PATHOGENESIS OF PORPHYRIN-SENSITIZED LETHAL AND SUBLETHAL LIGHT-INDUCED DAMAGE

R. D. Barabash, V. E. Normanskii, K. P. Andreeva, G. A. Galil-Ogly, and A. S. Kolobanov

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The mechanisms of skin damage in porphyrin-sensitized individuals exposed to local irradiation from a xenon lamp, over its whole range or in certain parts of its spectrum [5-9], and also to radiation from a laser on a dye with wavelength of 630 nm [4, 11, 12], have been described in detail in the literature, and there have been isolated reports of death of sensitized animals after irradiation of the abdominal or dorsal surface of the body with visible light after removal of the hair cover [1], but there have been virtually no investigations into general responses of the body under near-natural conditions, including cases of severe damage leading to death of the animals. This information is clearly necessary so that methods of intensive treatment and resuscitation can be developed for cases of severe photosensitized damage, in order to determine the range of therapeutic action of photosensitizers, to establish their toxic doses, and to draw up safe schedules of illumination for patients at various times after administration of photosensitizers and laser irradiation, in connection with photodynamic treatment of patients with cancer and other diseases.

EXPERIMENTAL METHOD

Experiments were carried out on 57 noninbred albino mice weighing 18-20 g. To reproduce direct solar irradiation of the experimental animals, a laboratory system was set up with a 2 kW xenon lamp, with various filters and mirrors, and a revolving multicompartment cage, into which air was blown, to simulate the sunlight, as regards power density (100 mW/cm^2) and spectral characteristics, on a bright day at the autumnal equinox at sea level, and at the latitude of Moscow, and also to ensure constancy of the microclimatic conditions and uniformity of the photic flux. The duration of irradiation varied from 1.5 to 8 h. A preparation of an oligomeric hematoporphyrin derivative (HPD), injected intraperitoneally immediately before irradiation, or 12 and 24 h before irradiation, in doses of 1-14 mg/kg, was used as the photosensitizer. Animals which died or which survived for more than 10 days after exposure to this combination of conditions served as the test object. Autopsy material was fixed in 10% neutral formalin and embedded in paraffin wax. Histological sections were stained with hematoxylin and eosin and the presence of intravascular fibrin was determined by the standard Mallory's staining method. The character of fatty degeneration in the liver tissue was studied in frozen sections stained with Sudan black. The glycogen concentration in the liver was determined by Best's method. The response of the mast cells was studied in film preparations of skin fixed in Carnoy's fluid and stained with 0.1% toluidine blue solution, pH 4.7. Material for electron microscopy was fixed in 2% glutaraldehyde in cacodylate buffer, pH 7.4, and embedded in Epon. Ultrathin sections were cut on an LKB Ultrotome and examined in the 100 AK electron microscope.

EXPERIMENTAL RESULTS

The intensity of photodynamic injury leading to death of the animals was found to depend directly on the dose of photosensitizer injected (in mg/kg) and the total energy

