Original

Dermatotoxicity in the Hypotrichotic (*htr*) Rabbit Derived from the Japanese White (JW-NIBS) Strain Rabbit

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Abstract: Utility of Japanese White rabbit mutants with hypotrichosis (*htr* strain) was investigated in dermal toxicity studies. In the primary irritation study, the primary irritation indices (P.I.I.) by application of hexachlorophene and 3, 3', 4', 5-tetrachlorosalicylanilide (TCSA) were higher in the *htr* rabbits than in the control, haired rabbits. The P.I.I. by application of Tween 80 and sodium lauryl sulfate (SLS) were similar between the *htr* rabbits and the control rabbits. In the 16-day cumulative irritation study, irritation scores induced by SLS and sodium hydroxide were lower in the *htr* rabbits than in the control rabbits throughout the observation periods. However, approximately 30% of the dorsal skin of the control rabbits underwent the anagen phase of the hair cycle during the cumulative periods, resulting in difficulty to apply the chemicals and to estimate the skin reactions. In the phototoxicity study, irritation scores of the *htr* rabbits treated with 8-methoxypsoralen and TCSA were similar to those of the control rabbits. These results revealed advantages of the *htr* rabbits in the administration of test chemicals and the evaluation of skin reactions, suggesting the usefulness of *htr* rabbits for dermal toxicity studies. (J Toxicol Pathol 2002; 15: 153–159)

Key words: congenital hypotrichosis rabbit, primary dermal irritation study, cumulative dermal irritation study, phototoxicity study

Introduction

Animal skin tests are widely used to predict human skin response to the potential irritativeness of toiletries and cosmetics^{1,2}. The skin of the rabbit is especially sensitive to various kinds of cosmetic compounds, compared with that of guinea pig, rat, miniature pig, and human^{3,4}. The sensitivity of the skin is known to be affected by the hair cycle^{5,6}. In the rabbit, extensive portions of the trunk skin have hair follicles undergoing a synchronous cycle⁷. After clipping the trunk hair of rabbit, it is possible to define areas of active hair growth (anagen phase) and areas of inactive hair growth (telogen phase)^{7,8}. In the anagen phase, it is difficult to evaluate skin reactions to irritants⁶. In addition, haired animals including rabbits have the technical disadvantage that the test site of the skin has to be clipped before application of test compounds in the dermal toxicity studies. On the contrary, hairless animals may have an advantage in that their skin response to various chemicals are similar to or more sensitive than that of haired animal skin. Various kinds of hereditary hairless animals have been reported in

micropigs⁹, rats^{10,11}, mice^{12–14}, dogs¹⁵, and rabbits¹⁶, and some of them were indicated to be useful for phototoxicity studies^{11–15}. Recently mutant rabbits with hypotrichosis (*htr*) have been found in a closed colony of Japanese White (JW-NIBS) strain and successfully bred¹⁶. The *htr* rabbits have extremely few and sparsely developed short and delicate primary hairs in the trunk throughout life¹⁶. In this paper, the results of the primary dermal irritation study, cumulative dermal irritation study, and phototoxicity study of several chemicals in the *htr* rabbits were described and the utility of the rabbits was discussed.

Materials and Methods

Animals

Female *htr* rabbits and female Japanese White (JW-NIBS) rabbits with a normal hair coat, used as the controls, were obtained at 3 to 6 months of age from the Laboratory Animal Research Station of Nippon Institute for Biological Science (Yamanashi, Japan). They were housed individually in aluminum wire mesh floor cages (44 × 30 × 35 cm) in a barrier-sustained room controlled at 18–26°C, 30–70% relative humidity, more than 10 times/hr ventilation, and a 12-hr light-dark cycle. The animals had free access to standard laboratory diet (NRT-1, Nisseiken Co., Ltd., Tokyo, Japan) and tap water. Six *htr* and 6 age-matched control rabbits were used in each test. In all tests,

Received: 30 April 2002, Accepted: 5 August 2002

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the skin of the dorsal to lumbar regions of the *htr* rabbits was used without clipping. Body weights of the rabbits ranged from 1.9 to 2.8 kg on the beginning of the experiments.

