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## Induction of Experimental Protoporphyrin in Hairless Mice by Griseofulvin

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**Abstract:** To investigate whether or not protoporphyria can be induced in hairless mice, feed containing 0.5% griseofulvin (GF) was given to hairless Hos<sup>®</sup> HR/De strain mice for two to four months. Administration of GF caused protoporphyria in the hairless mice, that is, protoporphyrin levels in the erythrocytes and the liver were extremely elevated. Acute skin changes such as erythema, edema, blister and crust formation were produced by exposing the hairless mice with protoporphyria to 400 nm of light using the metal halide lamp.

From these results, we confirmed that hairless mice of HR/De strain can be utilized as a model for protoporphyria. These mice are useful for macroscopic and histopathological observations of skin changes and are also convenient for photosensitivity experiments.

**Key words:** Griseofulvin-induced protoporphyria, Strain difference, Hairless mice, Experimental porphyria, Drug-induced porphyria.

### INTRODUCTION

Many cases of porphyrias have been reported in tropical regions of the world, especially in Africa and Asia. These diseases are very important for tropical dermatology because cutaneous manifestations are induced by exposure to sunlight. There is a wide difference in the skin changes of porphyrias between tropical regions and other areas. For example, severe cutaneous changes in porphyrias are reported in India (Kaur I *et al.*, 1984).

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These patients showed severe photosensitivity and even acral deformities in spite of their young age. One of the most important symptoms in porphyrias, especially in erythropoietic protoporphyrias (EPP), is photosensitivity. Experimental studies concerning photosensitivity in porphyria were performed using mice with protoporphyria by Konrad *et al.* (1975), and Hönigsmann *et al.* (1976). This experimental protoporphyria was induced by large amounts of griseofulvin (GF), which is an antifungal drug produced from a penicillin species. We also confirmed the presence of photosensitivity and an elevation of skin porphyrin levels in mice with protoporphyria (Nonaka *et al.*, 1977).

Previously, mice with hair have always been used in experiments, and observations of skin changes or the irradiation of light have been restricted to the ears or tail. The existence of the hair was always an obstacle in studies using these mice, but the restrictions and difficulties disappear in experiments carried out on hairless mice. There has been a report by Baart *et al.* (1980) about the use of diethyl-dinitro carbamazine (DDC) on hairless mice, but reports concerning the induction of protoporphyria by GF are still unavailable. The purpose of this study is to investigate whether or not protoporphyria can be induced in the hairless mice by GF, and whether or not these mice are suitable for the study of porphyric photosensitivity.

## MATERIALS AND METHODS

### 1) Animals

Adult male Hos<sup>®</sup> HR/De strain hairless mice were purchased from Hoshino experimental animals (Yashio, Japan). Twenty-four mice were used for this study.

### 2) Chemicals

Griseofulvin (GF) was provided by Shionogi Pharmaceutical Co., Osaka, Japan. Ordinary chemicals for porphyrin analysis were purchased from Nakarai Pure Chemical Co., Kyoto, Japan.

### 3) Feeds and Experimental Designs

Normal MF pellet type feeds for rats and mice were purchased from Oriental Yeast Co., Tokyo, Japan. GF was mixed into the normal feeds in a concentration of 0.5%. The feeds containing GF were given to 19 hairless mice for two to four months. Normal feeds were given to 5 hairless mice for one month as controls.

### 4) Quantitative Analysis of Coproporphyrin and Protoporphyrin

The quantitative analysis of coproporphyrin (CP) and protoporphyrin (PP) and the observation of red fluorescence in the liver and erythrocytes were carried out using the same procedures as in previous reports (Honda *et al.*, 1983; Sano and Granick, 1961).

### 5) Light Source and Irradiation Method

Two 700 W metal halide (MH) lamps (Mitsubishi, M-700 G-D, Stabilizer: HGN. 700 HA 100) were used in this study. The spectral irradiance and intensity of energy in this lamp have been described in a previous report (Honda *et al.*, 1983). The irradiation was performed on the mice for 2 hours at a distance of 90 cm (7.9 J/cm<sup>2</sup>), or for 1 hour at a

distance of 30 cm (22.0 J/cm<sup>2</sup>) using a glass filter (5 mm thickness) to avoid UVB. The intensity of energy from the light source was measured at 405 nm using diode photometers (Topcon UVR-405). Repeated irradiation was carried out only on the mice which did not show any macroscopic change by the first irradiation. Macroscopically, we observed cutaneous changes such as erythema, edema, erosion and crust, and compared these with the control mice. The severity of skin changes was graded from (-) to (+++); with (-) indicating no change, (+) slight erythema, (++) severe edema, erythema and vesicles, and (+++) necrosis and crust formation.

## RESULTS

The results are shown in Table 1 and 2.

### 1) Liver Weight and Liver/Body Weight Ratio

The average body weight was 22.8g and average liver weight was 1.2g in the 5 normal hairless mice, the average liver/body weight ratio being 5.3%. In the group treated with GF, the average body weight was 23.8g, the average liver weight was 3.5g, and the average liver/body weight ratio was 14.7%.