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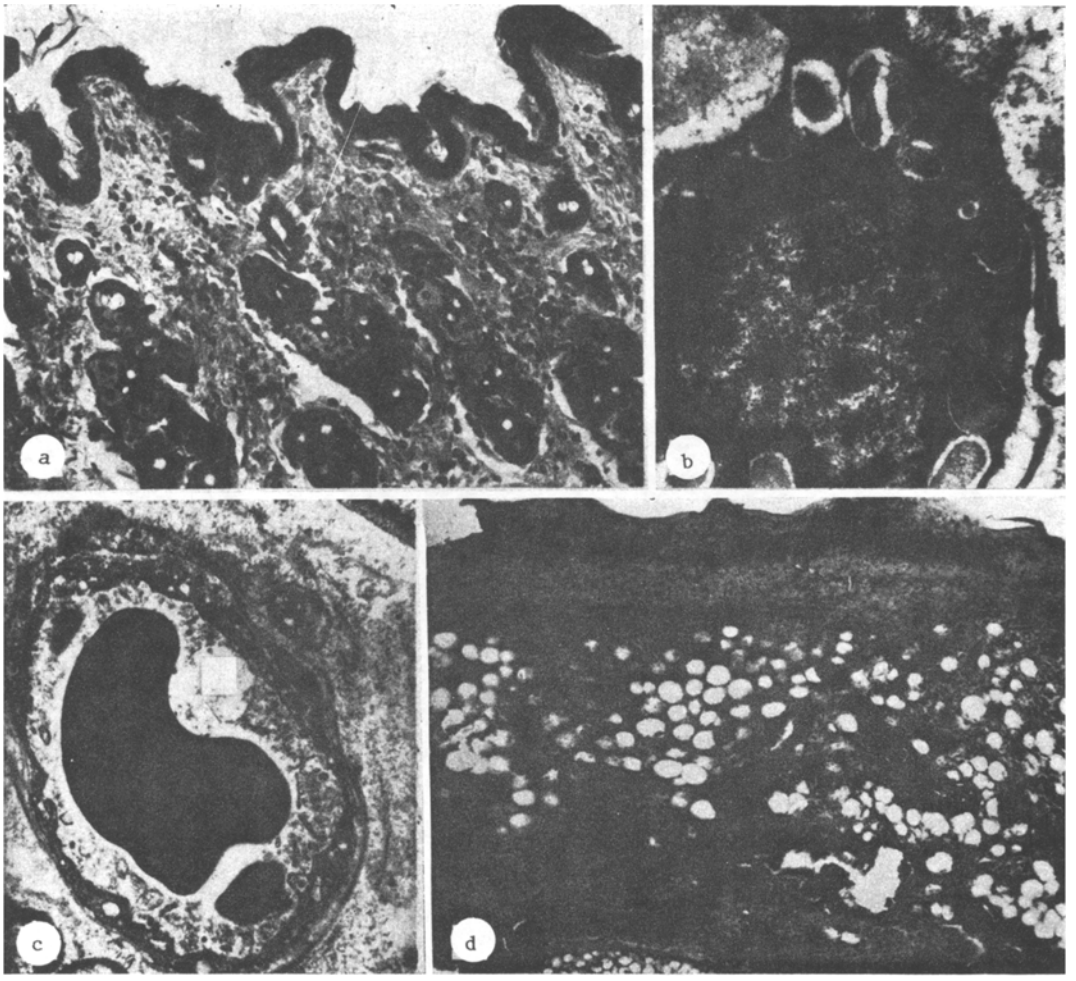
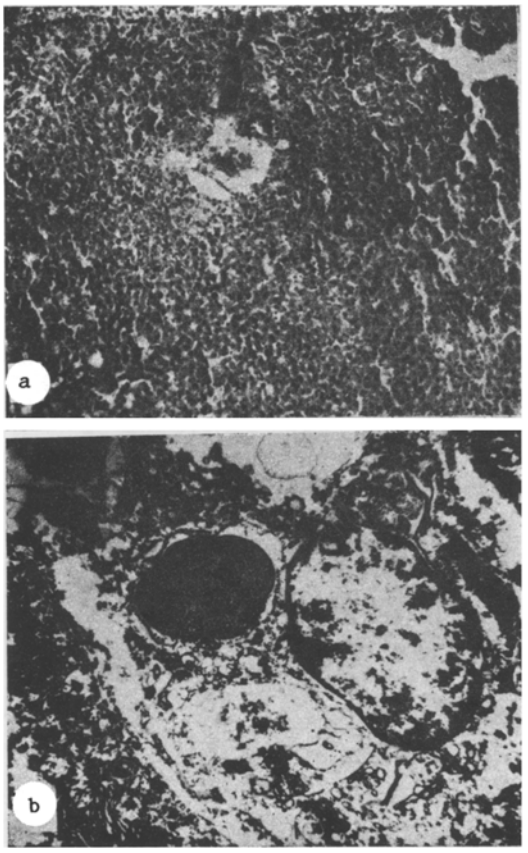