All animal procedures in this study complied with the Nippon Institute for Biological Science's Guidelines for Care and Use of Laboratory Animals (1999).

Primary dermal irritation study

The skin of the dorsal to lumbar regions of the haired control rabbits was clipped one day before application of test chemicals. The test region of the skin in each animal was divided into 8 sites according to the Draize's test method¹⁷. Four of the 8 sites were used intact and the remaining 4 sites were used as the abraded skin after receiving minor incisions through the stratum corneum by using a sterile needle. The test compounds used were 0.1 and 1% Hexachlorophene (HCP; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) in white petrolatum, 0.1 and 1% 3,3',4',5-Tetrachlorosalicylanilide (TCSA; Kanto Chemical Co., Inc., Tokyo, Japan) in ethanol, 1 and 5% sodium lauryl sulfate (SLS; Nacalai Tesque Inc., Kyoto, Japan) in white petrolatum, and Tween 80 (Kanto Chemical Co., Inc., Tokyo, Japan). 0.15 mL of each test compound were applied to both intact and abraded sites for 24 hrs by using a lint patch (2 × 2 cm), polyethylene film tape (Blenderm, 3M Pharmaceutical Co., Tokyo, Japan), a double faced adhesive tape, a strip of bleached cloth, and an elastic type bandage tape (Vetrap, 3M Pharmaceutical Co.). The sites were observed 24 and 72 hrs after application. The skin reactions were scored according to the Draize's evaluation standard and estimated by calculating the primary irritation index (P.I.I.)¹⁷.

16-day cumulative dermal irritation study

The dorsal skin of the control rabbits was clipped two days before the first application of the test chemicals, and 6 and 13 days after the first application. The clipped region was divided into 8 sites in each animal. The test compound used were 0.05, 0.25, 1, and 4% SLS in distilled water and 0.1, 0.25, 0.5, and 1% sodium hydroxide (SH; Kanto Chemical Co., Inc., Tokyo, Japan) in distilled water. 0.15 mL of each test compound were applied to the test sites (2 × 2 cm) of the skin 14 times during the study (day-1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 15, 16, 17, and 18). The reactions were graded as follows: score 0, no erythema; score 1, barely perceptible erythema; score 2, well-defined erythema; score 3, moderate to severe erythema; and score 4, beet red erythema with or without slight eschar formation or disruption of epidermal intactness. The skin reaction was observed for 16 days (day-2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, and 19) during the test period and the mean irritation scores for 6 rabbits in each day were calculated. Finally, the mean cumulative irritation score of 16 evaluations in each group was calculated.

Phototoxicity study

The hair covering those regions of the control rabbits

was clipped one day before application of the test chemicals. The test regions were divided into 12 sites in each animal. The test compounds used were 0.00025, 0.0025, and 0.025% 8-methoxypsoralen (8-MOP; Nacalai Tesque Inc., Kyoto, Japan) in acetone and 0.00025, 0.0025, and 0.025% TCSA in ethanol. Two test sites (1.5 × 1.5 cm) of the skin were applied with 0.02 mL of each test compound. After application, one site was covered with the aluminum foil to evaluate only the primary irritant reaction. The other site was irradiated once with long wavelength ultraviolet light (FL-BLB, Black light, Toshiba Co., Ltd., Tokyo, Japan, UVA: 320–400 nm) at a dose of 10.2 J/cm². The skin reactions of erythema and edema at the test sites were graded as follows: (1) Erythema and eschar formation: score 0, no erythema; score 1, very slight erythema; score 2, well-defined erythema; score 3, moderate to severe erythema; score 4, beet red erythema with or without slight eschar formation. (2) Edema formation: score 0, no edema; score 1, very slight edema; score 2, well-defined edema; score 3, moderate edema; score 4, severe edema. The sites were evaluated 24 hrs after irradiation. The mean phototoxic scores in each group were defined as the sum of the individual score.

