statistical Analysis** The results were expressed as means±S.E., except for the correlation between the \(a^*\) values and the visual evaluation which was expressed as a mean±2 S.D. The level of significance between the values in each group at the same application time was analyzed by the unpaired Student’s t-test (p<0.05). When the correlation between the values was calculated, linear regression was used.

**RESULTS**

Relationship between \(a^*\) Values and Visual Scores When PRL Was Applied Transdermally to the Skin The relationship between \(a^*\) values and visual scores made according to the criteria of Draize et al. \(^{18}\) when the ethanol solution of each concentration of PRL was applied to the skin of guinea pigs is shown in Fig. 1. Erythema associated with formation of edema was included in the visual evaluation. This figure shows the high correlation between the \(a^*\) values and erythema scores due to visual evaluation (r = 0.988).

Adhesion of PRL/AP to Guinea Pig Skin The peeling force of PRL/AP to the skin of guinea pigs was 29.7 g/cm width for 0-PRL/AP, 22.5 g/cm width for 0.4-PRL/AP, 9.4 g/cm width for 0.8-PRL/AP and 9.5 g/cm width for 1.2-PRL/AP. PRL crystals were observed in the 0.8-PRL/AP and 1.2-PRL/AP.

Cumulative Amount of PRL Released from PRL/AP Changes in the cumulative amounts of PRL released from PRL/AP and PRL/P with time are shown in Fig. 2. The
cumulative amount of PRL released from PRL/AP (except 0.4-PRL/AP) increased significantly when the PRL content in the adhesive patches was greater. The cumulative amounts released 24 h after application were 35 μg/cm² for 0.4-PRL/AP, 270 μg/cm² for 0.8-PRL/AP and 640 μg/cm² for 1.2-PRL/AP. PRL was scarcely released from PRL/P.

**PRL Content in the Stratum Corneum** Changes in the PRL content in the stratum corneum after application of PRL/AP and PRL/P with time are shown in Fig. 3. The PRL content of the stratum corneum after application of 0.4-PRL/AP was almost the same as that after application of PRL/P and the respective PRL contents of the stratum corneum at 24 h after application were 16 and 21 μg/cm². The PRL content of the stratum corneum after application of 0.8-PRL/AP and 1.2-PRL/AP increased from 1 to 4 h after application, and peaked at 73 and 160 μg/cm², respectively, 16 h after application. The PRL content of the stratum corneum after application increased significantly with increasing PRL content in the adhesive patches.

**PRL Content in the Stripped Skin** Changes in the PRL content of the stripped skin after application of PRL/AP and PRL/P are shown in Fig. 4. The PRL content of the stripped skin increased from 4 h after application of 0.4-PRL/AP and was 10 μg/cm² 24 h after application. The PRL content in the stripped skin increased linearly from 1 to 8 h after application of 0.8-PRL/AP and 1.2-PRL/AP and was 45 and 65 μg/cm², respectively, 8 h after application. The PRL content of the stripped skin was 4 μg/cm² 24 h after application of PRL/P. The PRL content of the stripped skin increased significantly as the PRL content of the adhesive patches increased.

**Redness of the Skin** Changes in the a* values at the application site of PRL/AP and PRL/P with time are shown in Fig. 5. The a* values were as high as 7.7 h after application of 0-PRL/AP and as high as 7.9 h after application of 0.4-PRL/AP, and thereafter the a* values scarcely increased. The a* value was 6.8 h after application of 0.8-PRL/AP and thereafter slightly increased. The a* value increased linearly from 7.3 h after application to 11 24 h after application of 1.2-PRL/AP. No clear-cut increase in a* was observed for PRL/P.

**Visual Observation** The results of the evaluation of the skin irritation reaction due to PRL/AP and PRL/P according to the criteria of Draize et al. are shown in Table 2. Very slight erythema was observed in all the
Fig. 4. Changes in PRL in the Stripped Skin of Guinea Pigs after Application to the Skin of Guinea Pigs

PRL/AP containing 0.4 (●), 0.8 (△) and 1.2 (■) mg/cm² PRL and PRL/P (□) were applied to the respective sites for 1, 2, 4, 8, 16 and 24 h. The amount of PRL in the stripped skin means that in the skin at the application site obtained after peeling 10 times with cellophane tape. Each point and bar represent the mean ± S.E. of 4 animals. *p < 0.05, **p < 0.01 vs. 0.4-PRL/AP, *p < 0.05, **p < 0.01 vs. 0.8-PRL/AP determined by the unpaired Student’s t-test.

Fig. 5. Changes in $a^*$ Values at the Site of PRL/AP Application to the Skin of Guinea Pigs

PRL/AP containing 0.4 (●), 0.8 (△) and 1.2 (■) mg/cm² PRL, the placebo adhesive tape (○) and PRL/P (□) were applied to the respective sites for 1, 2, 4, 8, 16 and 24 h. The $a^*$ value indicates the value measured with a Minolta chromameter CR-200 and reflects the redness of the skin. Each point and bar represent the mean ± S.E. of 4 animals.

animals 2 and 4 h after application of 0-PRL/AP, and erythema was clearly observed from 8 h after application. The intensity of erythema after application of 0.4-PRL/AP was almost the same as that after application of 0-PRL/AP. Very slight erythema was observed from 1 to 2 h after application of 0.8-PRL/AP, and clear-cut erythema was observed 4 h after application and was observed in all the animals 24 h after application. The intensity of erythema after application of 1.2-PRL/AP was almost the same as that after application of 0.8-PRL/AP. Very slight edema was observed in 1 animal 24 h after application of 1.2-PRL/AP. Very slight erythema was observed in 1 animal 4 and 16 h after application of PRL/P.

