SKIN TOXICITY OF PROPRANOLOL IN GUINEA PIGS

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ABSTRACT — The skin toxicities of propranolol were studied in guinea pigs. In the primary and cumulative skin irritation studies, the skin reactions and the histopathological changes were observed in all animals treated with propranolol, and those tended to increase with the increase of propranolol dosage. The skin reactions increased with the application times of propranolol up to 7 days in the cumulative skin irritation study. In the skin sensitization, the phototoxicity and the skin photosensitization studies, no skin reactions were observed in any animals used in the studies. These results indicate that propranolol caused skin irritation, but was negative for skin sensitization, phototoxicity and skin photosensitization in guinea pigs.

KEY WORDS: Propranolol, Skin toxicity, Guinea pig, Skin irritation, Skin sensitization, Phototoxicity, Photosensitization

INTRODUCTION

Propranolol, a β -adrenoceptor blocking agent, is widely used for the treatment of hypertension and heart diseases such as arrhythmia, and angina pectoris. In general, propranolol has been used as an oral or injectable drug. Recently, studies on the skin permeability of propranolol have been conducted (Corbo *et al.*, 1989; Thacharodi and Rao, 1996). Krishna and Pandit (1994 and 1996) and De Mey *et al.* (1989) have reported that the transdermal delivery system (TDS) for propranolol causes inflammatory responses at the application site. We have demonstrated that the skin irritation correlated with the percutaneous absorption of propranolol (Kobayashi *et al.*, 1996). However, there are very few reports which have described the skin toxicity induced by transdermal administration of propranolol. We investigated in the present studies the primary skin irritation, the cumulative skin irritation, the skin sensitization, phototoxicity and skin photosensitization of propranolol to utilize the results of those studies for the development of TDS for propranolol.

MATERIALS AND METHODS

Materials

dl-Propranolol hydrochloride (PRL-HCl), 2,4-dinitrochlorobenzene (DNCB), sodium lauryl sulfate (SLS), olive oil, ethanol and acetone (reagent grade) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Propranolol base (PRL) was prepared from *dl*-propranolol hydrochloride by alkalization with sodium hydroxide according to the method described in the Japanese Pharmacopoeia (JP). 8-Methoxypsoralen (8-MOP) was purchased from Sigma Inc. (MO, USA). 3,5,4'-Tribromosalicylanilide (TBS) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Freund's complete adjuvant (FCA) was purchased from Difco Laboratory (MI, USA). Distilled water (d-water) for injection (JP grade) was purchased from Fuso Pharmaceutical Industries, Ltd. (Osaka, Japan).

Animals

Four to five-week-old male Std: Hartley guinea

pigs were purchased from Japan SLC, Inc. (Hamamatsu, Japan). After 10-18 days for acclimatory rearing, those weighing 346-487 g were used. The animal room was maintained at $22 \pm 2^\circ\text{C}$ and $55 \pm 10\%$ humidity with a 12-hr light(7:00~19:00)/12-hr dark (19:00~7:00) cycle. The animals were individually housed in stainless steel hanging cages (W260×L400×H200 mm) and given solid food (Labo G standard, Nihon Nosan Kogyo, Yokohama, Japan) and tap water *ad libitum* throughout the study.

Primary irritation study

The animals were assigned to 3 groups consisting of 8 animals each. In the first group, one side of the previously shaved bilateral flank of each animal had an adhesive bandage applied for a patch test (Torii Co., Ltd., Tokyo, Japan, hereafter designated as patch) which contained 0.1 ml of 2.5% PRL in ethanol (dose of PRL:1.25 mg/cm²), and the other side had a patch applied containing 0.1 ml of 5% PRL in ethanol (dose of PRL:2.5 mg/cm²) for 24 hr. In the second group, the same procedure was carried out with 10% PRL in ethanol (dose of PRL: 5 mg/cm²) and ethanol. In the third group, the same procedure was carried out with 5% SLS in d-water (dose of SLS : 2.5 mg/cm²) and d-water. At 24, 48 and 72 hr after patch application, the conditions of the skin were observed visually and evaluated according to the criteria of Draize *et al.* (1944) as shown in Table 1. After the patch application, two animals at 24 hr and six animals at 72 hr were bled to death, and the skins of the application sites were cut out for histopathological examination. The sections of the skin were stained with hematoxylin and eosin by the ordinary method, and subjected to histopathological examination. The scores of changes in histopathological findings were: 0; no marked change, 1; very slight changes, 2; slight changes, 3; moderate changes, and 4; severe changes. The histopathological examination was subcontracted to Nippon Experimental Medical Research Institute Co., Ltd. (Gunma, Japan).

