Histopathological Studies on Sun-Exposed Hexachlorobenzene-Induced Porphyric Rat Skin

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Hexachlorobenzene (HCB) is a porphyrogenic agent. The inducement of skin changes was attempted through repeated exposure of the skin of HCB-induced porphyric rats to sunlight. The following skin changes were produced in the porphyric rats: erythema, erosion, crust, skin thickening and scarring. Histopathological examination revealed the presence of acanthosis, vacuolization of the malpighian cells, subepidermal vesicle, fibrosis, dilatation and increase of the blood vessels and perivascular cell infiltration composed of lymphocytes, histiocytes and mast cells. The PAS stainability of blood vessel walls was slightly intensified. The assumption is that photosensitive flares were elicited within the short 2-month period though destruction of endothelial cells was not prominent. There were no distinct skin changes, clinically or histopathologically, in any of the three control groups.

Porphyria cutanea tarda (PCT), a very common type of porphyria, exhibits skin photosensitivity on exposed areas and is characterized by excess excretion of uroporphyrin in the urine. Ockner and Schmid (1961) reported that hexachlorobenzene (HCB) could induce experimental porphyria in rats which then excreted a high level of urinary uroporphyrin. Because the metabolic pattern in rats seems to resemble that in human PCT (Stonard 1974), we have used them as a model for PCT.

In the present paper, we analyzed acute cutaneous changes after sun exposure of HCB-induced porphyratic rats, both clinically and histopathologically.

Materials and Methods

Rats. Sprague-Dawley albino female rats initially weighing 150–200 g were used for this study.

Induction of porphyratic rat. Twenty rats were fed 0.25% (W/W) HCB-diet for two months and then normal diet for two months. Another 20 rats were kept on normal diet.

Porphyrin analysis of urine. Urinary porphyrins in individual excreta were determined by Rimington’s method (Rimington 1971).

Sunlight exposure. Rats were divided into four groups; (1) 10 normal rats kept in the dark, (2) 9 sun-exposed normal rats, (3) 6 porphyratic rats kept in the dark, and (4) 8
sun-exposed porphyric rats. Hair was shorn with an electric clipper once every week. The backs of the rats were exposed to sunlight twice a week for two months during August and September 1979, for 2 to 3 hr in the early afternoon. Skin biopsies of the back were performed both before and after peak sun-exposure. Specimens were fixed in 10% formalin, stained with hematoxylin-eosin, periodic acid-Schiff and toluidine blue (0.05%).

RESULTS

Urinary porphyrin. Data obtained are summarized in Table 1. Analysis, made 60 days after starting HCB, showed the expected increase in urinary coproporphyrin and uroporphyrin as contrasted with the normal rats (for both porphyrins, 0.05 > p > 0.01).

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<th>Table 1. Urinary porphyrins in porphyric and normal rats</th>
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<td>Coproporphyrin (µg/liter)</td>
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Mean±s.d.

Group’s composition is described in Material and Methods.

Clinical and histopathological findings on porphyric and normal rats.

Sun exposure was started after confirming that a high level of urinary porphyrin was successfully induced in the porphyric rats. The rats of the first three groups did not exhibit any skin changes, while those of the fourth group (sun-exposed porphyric rats) showed the following successive changes; erythema, erosion, crust, skin thickening and scar formation on the exposed skin area.

Histopathology of the skin specimens obtained from the first three groups was normal; epidermis and dermis were intact (Fig. 1). However, skin specimens obtained from rats in the fourth group revealed hyperkeratosis, acanthosis, vacuolization of the malpighian cells, swelling and thickening of collagen fibers and fibrosis, as shown in Fig. 2. In addition, subepidermal vesicles were also observed (Fig. 3). The middle and/or lower dermis showed dilatation and formation of new blood vessels containing red blood cells and increased perivascular cell infiltration composed chiefly of lymphocytes and histiocytes (Fig. 4). Furthermore, we observed large elliptical cells with numerous granules in the cytoplasm around the blood vessels (Fig. 5). These cells are thought to be mast cells because their granules showed metachromasia with toluidine blue. The PAS stain appeared to be slightly intensified in the blood vessel walls, but PAS-positive substances were not clearly observed around the vessels or dermal papillae.