Table 1. Body weight, liver weight, liver/body weight ratio, hepatic and erythrocytic porphyrin levels in the hairless mice

Group	Body weight (g)	Liver weight (g)	Liver/Body weight ratio (%)	Hepatic porphyrins ( $\mu\text{g/g}$ wet weight)			Erythrocytic porphyrins ( $\mu\text{g/dl}$ pcv)	
				UP	CP	PP	CP	PP
GF (n=5)	22.8	1.2	5.3	0.01	0.05	0.15	2.18	6.60
	$\pm$ 0.6	$\pm$ 0.02	$\pm$ 0.2	$\pm$ 0.005	$\pm$ 0.017	$\pm$ 0.072	$\pm$ 0.8	$\pm$ 2.82
GF (n=19)	23.8	3.5	14.7	2.21	41.93	746.76	178.76	1095.60
	$\pm$ 0.8	$\pm$ 0.3	$\pm$ 1.0	$\pm$ 0.28	$\pm$ 22.42	$\pm$ 80.11	$\pm$ 13.51	$\pm$ 147.08

abbreviation: pcv=packed cell volume, UP=uroporphyrin

CP=coproporphyrin, PP=protoporphyrin

Table 2. Skin reactions due to metal halide lamp irradiation in the hairless mice treated with or without GF

Group of mice	Liver/ Body weight ratio (%)	Hepatic porphyrins ( $\mu\text{g/g}$ wet weight)			Erythrocytic porphyrins ( $\mu\text{g/dl}$ pcv)		Skin reaction	No. of mice
		UP	CP	PP	CP	PP		
Normal	5.3	0.01	0.05	0.15	2.18	6.60	(-)	5
GF	7.9	2.22	10.05	733.82	200.58	512.32	(-)	2
	10.6	1.74	14.47	531.43	149.75	576.70	(+)	4
	12.6	2.82	29.62	544.72	184.44	809.17	(++)	3
	17.6	2.22	22.90	896.08	184.32	1505.73	(+++)	10

## 2) Hepatic and Erythrocytic Porphyrin Levels

In the normal control group, the mean value for hepatic UP was 0.01  $\mu\text{g/g}$  wet weight, that for CP was 0.05  $\mu\text{g/g}$  wet weight, and that for PP was 0.15  $\mu\text{g/g}$  wet weight. The mean value for erythrocytic CP was 2.2  $\mu\text{g/dl}$  and that for PP was 6.6  $\mu\text{g/dl}$ .

In the GF group, the mean value for hepatic UP was 2.2  $\mu\text{g/g}$  wet weight, which is 220 times the normal value; the mean value for hepatic CP was 41.9  $\mu\text{g/g}$  wet weight, which is 840 times the normal value; and that for hepatic PP was 746.8  $\mu\text{g/g}$  wet weight, which is 5,700 times the normal value. The mean value for erythrocytic CP was 178.8  $\mu\text{g/dl}$  pcv, which is 80 times the control level, and that for PP was 1095.6  $\mu\text{g/dl}$  pcv, which is 165 times the control level. These results were consistent with the biochemical features of protoporphyria.

## 3) Provocation of Skin Reaction

The results are shown in Table 2. Strong reactions occurred in the animals that showed high levels of liver/body weight ratio and erythrocytic PP. Namely, strong edema occurred immediately on one mouse after exposure to MH lamps. A blister could be seen on part of the exposed site.

## DISCUSSION

In this study, we attempted to induce protoporphyria in HR/De strain hairless mice by treatment with GF. When the feeds mixed with GF were given to the HR/De strain mice for 2 months, clearly distinguished protoporphyria was induced in all the mice.

Since Hurst and Paget (1963) reported the induction of protoporphyria, there have been many experiments. We also performed a biochemical and histopathological investigation using GF in a concentration of 0.5% (Shimoyama *et al.*, 1984). We were able to induce protoporphyria in the HR/De strain hairless mice by the administration of GF in the concentration of 0.5%. When compared with the results of a previous report carried out the same way using the dd strain mice, all values of liver/body weight ratio, hepatic UP, CP and PP, erythrocytic CP and PP in the HR/De strain hairless mice were higher than those in the dd strain mice (Shimoyama *et al.*, 1984). There seems to be a different porphyrinopathic sensitivity to the GF between the two species of mice. Of course, there were also some differences in the abnormal porphyrin metabolism induced by GF among the individual animals of the same species. It is still unclear whether those differences are based on the different intake volume of feeds or on a different reactivity to GF. From the present results, we felt that the differences of porphyrin metabolism between dd strain mice and HR/De strain hairless mice were in keeping with our original expectations.