Cumulative irritation study

The animals were assigned to 4 groups of 6 animals each. One side of the previously shaved bilateral flank of each animal had a patch applied which contained 0.1 ml of 2.5, 5 or 10% PRL in ethanol, and the other side had a patch applied which contained 0.1 ml of ethanol for 24 hr. After patch application, the conditions of the skin were observed visually and evaluated according to the criteria of Draize *et al.* (1944). The patch application and the evaluation of the skin reac-

tion were repeated every day for 14 days. In the control group, the same procedure was carried out with 2.5% SLS in d-water (dose of SLS : 1.2 mg/cm²) and d-water. After the final evaluation of skin reaction, the animals were bled to death, and the skins of the application sites were subjected to histopathological examination in the same manner as the methods of the primary irritation study.

Skin sensitization study

The study was conducted according to the modified method of the Maximization Test (Magnusson and Kligman, 1969). The animals were assigned to PRL sensitization groups of 10 animals and PRL non-sensitization, DNCB sensitization and DNCB non-sensitization groups of 5 animals each. The previously shaved dorsal neck region (approximately 2×4 cm) was used as the injection site, with three sites each on the left and right sides of the back median line as the axis of symmetry. Each 0.1 ml of FCA emulsion (equi-volume mixture of d-water and FCA), 0.1 ml of 10% PRL-HCl in d-water and 0.1 ml of a mixed emulsion of 20% PRL-HCl in d-water and FCA was intradermally injected to the injection sites symmetrically on the left and right sides. After 1 week, the previous injection sites

Table 1. Evaluation of skin reactions.

a)Erythema and Eschar Formation	
No erythema	0
Very slight erythema(barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema(beet redness) to slight eschar formation(injuries in depth)	4
Total possible erythema score	4
b)Edema Formation	
No edema	0
Very slight edema(barely perceptible)	1
Slight edema(edges of area well defined by definite raising)	2
Moderate edema(area raised approximately 1 mm)	3
Severe edema(raised more than 1 mm and extending beyond area of exposure)	4
Total possible edema score	4
$\Sigma [(a)+(b)]$	
Mean total score = $\frac{\Sigma [(a)+(b)]}{N}$	
N : Number of animals	

had a 10% mixture of SLS in vaseline applied topically. On the next day, 0.2 ml of 10% PRL in ethanol was applied closely to the same site for 48 hr. After two weeks of the closed patch application, each 50 μ l of 0.1, 1 and 10% PRL in acetone was applied to the skin of the previously shaved flank. At 24, 48 and 72 hr after PRL application, the conditions of the skin were observed visually and evaluated according to the criteria of Draize *et al.* (1944). As a positive control, DNCB was used in the same manner as the methods of PRL except for induction and challenge concentration (0.1% in olive oil and 0.1% in acetone, respectively).

Phototoxicity study

The animals were assigned to the PRL group and the 8-MOP group of 10 animals each. Six previously shaved sites of 1.5×1.5 cm of the dorsal region of each animal were used as the application sites; three sites each on the left and right sides of the back median line as the axis of symmetry. The application sites symmetrically had 0.02 ml of 1, 5 and 10% PRL in acetone applied. After 30 min of PRL application, the application sites on one side of the back median line were covered with aluminum foil and rubber sheet and then long-wavelength UV-A radiation of approximately 10 Joules/cm² was irradiated with 7 fluorescent chemical lamps (FL20S-BL, Toshiba Co., Ltd., Tokyo, Japan) through a glass-plate filter 5 mm thick. At 24, 48 and 72 hr after irradiation, the skin reactions were observed visually and evaluated according to the criteria of Draize *et al.* (1944). As a positive control, 8-MOP was used in the same manner as the methods of PRL except for the number of the application sites (8-MOP:2 sites).