Fig. 1. Morphological changes in skin on dorsal surface of body of irradiated mice. a) Edema of dermis in animals of group 1, after 1 h of irradiation. Hematoxylin-eosin, 400 ×; b) rarefaction of matrix of osmiophilic granules in cytoplasm of mast cells in animals of group 1 after irradiation for 3 h. 50,000 ×; c) concentrations of photocoagulate (Ph) of plasma proteins in capillary lumen of zona reticularis of dermis in animals of group 1 after irradiation for 3 h. 39,000 ×; d) necrosis of epidermis and dermis in animals of group 3 on 10th day after irradiation. Hematoxylin-eosin, 200 ×.

of photic flux, calculated per unit area of body surface (J/cm^2). For example, LD_{50} for irradiation energy was 821 J/cm^2 if begun immediately after administration of the standard dose of 5 mg/kg but, conversely, LD_{50} of HPD was 5.2 mg/kg with a standard irradiation dose of 900 J/cm^2. If irradiation began a considerable time (12-24 h) after administration of HPD, the photodynamic effect was significantly reduced and LD_{50} for irradiation increased accordingly, directly proportionally to the increase in the interval between administration of HPD and the beginning of irradiation, as a result of increasing catabolism and elimination of the preparation on the body.

Depending on the magnitude of the photodynamic effect, early (hours), delayed (days), or remote (weeks) death of the animals was observed (groups 1, 2, and 3, respectively). As regards the probability and frequency of death, prognosis of the outcome in each of the three groups can be characterized as lethal, semilethal, and sublethal, respectively. The mechanisms of the lethal process and of terminal states were distinguished, not only by the familiar pathological features, but also by marked specificity in each group. In group 1 the characteristic clinical picture was observed with a lightening course of agony, with convulsions, Cheyne-Stokes respiration, and signs of vaso-respiratory insufficiency. In group 2 the agonal period was characterized by a subacute course, with clinical



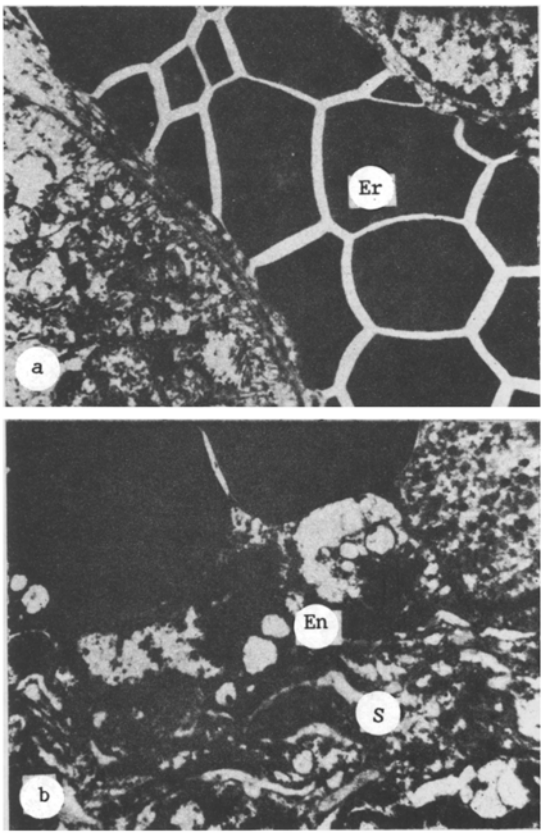


Fig. 2 Fig. 3

Fig. 2. Toxic damage to liver in animals of group 1. a) Centrolobular necrosis of hepatocytes. Hematoxylin-eosin. 100 ×; b) colliquative necrosis of Kupffer cells. 22,000 ×.

Fig. 3. Ultrastructural changes in kidney and lungs of laboratory animals. a) Stasis of erythrocytes (Er) in lumen of renal venule. 30,000 ×; b) Partial necrosis of endotheliocyte (En), edema of interalveolar stroma (S). 35,000 ×.

manifestations of general intoxication: depression of defensive reflexes, torpor, paresis of the limbs. Individual animals in group 3 died as a result of pyoresorptive toxicosis against a background of ulceration and necrosis of the dorsal surface of the body of the experimental animals.