Histopathology

All rabbits were euthanized under deep anesthesia with sodium pentobarbital (Ravonal®, Tanabe Seiyaku Co., Ltd., Osaka, Japan) and subjected to a complete necropsy 72 hrs after application of test materials in the primary dermal irritation study, soon after the last evaluation in the 16-day cumulative dermal irritation study, and 72 hrs after irradiation in the phototoxicity study. The skins of all test sites were removed, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned sagittally, and stained with hematoxylin and eosin (HE) for histopathological examination.

Statistical analysis

The statistical significance was assessed by the Wilcoxon rank sum test for differences between the *htr* and control rabbits.

Results

Primary dermal irritation study

As shown in Fig. 1, the P.I.I. in the *htr* and control rabbits were 0.7 and 0.4 in the 0.1% HCP group, 3.7 and 2.0 in the 1% HCP group, 0.5 and 0.1 in the 0.1% TCSA group, 3.4 and 0.8 in the 1% TCSA group, 2.9 and 2.8 in the Tween 80 group, 5.9 and 6.4 in the 1% SLS group, and 7.3 and 7.6 in the 5% SLS group, respectively. In the 1% HCP and 1% TCSA groups, the P.I.I. of the *htr* rabbits was significantly ($p < 0.05$ in 1% HCP and $p < 0.01$ in 1% TCSA) higher than that of the control rabbits. There were no significant differences in the P.I.I. of the *htr* and control rabbits in the other groups. In one of 6 control rabbits, the skin reactions of the 0.1 and 1% HCP and 0.1 and 1% TCSA application

Fig. 1. The primary irritation index (P.I.I.) of the skin reactions in the *htr* and control rabbits treated with 0.1 and 1% hexachlorophene (HCP), 0.1 and 1% 3,3',4',5-tetrachlorosalicylanilide (TCSA), Tween 80, and 1 and 5% sodium lauryl sulfate (SLS) in a primary dermal irritation study. 0.1 and 1% HCP and TCSA application sites (#) in one control rabbit were not evaluated because of the anagen phase. Values represent mean $\pm$ SD. Significant differences: *: $p < 0.05$ and **: $p < 0.01$ from the control rabbits.

sites were not evaluated, because the hair cycle of those sites was in the anagen phase.

Histopathologically, crust formation with epidermal hyperplasia was observed in the application sites of both *htr* and control rabbits treated with 1% HCP and 1% TCSA. The lesions were severe in the *htr* rabbits compared to the control rabbits. In the application sites of 1 and 5% SLS, crust formation with epidermal hyperplasia and necrotic foci in the dermis were observed in both *htr* and control rabbits. The degree of these lesions, however, was slightly lower in the *htr* rabbits than in the control rabbits. In the application sites of 1 and 5% SLS, and Tween 80, infiltration of pseudo eosinophils and lymphocytes was marked in the dermis of the *htr* rabbits compared with the control rabbits.

16-day cumulative dermal irritation study

As shown in Figs. 2 and 3, the irritation scores of the *htr* rabbits treated with 4% SLS and 0.5% SH were lower than those of the control rabbits throughout the study. When treated with 1% SLS, the irritation scores of the *htr* rabbits were higher than those of the control rabbits at application day-3 and 4 and lower from application day-5 to 18. The irritation scores of the *htr* rabbits treated with 1% SH were higher than those of the control rabbits at application day-3 and 4 and were lower from application day-6 to 19. The skin reactions of all treated rabbits were dose-dependent: barely perceptible erythema was seen in the application sites of *htr* and control rabbits treated with 0.05% SLS and *htr* rabbits treated with 0.25% SLS or 0.5% SH, and control rabbits treated with 0.1 and 0.25% SH; well-defined erythema in the *htr* rabbits treated with 1% SLS or 1% SH, and the control rabbits treated with 0.25% SLS or 0.5% SH; moderate to severe erythema in the *htr* rabbits treated with 4% SLS (Fig. 4A) and in the control rabbits treated with 1 and 4% SLS or 1% SH (Fig. 4B). The skin reactions in the control rabbits were not evaluated in the application sites of each rabbit

treated with 4% SLS and 0.1 and 0.25% SH, and in those of 2 rabbits treated with 0.5% SH, because the hair cycle of those sites was in the anagen phase.