Skin photosensitization study

The animals were assigned to the PRL group of 10 animals and the TBS group of 5 animals. The study was conducted according to the Adjuvant and Strip Test (Ichikawa *et al.*, 1981). Briefly, 0.1 ml each of FCA emulsion was injected intradermally into the 4 corners of the previously shaved dorsal neck region (approximately 2×4 cm) of each animal. The stratum corneum at the induction site was stripped with cellophane tape. The induction site had 0.1 ml of 2% PRL in ethanol applied. After 30 min of PRL application, the site was irradiated with long-wavelength UV-A radiation of approximately 10 Joules/cm² through a glass-plate filter 5 mm thick. The steps from tape stripping to UV irradiation were repeated once a day for 5 consecutive days. After 3 weeks of initial sensitization, six previously shaved sites of 1.5×1.5 cm of the dorsal

region of each animal were used as the application sites; three sites each on the left and right sides of the back median line as the axis of symmetry. The sites had 0.02 ml of 0.1, 1 and 10% PRL in acetone symmetrically applied. After 30 min of PRL application, long-wavelength UV-A radiation was the same as in the phototoxicity study. At 24 and 48 hr after irradiation, the skin reactions were observed visually and evaluated according to the criteria of Draize *et al.* (1944). As a positive control, 3, 5, 4'-tribromosalicylanilide (TBS) was used in the same manner as the methods of PRL.

Statistical analysis

In the primary skin irritation and cumulative skin irritation studies, the statistical analysis of the skin reactions between PRL group and solvent group was carried out by the Mann-Whitney U-test (Mann and Whitney, 1947). The results were expressed as the mean $\pm$ S.E.

RESULTS

Primary irritation study

The results of the primary irritation study are shown in Table 2 and the histopathological findings are shown in Table 3 and Photo 1. In the PRL groups, formation of very slight to well-defined erythema and very slight to slight edema was observed after PRL application. Histopathological examination revealed pseudoeosinophil infiltration, degeneration/necrosis and hyperplasia in the epidermis at 24 hr of PRL application, and hyperkeratosis of the epidermis at 72 hr of PRL application. Furthermore, mononuclear cell infiltration and pseudoeosinophil infiltration in the dermis were observed at 24 and 72 hr of PRL application. These skin reactions and histopathological changes tended to increase as the applied amount of PRL was increased. In the ethanol group, very slight erythema formation was observed after ethanol application. Histopathological examination revealed mononuclear cell infiltration in the dermis at 24 hr of ethanol application, and hyperplasia of the epidermis and mononuclear cell infiltration in the dermis at 72 hr of ethanol application. In the SLS group, formation of very slight to well-defined erythema, slight eschar and slight to moderate edema were observed after SLS application. Histopathological examination revealed moderate to severe degeneration/necrosis in the epidermis and mononuclear cell infiltration and pseudoeosinophil infiltration in the dermis at 24 hr of SLS application. Furthermore, scab formation, regeneration of the epi-

Table 2. Primary skin irritation of propranolol in guinea pigs.