In all groups the primary target for sensitized photodynamic injury was the skin on the dorsal surface of the body. On morphological investigation of the dermis marked edema was found in the first two groups after 1 h of irradiation (Fig. 1a). A very small increase in the degranulation coefficient was observed in the mast cells of the subcutaneous stratum adiposum and stratum reticulare of the dermis, from 0.3 in the control group to 0.5 after lethal irradiation for 3 h. Ultrastructural investigation revealed a rim of translucency around the osmiophilic granules in the cytoplasm of the mast cells (Fig. 1b), a morphological sign of the initial phase of mast cell secretion [2]. Concentrations of loose osmiophilic microgranular substance were seen in the lumen of the dilated capillaries and venules (Fig. 1c). Photocoagulation of the blood plasma proteins in the vessels of the microcirculatory bed in animals of groups 1 and 2 was observed only in the dermis, usually down to a depth of a few millimeters, but was absent in the vessels of the muscles and internal organs and corresponded to the depth of penetration of the red region of the solar spectrum in the 620-630 nm band, characteristic of the last peak of absorption of HDP [1]. Marked pathological changes were found in the skin of the animals of group 3 on the 3rd-4th days, no longer due to photocoagulation of the plasma proteins, but to the direct sensitized light-induced injury to the epidermic, skin appendages, endotheliocytes, and connective tissue cells. In doses below LD_{50} the zone of necrosis was confined to the epidermis, skin appendages, and stratum papillare of the dermis; with larger doses, necrosis extended to all layers of the dermis and to the subcutaneous cellular tissue (Fig. 1d).

Evidence of the toxemic pathomorphology of the lethal outcome in the animals of group 1 is given by the earlier involvement of the liver with colliquative necrosis of hepatocytes in the central zones of the hepatic lobule (Fig. 2a) and of the Kupffer cells (Fig. 2b), which are most exposed to the action of blood-borne poisons. In the case of delayed death of the animals of group 2, besides the changes described above, more marked polymorphism of the hepatocytes was observed due to an increase in the number of swollen cells with pale, vacuolated cytoplasm. In addition, severe microcirculatory disturbances were discovered in the circulatory system of the animals of the first two groups, by contrast with group 3. Stasis of erythrocytes was present in the lumen of the microvessels of the kidneys (Fig. 3a), lungs, and brain; edema of the brain developed. Further, as a result of disturbances in the microcirculatory system, a shock reaction developed, and was one of the main causes of early and delayed death of all the animals of groups 1 and 2. For instance, in the case of early death of animals "under the beam" morphological features of shock were most marked in the lungs, where the endothelium of the interalveolar capillaries was most affected: large vacuoles and foci of partial necrosis were visible in the cytoplasm of the endotheliocytes. Marked interstitial edema was present in the stroma of the interalveolar capillaries (Fig. 3b). In the case of delayed death of the animals on the 2nd-3rd days after irradiation, alveolar macrophages and granulocytes were more numerous in the lungs, in addition to the changes described above.

Differences in the mechanisms and times of death of the animals in the first two groups were due chiefly to differences in the degree of severity of disturbances in the microcirculatory system, which depended directly on radiation absorbed by the sensitizer. The more marked cellular polymorphism in the hepatic parenchyma, the increase in the number of alveolar macrophages and granulocytes in the lungs, and the appearance of foci in infiltration in the dermis in the animals of group 2 can be explained, first, by intensification of necrotic processes in the tissues with the development of cellular necrosis and, second, by reactive processes of exudation and activation of inflammatory cells. In animals of group 3 which survived more than 2 weeks after irradiation, morphological signs of general toxicity were confined to small foci of lymphohistiocytic infiltration and an increase in the number of reticuloendothelial cells in the liver. The mildness of the signs of toxemic pathology in these animals can be explained by the rapid fall of the HDP concentration in the blood plasma, on account of which the considerable photocoagulation of the blood plasma proteins, which leads to marked toxemia, did not take place in them during irradiation. This explanation is in good agreement with data in the literature [1, 3, 10], according to which only 50% of its initial concentration can be found in the blood of laboratory animals as early as 3 h after injection of the photosensitizer.

Sensitized light-induced injury is thus a pathological process with characteristic patterns of pathogenesis arising after the use of lethal or sublethal doses of photosensitizers and irradiation.

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