The mean cumulative irritation scores of the skin reactions in the *htr* and control rabbits are shown in Fig. 5. The mean cumulative irritation scores of the *htr* rabbits treated with 0.05, 0.25, 1, and 4% SLS were 1.2, 0.3, 5.3, and 21.2, respectively, and those of the control rabbits were 0.5, 2.8, 12.0, and 33.4, respectively. There were no significant differences of the mean cumulative irritation scores between the *htr* and control rabbits treated with SLS. The mean cumulative irritation scores of the *htr* rabbits treated with 0.1, 0.25, 0.5, and 1% SH were 0, 0, 0.3, and 9.0, respectively, and those of the control rabbits were 1.4, 1.6, 11.0, and 15.8, respectively. The mean cumulative irritation scores of the *htr* rabbits treated with 0.25 and 0.5% SH were significantly ($p < 0.05$ and $p < 0.01$, respectively) lower than those of the control rabbits.

Histopathologically, epidermal hyperplasia was observed in the application sites of all rabbits treated with 1 and 4% SLS and a little marked in the *htr* rabbits with application of 1% SLS. In rabbits treated with 4% SLS, dermal edema was observed in the application sites and the degree of the lesion was slightly lower in the *htr* rabbits than in the control rabbits. Slight epidermal hyperplasia was observed in the application sites of the *htr* rabbits treated with 1% SH and the control rabbits treated with the 0.5 and 1% SH. With application of 1% SH, slight inflammatory cell infiltration was observed in the dermis of the *htr* rabbits.

Phototoxicity study

The mean phototoxic scores of the skin reactions in the *htr* and control haired rabbits 24 hrs after irradiation are shown in Fig. 6. The mean scores of the *htr* rabbits treated with 0.00025, 0.0025, and 0.025% 8-MOP were 0, 1.7, and 7, respectively, and those of the control rabbits were 0, 3.3,

Fig. 2. Daily irritation scores of the skin in the *htr* and control rabbits treated with 1 and 4% SLS in a 16-day cumulative dermal irritation study.

Fig. 3. Daily irritation scores of the skin in the *htr* and control rabbits treated with 0.5 and 1% sodium hydroxide (SH) in a 16-day cumulative dermal irritation study.

Fig. 4. External appearances of the application sites with 4% SLS on day-5 in the *htr* (A) and control (B) rabbits in a 16-day cumulative irritation study. Note moderate to severe erythema (score 3) in both application sites.

Fig. 5. Mean cumulative irritation scores of the skin reactions in the *htr* and control rabbits in a 16-day cumulative dermal irritation study. 4% SLS, 0.1 and 0.25% SH application sites in one control rabbit (#) and 0.5% SH application sites in 2 control rabbits (##) were not evaluated because of the anagen phase. Values represent mean $\pm$ SD. Significant differences: *: $p < 0.05$ and **: $p < 0.01$ from the control rabbits.

Fig. 6. Mean phototoxic score of the skin reactions in the *htr* and control rabbits 24 hrs after irradiation in a phototoxicity study. Values represent mean $\pm$ SD.