Test article	Conc. (%)	Judgement time (hr)	Score										Mean total score
			Erythema&Eschar					Edema					
			0	1	2	3	4	0	1	2	3	4	
PRL	2.5	24	0	5	3	0	0	8	0	0	0	0	1.4*
		48	0	1	5	0	0	6	0	0	0	0	1.8**
		72	0	2	4	0	0	6	0	0	0	0	1.7*
	5	24	0	0	8	0	0	7	1	0	0	0	2.1**
		48	0	0	6	0	0	2	4	0	0	0	2.7**
		72	0	0	4	2	0	6	0	0	0	0	2.3**
	10	24	0	1	7	0	0	3	4	1	0	0	2.6**
		48	0	0	2	4	0	2	4	0	0	0	3.3**
		72	0	0	2	4	0	4	2	0	0	0	3.0**
Ethanol		24	3	5	0	0	0	8	0	0	0	0	0.6
		48	5	1	0	0	0	6	0	0	0	0	0.2
		72	3	3	0	0	0	6	0	0	0	0	0.5
SLS	5	24	0	1	7	0	0	0	0	6	2	0	4.1##
		48	0	0	0	0	6	0	4	2	0	0	5.3##
		72	0	0	0	0	6	6	0	0	0	0	4.0##
d-water		24	8	0	0	0	0	8	0	0	0	0	0.0
		48	6	0	0	0	0	6	0	0	0	0	0.0
		72	6	0	0	0	0	6	0	0	0	0	0.0

**p<0.01,*p<0.05 Significant difference from ethanol at Erythema & Eschar and Edema formation by U-test.

##p<0.01,*p<0.05 Significant difference from d-water at Erythema & Eschar and Edema formation by U-test.

Table 3. Histopathological findings in guinea pigs following primary dermal application of PRL.

Judgement time (hr)	Findings	2.5% PRL					5% PRL					10% PRL					Ethanol					5% SLS					d-water				
		-	1	2	3	4	-	1	2	3	4	-	1	2	3	4	-	1	2	3	4	-	1	2	3	4	-	1	2	3	4
24	Epidermis																														
	pseudoeosinophil infiltration	1	1	0	0	0	0	1	1	0	0	0	2	0	0	0	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
	degeneration / necrosis	0	2	0	0	0	0	0	1	1	0	0	0	0	2	0	1	1	0	0	0	0	0	0	2	2	0	0	0	0	0
	hyperplasia	0	2	0	0	0	0	0	2	0	0	0	2	0	0	0	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
	Dermis																														
	mononuclear cell infiltration	0	1	1	0	0	0	0	1	1	0	0	0	0	2	0	0	2	0	0	0	0	0	0	1	1	2	0	0	0	0
72	pseudoeosinophil infiltration	1	1	0	0	0	0	0	1	1	0	0	0	0	2	0	2	0	0	0	0	2	0	0	1	1	2	0	0	0	0
	Scab formation	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	0	0	1	4	1	6	0	0	0	0
	Epidermis																														
	degeneration / necrosis	5	1	0	0	0	6	0	0	0	0	5	1	0	0	0	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0
	hyperplasia	0	0	2	4	0	0	0	1	5	0	0	0	0	6	0	0	1	5	0	0	6	0	0	0	0	6	0	0	0	0
	hyperkeratosis	1	4	1	0	0	0	3	3	0	0	0	0	6	0	0	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0
	regeneration	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	0	0	1	5	0	6	0	0	0	0
	Dermis																														
	mononuclear cell infiltration	1	3	2	0	0	0	3	3	0	0	0	1	5	0	0	0	6	0	0	0	1	5	0	0	0	6	0	0	0	0
	pseudoeosinophil infiltration	2	4	0	0	0	3	3	0	0	0	1	5	0	0	0	4	2	0	0	1	5	0	0	0	6	0	0	0	0	0
	hemorrhage in superficial area	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	0	3	3	0	0	6	0	0	0	0

The scores of changes of histopathological findings are: -, no marked change, 1; very slight change, 2; slight change, 3; moderate change and 4; severe change. Numbers in the columns of findings represent the number of corresponding animals.

dermis and hemorrhage in the superficial area of the dermis were observed at 72 hr of SLS application. In the d-water group, no marked visual and histological changes were observed after application.