DISCUSSION

PCT appears to be an error of porphyrin metabolism characterized by the excretion of excessive uroporphyrin in both urine and feces, with symptoms
occurring in adult life. Elder et al. (1978) biochemically measured uroporphyrinogen decarboxylase activity in liver of PCT patients and found it was lower than that of the controls. On the other hand, HCB can induce experimental porphyria in rats which in turn excrete a high level of uroporphyrin in the urine (Ockner and Schmid 1961), and recently San Marrtin de Viale et al. (1977) found a marked decrease (>90%) of hepatic uroporphyrinogen decarboxylase in rats with HCB-induced porphyria. Based on this evidence, rats with HCB-induced porphyria can be used as a PCT model.

Cutaneous changes after sun exposure were studied on HCB-induced porphyric rats. The sun-exposed porphyric rats exhibited such successive skin changes as erythema, erosion, scar, crust and skin thickening as seen in PCT. Similar cutaneous changes were observed on peritoneally hematoporphyrin injected rats (Megovern 1961) and griseofulvin administered mice (Nonaka et al. 1977). Nonaka et al. (1977) emphasized that there were destruction and/or degeneration of endothelial cells and an increase of mast cells around the dermal vessels. In
our observation as shown in Figs. 2–5, mast cell infiltration was observed, but prominent endothelial damage was not clearly shown.

Appearance of mast cells in porphyria has already been observed by Mcgovern

Fig. 3. Subepidermal vesicle in a sun-exposed porphyric rat (H. & E. × 320).

Fig. 4. Cutaneous lesion biopsy of a sun-exposed porphyric rat showing dilatation and renewal of dermal vessels and perivascular cell infiltration (H. & E. × 200).
Skin Changes on Porphyric Rat

(1961) and Tsuyuki (1965), and it was postulated that, in the photosensitive state, contact with light causes an increase in permeability through an emission of a substance causing a "histamine-release" type of reaction. It is well known that histamine released from mast cells produces teleangiectasia, an increase in permeability, and a skin reaction which consists of redness, edema and itching. Rimington et al. (1967) thought that histamine would probably be one of the mediators of urticaria formation produced with 400 nm irradiation in a patient with porphyria. The exact role of mast cells in the pathophysiology of porphyria remains to be investigated.

In human porphyria, histological studies with conventional light microscopy showed the presence of a homogeneous thickening of the vessel walls composed of a PAS-positive diastase resistant material, and electron microscopy demonstrated reduplication of the basal lamina around vessels and the presence of masses of fine fibrillar material, most notably around the same blood vessels (Epstein et al. 1973). In addition, Höögsmann et al. (1976) observed endothelial damage and excessive, concentric, tube-like basal laminae around dermal vessels which appeared as PAS-positive hyaline materials under the light microscope in the mouse model for protoporphyria. Biopsy specimens of our experimental rats showed that the vessel walls were slightly positive in PAS-stain and less striking than those noted in PCT by Epstein. The mean duration of investigation for Epstein’s patients with active PCT was 5.2 years, and for Höögsmann’s mice a 10-month period of
irradiation, while for our rats it was only a 2-month period of eliciting photosensitivity flares. Therefore, it appears likely that endothelial damage was not prominent and PAS stainability was slight in our rats due to the shorter time factor.

Hönigsmann et al. (1976) hypothesized that, in murine protoporphyria and human erythropoietic protoporphyria, endothelial cells are photosensitized by protoporphyrin circulating in the serum and that photosensitized endothelium represents the primary cellular target of the photochemical reaction induced by long-wave ultraviolet light. Even in PCT, the cutaneous manifestations seem to occur by the same mechanism as hypothesized by Hönigsmann et al.

References