Fig. 7. Dorsal skin treated with 0.025% 8-methoxypsoralen 72 hrs after UV irradiation in a phototoxicity study. Note edema and mild infiltration of lymphocytes and pseudoeosinophils in the superficial layer of dermis in the *htr* rabbit (A) and the similar lesion extending to the deep portion of dermis in the control rabbit (B). HE, $\times 100$.

and 6.8, respectively. The skin reactions scored 1.7 in the *htr* rabbits consisted of very slight to slight erythema and edema and those scored 3.3 in the control rabbits consisted of slight to moderate erythema and edema. The skin reactions scored 7 in the *htr* rabbits and 6.8 in the control rabbits were moderate to severe erythema, eschar formation and edema. The mean scores of the *htr* rabbits treated with 0.00025, 0.0025, and 0.025% TCSA were 0, 0, and 0.2, respectively, and those of the control rabbits were 0, 0, and 1.2, respectively. The skin reaction scored 0.2 in one of 6 *htr* rabbits consisted of very slightly erythema and those scored 1.2 in 4 of 6 control rabbits were very slight to well defined erythema and edema. There were no significant differences of the mean phototoxic scores between both rabbits treated with 8-MOP and TCSA. No skin reactions were observed in any application sites of any doses of 8-MOP and TCSA in all rabbits without UV irradiation.

Histopathologically, epidermal hyperplasia with edema and lymphocytes and pseudoeosinophil infiltration in the dermis were observed in the application sites of all rabbits treated with 0.0025% 8-MOP, and moderate ulcer was present in the control rabbits. With application of 0.025% 8-MOP, similar changes were observed in the superficial portion of the dermis of *htr* rabbits, and edema in the control rabbits extended to the deep portion of the dermis with mild infiltration of those inflammatory cells (Fig. 7).

Discussion

Hair growth cycles are generally divided into three stages: an anagen phase (growing period), a catagen phase (transient period), and a telogen phase (resting period)^{7,8}. The vascular bed surrounding hair follicles is a functionally distinct entity, which undergoes cyclic changes associated with the hair growth cycle⁷. The sensitivity of the skin to chemicals is known to be affected by these hair cycle stages. When the skin in the growing period was used, it has been described difficult to evaluate the reactions induced by test chemicals because of the reddish skin color and hair growth⁶. Therefore, it is recommended to use rabbits with the hair coat in the resting period for dermal toxicological studies to obtain reproducible data⁶. The *htr* rabbits used here show hypotrichosis in the trunk, in which the skin appears to remain at a phase like a resting period¹⁶.

In the primary irritation study, the P.I.I. of HCP and TCSA in the *htr* rabbits were significantly higher than those in the haired control rabbits, although the P.I.I. of Tween 80 and SLS in the *htr* rabbits were similar to those in the control rabbits. These results suggest that the skin of the *htr* rabbits may be more sensitive to certain irritants such as sterilization drugs than that of the control rabbits. In the 16-day cumulative irritation study, the irritation scores on each application day and the mean cumulative irritation scores of SLS and SH increased with the dosage of those chemicals in

the *htr* rabbits as well as in the control rabbits. These results showed that dose-related cumulative skin reactions to chemicals could be evaluated in the *htr* rabbits. In the phototoxicity study using 8-MOP and TCSA, there were no apparent differences in phototoxicity skin reaction between the *htr* and control rabbits, suggesting that phototoxic sensitivity of the skin was essentially the same between the *htr* and control rabbits.

Histopathological observation in the present study revealed that histologic findings of the skin reactions against chemicals in the *htr* rabbits were essentially similar to those in the control rabbits, suggesting a potential usefulness of the *htr* rabbits for dermatotoxicity studies.

The *htr* rabbits with hypotrichotic skin is of great advantage for the administration of the chemicals and the evaluation of the skin reactions. The skin of their trunk remains to be in the telogen phase and no clipping is required in the testing sites of the skin. On the contrary, approximately 30% of the dorsal skin of the haired control rabbits entered the anagen phase during the 16-day cumulative irritation study, resulting in difficulty to apply the chemicals and to estimate the skin reactions.

The facts that a high dermal irritability to several chemicals, arrested hair cycle remained at a phase like the resting period and essential advantages in the administration and the evaluation of test chemicals suggest that the *htr* rabbits are useful laboratory animals for dermal toxicity studies.

Acknowledgements: We thank T. Fujii, M. Kaneko, Y. Ogata, and M. Yamazaki for their technical assistance in this study.

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