Cumulative irritation study

The results of cumulative irritation study are shown in Table 4 and the histopathological findings are shown in Table 5 and Photo 2. In the PRL groups, very slight to slight erythema formation was observed at the first day after PRL application in all animals and then edema and scab formation were observed with the repeated application of PRL. Histopathological examination revealed granulation tissue in the subcutis and either scab formation or ulceration in all animals. In addition, hyperplasia and regeneration of the epidermis, mononuclear cell infiltration and pseudoeosinophil infil-

tration in the dermis, and hemorrhage/edema in the subcutis were observed. In the ethanol group, very slight to well-defined erythema formation was observed up to 14 days after ethanol application. Histopathological examination revealed hyperplasia and hyperkeratosis of the epidermis and mononuclear cell and pseudoeosinophil infiltration in the dermis. However, regeneration of the epidermis, and granulation tissue and hemorrhage/edema in the subcutis, which was observed in the PRL groups, was not observed in the ethanol group. In the SLS group, very slight erythema formation was observed at the first day after SLS application. Thereafter, formation of very slight to well-defined erythema and very slight edema was observed up to 14 days after SLS application. Histopathological examination revealed hyperplasia and hyperkeratosis of the epidermis and mononuclear cell and pseudoeosinophil infiltration in

Table 4. Cumulative skin irritation of propranolol in guinea pigs.

Test article	Conc. (%)	Mean total score													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14(day)
PRL	2.5	1.2**	1.3	1.7*	1.7*	1.8**	2.5**	4.0**	4.0**	4.2**	4.0**	4.0**	4.0**	4.0**	4.0**
	5	1.8**	2.0**	3.0**	3.7**	4.7**	5.7**	6.0**	5.3**	5.2**	5.3**	5.3**	5.0**	4.2**	4.0**
	10	1.8**	1.8*	3.5**	4.7**	5.8**	5.8**	6.0**	4.8**	5.3**	5.5**	5.2**	4.8**	4.0**	4.0**
Ethanol		0.3	1.1	1.0	1.0	1.0	1.0	0.8	0.4	0.4	0.4	0.8	0.7	0.4	0.6
SLS	2.5	0.7	1.7 [#]	2.0 ^{##}	2.2 ^{##}	2.0 ^{##}	2.0 ^{##}	2.0 ^{##}	1.8 ^{##}	1.5 [#]	1.7 [#]	1.5 [#]	2.0 ^{##}	2.2 ^{##}	1.8 ^{##}
d-water		0.0	0.3	0.3	0.5	0.3	0.3	0.3	0.5	0.3	0.3	0.3	0.3	0.2	0.2

**p<0.01, *p<0.05 Significant difference from ethanol at Erythema & Eschar and Edema formation by U-test.

^{##}p<0.01, [#]p<0.05 Significant difference from d-water at Erythema & Eschar and Edema formation by U-test.

Table 5. Histopathological findings in guinea pigs following cumulative dermal application of PRL.

Findings	2.5% PRL					5% PRL					10% PRL					Ethanol					2.5% SLS					d-water					
	-	1	2	3	4	-	1	2	3	4	-	1	2	3	4	-	1	2	3	4	-	1	2	3	4	-	1	2	3	4	
Scab formation	1	0	0	3	2	4	0	0	2	0	3	0	0	1	2	17	0	1	0	0	5	0	1	0	0	6	0	0	0	0	
Ulceration	2	0	2	1	1	0	0	0	1	5	1	0	0	1	4	18	0	0	0	0	6	0	0	0	0	6	0	0	0	0	
Epidermis																															
pseudoeosinopil infiltration	4	0	2	0	0	4	0	2	0	0	6	0	0	0	0	17	1	0	0	0	6	0	0	0	0	6	0	0	0	0	
degeneration / necrosis	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0	14	4	0	0	0	6	0	0	0	0	6	0	0	0	0	
hyperplasia	0	0	0	6	0	0	0	0	6	0	0	0	2	3	1	0	0	12	6	0	0	0	0	5	1	0	1	5	0	0	
hyperkeratosis	6	0	0	0	0	6	0	0	0	0	5	0	1	0	0	1	1	14	2	0	0	0	2	4	0	0	2	4	0	0	
regeneration	1	1	0	4	0	4	0	0	2	0	3	0	1	2	0	18	0	0	0	0	6	0	0	0	0	6	0	0	0	0	
Dermis																															
mononuclear cell infiltration	1	0	5	0	0	4	0	2	0	0	3	0	3	0	0	0	2	16	0	0	0	6	0	0	0	6	0	0	0	0	
pseudoeosinopil infiltration	4	1	1	0	0	4	1	1	0	0	4	1	1	0	0	14	3	1	0	0	0	6	0	0	0	6	0	0	0	0	
Subcutis																															
granulation tissue	0	0	5	1	0	0	0	2	3	1	0	1	0	4	1	18	0	0	0	0	6	0	0	0	0	6	0	0	0	0	
hemorrhage/edema	5	0	1	0	0	4	0	0	2	0	5	0	0	1	0	18	0	0	0	0	6	0	0	0	0	6	0	0	0	0	