### DISCUSSION

Generally, the formation of erythema is evaluated by visual observation. However, the formation of erythema is more objectively evaluated by $a^*$ values measured with a chromameter than by visual scores, in which subjective judgement may possibly be involved. In addition, $a^*$ values can be expressed as serial figures for the redness of the skin and are considered to be effective for a theoretical analysis of skin irritation reactions. Rohold et al. 13) have reported the relationship between $a^*$ values and visual scores in the contact sensitization of nickel sulfate. Hirvonen et al. 10) have reported the relationship between the permeability coefficient and E values indicating color changes in the skin obtained from $L^*a^*b^*$ color space parameters in a primary irritation study of propranolol hydrochloride and timolol tartrate. They also suggested...
that when $E$ values increased, skin irritation (redness) by visual observation increased.

When we investigated the relationship between $a^*$ values and visual scores according to Draize’s criteria in primary skin irritation by PRL, a high correlation was clearly observed as shown in Fig. 1. We used mainly the $a^*$ value for the intensity of erythema thereafter.

In PRL/P, considered not to have any skin irritation due to the adhesive, a clear-cut increase in $a^*$ values was not observed in guinea pigs even 24 h after application. This result is supported by the small amount of PRL absorbed into the skin.

In 0.8-PRL/AP and 0.4-PRL/AP, there was little or no penetration of PRL from the adhesive base to the skin of the guinea pigs. The amount of PRL permeating through the stratum corneum was little if any. However, the $a^*$ values from 1 to 24 h after application were slightly higher than the initial value, i.e., about 8. Since the peeling forces of these adhesive patches were 29.7 g/cm width and 22.5 g/cm width, respectively, and there was sufficient adhesion to the skin, the increase in $a^*$ values is considered to be mainly due to physical factors such as peeling.

In 0.8-PRL/AP and 1.2-PRL/AP, the cumulative amount of PRL released from the adhesive base increased with time (Fig. 2). The PRL content in the stratum corneum became constant from 4 h after application and tended to decrease 24 h after application (Fig. 3). The PRL content in the stripped skin reached a maximum 8 h after application and decreased 16 h after application and, thereafter, the levels tended to remain constant up to 24 h after application (Fig. 4). The decrease in the PRL content of the stratum corneum 24 h and the stripped skin 16 h and 24 h after application is considered to be mainly due to a decrease in the amount of PRL released per hour. The cumulative amount of PRL permeating through the stratum corneum, calculated from the cumulative amount of PRL released and its content in the stratum corneum, increased almost linearly up to 24 h after application of 0.8-PRL/AP and 1.2-PRL/AP (Fig. 6). These results suggest that PRL is absorbed mainly into the skin tissues up to 8 h after application and the PRL transported to the blood stream increases thereafter. The $a^*$ values increased almost linearly from 1 to 24 h after application (Fig. 5). In these PRL/AP, the peeling force was 9.4—9.5 g/cm width and was lower than that of 0.0-PRL/AP and 0.4-PRL/AP. Adhesion of PRL/AP to the skin was rare. This is considered to be due to crystallization of PRL in the adhesive base. From these findings, it is clear that the increase in $a^*$ values after application of 0.8-PRL/AP and 1.2-PRL/AP was scarcely attributable to physical factors such as peeling, but mainly due to percutaneous absorption of PRL after application.

Hirvonen et al.\(^{10}\) have reported that there is a correlation between the changes in color of rabbit pinna skin and the penetration coefficient of propranolol hydrochloride or timolol tartrate. In addition, Kubota et al.\(^{11}\) have reported that there is a correlation between the intensity of erythema and plasma concentrations of timolol. We thought before the start of the study that the amount of PRL in the skin was highly correlated with the intensity of the skin irritation reaction. Then, we investigated the relationship between the PRL content of the stripped skin and the $a^*$ values after application of 0.8-PRL/AP and 1.2-PRL/AP which had scarcely any adhesion. As a result, the PRL content of the stripped skin increased linearly up to 8 h after application and showed a high correlation with the $a^*$ values ($r = 0.872$);
however, there was no clear correlation up to 24h after application. On the other hand, when the relationship between the cumulative amount of PRL permeating through the stratum corneum and $a^*$ value was investigated, there was a high correlation between the cumulative amount of PRL permeating through the stratum corneum and the $a^*$ values ($r = 0.928$), as shown in Fig. 7.

These results suggest that a test drug which permeates through the stratum corneum with time becomes a chemical irritation factor and leads to erythema, and there is a linear relationship between the amount of PRL permeating and the intensity of the erythema. This relationship will be further investigated for other $\beta$-blockers.

REFERENCES