Although a concentration of GF 0.5% in the feed was used in this study, higher concentrations of 1.0 to 2.5% in the feed were used in previous reports (Nonaka *et al.*, 1977). We reported that protoporphyria could be sufficiently induced in dd strain mice by a 0.5% concentration of GF (Shimoyama *et al.*, 1984). In this study using the hairless species, all the mice developed protoporphyria by ingesting a 0.5% concentration of griseofulvin, and

photosensitive skin changes were experimentally induced in almost all the mice. The concentration of 0.5% was sufficient to induce protoporphyria in HR/De strain hairless mice used for the photosensitive experiments. When the hairless mice were exposed to light from the MH lamps, some mice showed an acute reaction while others did not show any reaction in spite of large doses of irradiation. Although these differences may be due to erythrocytic protoporphyrin levels, the quantitative analysis of the light energy in our study is still not sufficient. Further investigation is needed.

The protoporphyria in HR/De strain hairless mice induced by a concentration of 0.5% GF may be useful for the investigation of porphyric photosensitivity because various skin changes are induced in almost all the mice by the irradiation of light. The use of these mice will also prove valuable in tropical dermatology. Moreover, we may need colored mice with experimental porphyria to investigate species differences in the skin changes of porphyria.

Generally, porphyriopathies differ according to different combinations of animal species and chemicals. Further investigations are needed to discover the best animal model for porphyric photosensitivity.

We reported that normal porphyric metabolism varied with animal species (Murayama *et al.*, 1986). Strik (1973) reported that there was a great variation among animal species in the induction of experimental porphyria by polyhalogenated aromatic compounds. This shows that it may be possible to induce a form of experimental porphyria that more closely resembles human porphyria. The difference in protoporphyria between HR/De strain hairless mice and dd strain mice may also show the existence of a difference among strains.

#### REFERENCES

- 1) Baart de la Faille, H., Woutersen, R. A., Van Weelden, H. & Baart de la Faille-Kuyper, E. H. (1980): Experimental protoporphyria in hairless mice: photodermatitis. pp. 603-615. In D. A. Willoughby & J. P. Giround (ed.). *Inflammation: Mechanisms and Treatment*. University Park Press, Baltimore, USA.
- 2) Dresel, E. I. B. & Falk, J. E. (1956): Studies on the biosynthesis of blood pigments, 2 Haem and porphyrin formation in intact chicken erythrocytes. *Biochem. J.* 63, 72-79.
- 3) Honda, T., Nonaka, S., Murayama, F., Ohgami, T., Shimoyama, T. & Yoshida, H. (1983): Effects of KC-400 (polychlorinated biphenyls) on porphyrin metabolism, Liver and blood porphyrin analysis in rats treated with KC-400. *J. Dermatol. (Tokyo)*, 10 (3), 259-265.
- 4) Hönigsmann, H. Gschnait, F., Konrad, K., Sting, G. & Wolff, K. (1976): Mouse model for protoporphyria, III. Experimental production of chronic erythropoietic protoporphyria-like skin lesions. *J. Invest. Dermatol.*, 66, 188-195.
- 5) Kaur, I., Kumar, B., Kaur, S. & Mohanty, D. (1984): Congenital erythropoietic porphyria. *Indian. Pediatr.*, 21, 903-908.

- 6) Konrad, K., Hönigsmann, H., Gschnait, F. & Wolff, K. (1975): Mouse model for protoporphyria, II. Cellular and sub-cellular events in the photosensitivity flare of the skin. *J. Invest. Dermatol.*, 65, 300–310.
- 7) Hurst, E. W. & Paget, G. E. (1963): Protoporphyrin, cirrhosis and hepatomata in the livers of mice given griseofulvin. *Br. J. Dermatol.*, 75, 105–112.
- 8) Murayama, F., Nonaka, S., Ohgami, T. & Yoshida, H. (1986): Analyses of  $\delta$ -aminolevulinic acid dehydratase activity and protoporphyria levels in erythrocytes among three different species. *J. Dermatol. (Tokyo)*, 13 (5), 351–355.
- 9) Nonaka, S., Shimoyama, T., Honda, T., Hirowatari, T., Hori, M. & Nogita, M. (1977): Cutaneous changes by sun-light on experimental porphyria --Porhyrin metabolism and cutaneous changes by sun light on griseofulvin administered mice--. *Jpn. J. Dermatol.*, 87 (10), 585–598. (in Japanese)
- 10) Sano, S., Granick, S. (1961): Mitochondrial coproporphyrinogen oxidase and protoporphyria formation. *J. Biol. Chem.*, 236, 1173–1180.
- 11) Shimoyama, T., Nonaka, S., Honda, T., Murayama, F., Ohgami, T. & Yoshida, H. (1984): A biochemical study of experimental porphyria, I. The influence of griseofulvin at various concentrations on porphyrin metabolism. *J. Dermatol. (Tokyo)*, 11 (1), 49–55.
- 12) Strik, J. J. T. W. A. (1973): Species difference in experimental porphyria caused by polyhalogenated aromatic compounds. *Enzyme*, 16, 224–229.