The scores of changes of histopathological findings are: -, no marked change, 1; very slight change, 2; slight change, 3; moderate change and 4; severe change. Numbers in the columns of findings represent the number of corresponding animals.

Photo 1. Skin of guinea pigs administered 10% PRL in ethanol in the primary skin irritation study.
(A) 24 hr of PRL application: very slight pseudoeosinophil infiltration in the epidermis, moderate degeneration/necrosis of the epidermis, and slight mononuclear cell and pseudoeosinophil infiltration in the dermis. (B) 72 hr of PRL application: moderate hyperplasia of the epidermis, slight hyperkeratosis of the epidermis, and very slight mononuclear cell and pseudoeosinophil infiltration in the dermis. $\times 63$.

Skin toxicity of propranolol.

Photo 1. Skin of guinea pigs administered 10% PRL in ethanol in the cumulative skin irritation study.
(A) severe ulceration. (B) moderate scab formation and regeneration of the epidermis. $\times 63$.

the dermis. In the d-water group, very slight erythema was observed up to 14 days after d-water application. Histopathological examination revealed hyperplasia and hyperkeratosis of the epidermis.

Skin sensitization study

The results of skin sensitization study are shown in Table 6. In the PRL sensitization and non-sensitization groups, none of the animals had skin reactions detected at any of the PRL-treated sites. In the DNCB sensitization group, formation of moderate erythema and slight to moderate edema were observed in all of the animals at 24 hr after application. The skin reactions induced by DNCB tended to increase up to 72 hr, and slight eschar formation was observed at 48 and 72 hr after application. But in the non-sensitization group, none of the animals had skin reactions detected at any of the DNCB-treated sites.

Phototoxicity study

The results of the phototoxicity study are shown in

Table 7. In the PRL groups, none of the animals had skin reactions detected at either irradiated sites or non-irradiated sites at any observation time. In the 8-MOP group, none of the animals had skin reactions detected at the non-irradiated sites, but formation of slight to moderate erythema and very slight to moderate edema were observed at the irradiated sites in all of the animals.

Skin photosensitization study

The results of the skin photosensitization study are shown in Table 8. In the PRL groups, none of the animals had skin reactions detected at either irradiated sites or non-irradiated sites at any observation time. In the TBS group, very slight erythema formation was observed only in 1 animal out of 5 at the non-irradiated site. But in the irradiated sites, very slight to moderate erythema formation was observed in all animals and very slight to moderate edema was observed in 1 ~ 3 animals out of 5. In the preliminary study, 0.5% TBS in acetone was negative for phototoxicity (data not shown).

Table 6. Skin sensitization of propranolol in guinea pigs.

Test article	Challenge conc. (%)	Time (hr)	Group	Score										Positive ratio (%)	
				Erythema&Eschar					Edema						
				0	1	2	3	4	0	1	2	3	4		
PRL	0.1	24	Sens.	10	0	0	0	0	10	0	0	0	0	0	
			Nons.	5	0	0	0	0	5	0	0	0	0		
		48	Sens.	10	0	0	0	0	10	0	0	0	0		
			Nons.	5	0	0	0	0	5	0	0	0	0		
		72	Sens.	10	0	0	0	0	10	0	0	0	0		
			Nons.	5	0	0	0	0	5	0	0	0	0		
		1	24	Sens.	10	0	0	0	0	10	0	0	0	0	
				Nons.	5	0	0	0	0	5	0	0	0	0	
			48	Sens.	10	0	0	0	0	10	0	0	0	0	
				Nons.	5	0	0	0	0	5	0	0	0	0	
			72	Sens.	10	0	0	0	0	10	0	0	0	0	
				Nons.	5	0	0	0	0	5	0	0	0	0	
	10	24	Sens.	10	0	0	0	0	10	0	0	0	0		
			Nons.	5	0	0	0	0	5	0	0	0	0		
		48	Sens.	10	0	0	0	0	10	0	0	0	0		
			Nons.	5	0	0	0	0	5	0	0	0	0		
		72	Sens.	10	0	0	0	0	10	0	0	0	0		
			Nons.	5	0	0	0	0	5	0	0	0	0		
		DNCB	0.1	24	Sens.	0	0	0	5	0	0	0	3	2	0
					Nons.	5	0	0	0	0	5	0	0	0	0
				48	Sens.	0	0	0	2	3	0	0	3	2	0
					Nons.	5	0	0	0	0	5	0	0	0	0
				72	Sens.	0	0	0	0	5	0	0	5	0	0
					Nons.	5	0	0	0	0	5	0	0	0	0
	Sens.			0	0	0	0	0	0	0	0	0	0		
	Nons.			5	0	0	0	0	5	0	0	0	0		

Sens. :Sensitization group

Nons. :Non-sensitization group

Skin toxicity of propranolol.

Table 7. Phototoxicity of propranolol in guinea pigs.

Test article	Conc. (%)	Time (hr)	UV	Score										Positive ratio (%)	
				Erythema&Eschar					Edema						
				0	1	2	3	4	0	1	2	3	4		
PRL	1	24	+	10	0	0	0	0	10	0	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0		
		48	+	10	0	0	0	0	10	0	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0		
		72	+	10	0	0	0	0	10	0	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0		
	5	24	+	10	0	0	0	0	10	0	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0		
		48	+	10	0	0	0	0	10	0	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0		
		72	+	10	0	0	0	0	10	0	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0		
	10	24	+	10	0	0	0	0	10	0	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0		
		48	+	10	0	0	0	0	10	0	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0		
		72	+	10	0	0	0	0	10	0	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0		
8-MOP	1	24	+	0	0	1	9	0	0	0	2	8	0	100	
			—	10	0	0	0	0	10	0	0	0	0		
		48	+	0	0	0	10	0	0	0	6	4	0	100	
			—	10	0	0	0	0	10	0	0	0	0		
		72	+	0	0	0	10	0	0	3	5	2	0	100	
			—	10	0	0	0	0	10	0	0	0	0		

UV+ :UV-A irradiated

UV- :not irradiated

Table 8. Skin photosensitization of propranolol in guinea pigs.

Test article	Challenge conc. (%)	Time (hr)	UV	Score										Positive ratio (%)
				Erythema&Eschar					Edema					
				0	1	2	3	4	0	1	2	3	4	
PRL	0.1	24	+	10	0	0	0	0	10	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0	
		48	+	10	0	0	0	0	10	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0	
	1	24	+	10	0	0	0	0	10	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0	
		48	+	10	0	0	0	0	10	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0	
	10	24	+	10	0	0	0	0	10	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0	
		48	+	10	0	0	0	0	10	0	0	0	0	0
			—	10	0	0	0	0	10	0	0	0	0	
TBS	0.5	24	+	0	2	2	1	0	4	1	0	0	0	100
			—	5	0	0	0	0	5	0	0	0	0	
		48	+	0	0	4	1	0	2	2	1	0	0	100
			—	4	1	0	0	0	5	0	0	0	0	

UV+ :UV-A irradiated

UV- :not irradiated

DISCUSSION

Krishna and Pandit (1994 and 1996) and De Mey *et al.* (1989) have showed the primary irritation of PRL-HCl or PRL patches. However, there are few reports that describe the skin toxicity of PRL-HCl or PRL by transdermal administration. We are trying to make the TDS of PRL. Thus, we have conducted primary and cumulative skin irritation, skin sensitization, phototoxicity and skin photosensitization studies in guinea pigs to evaluate the skin toxicity of PRL. In the primary and cumulative skin irritation studies, the skin reactions induced by PRL tended to increase with the increase of the PRL dosage, and the reactions were more severe than that induced by ethanol used as a solvent control. Moreover, in the cumulative skin irritation study, the skin reactions tended to increase with the application times of PRL up to 7 days. These results demonstrate that PRL has the potential of skin irritation. Valsecchi *et al.* (1994) and Pereira *et al.* (1996) have described a case of occupational contact dermatitis from propranolol in man. But in the present skin sensitization study, PRL did not show an obvious skin reaction. These results suggest that there may be a species difference in the skin sensitizing activity of PRL. In the phototoxicity and skin photosensitization studies, PRL did not evoke a skin reaction.

From these results, it became apparent that PRL had skin irritating activity, but did not have skin sensitizing, phototoxic or skin photosensitizing activity under the condition of the present studies. It was concluded from our study that the decrease of the skin irritation was important in the development of a PRL TDS.

REFERENCES

- Corbo, M., Lie, J.C. and Chien, Y.W.(1989): Transdermal controlled delivery of propranolol from a multilaminate adhesive device. *Pharm. Res.*, **6**, 753-758.
- Draize, J.H., Woodard, G. and Calvery, H.O.(1944): Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharm. Exp. Ther.*, **83**, 377-390.
- De, Mey, C., Enterling, D., Ederhof, M., Wesche, H. and Osterwald, H.(1989): Transdermal delivery of mepindolol and propranolol in normal man. *Arzneim.-Forsch./Drug Res.*, **39**, 1505-1508.
- Ichikawa, H., Armstrong, R.B. and Harber, L.C.(1981): Photoallergic contact dermatitis in guinea pigs. Improved induction technique using Freund's complete adjuvant. *J. Invest. Dermatol.*, **76**, 498-501.
- Kobayashi, I., Hosaka, K., Ueno, T., Maruo, H., Kamiyama, M., Konno, C. and Gemba, M.(1996): Relationship between the amount of propranolol permeating through the stratum corneum of guinea pig skin after application of propranolol adhesive patches and skin irritation. *Biol. Pharm. Bull.*, **19**, 839-844.
- Krishna, R. and Pandit, J.K.(1994): Transdermal delivery of propranolol. *Drug Dev. Ind. Pharm.*, **20**, 2459-2465.
- Krishna, R. and Pandit, J.K. (1996): Carboxymethylcellulose-sodium based transdermal drug delivery system for propranolol. *J. Pharm. Pharmacol.*, **48**, 367-370.
- Magnusson, B. and Kligman, A.M.(1969): The identification of contact allergens by animal assay. The guinea pig maximization test. *J. Invest. Dermatol.*, **52**, 268-276.
- Mann, H.B. and Whitney, D.R.(1947): On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Stat.*, **18**, 50-60.
- Pereira, F., Dias, M. and Pacheco, F.A.(1996): Occupational contact dermatitis from propranolol, hydralazine and bendroflumethiazide. *Contact Dermatitis*, **35**, 303-304.
- Thacharodi, D. and Rao, K.P.(1996): Collagen membrane controlled transdermal delivery of propranolol hydrochloride, *Int. J. Pharm.*, **131**, 97-99.
- Valsecchi, R., Leighissa, P., Piazzolla, S., Naldi, L. and Cainelli, T.(1994): Occupational contact dermatitis from propranolol., *Contact Dermatitis*, **30**, 177.