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EFFECT OF HEMATOPORPHYRIN AND RED LIGHT ON AH-130 SOLID TUMORS IN RATS

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The irradiation of malignant tumors with red light (600–700 nm) in the presence of a hematoporphyrin derivative (HpD, LIPSON et coll. 1961) represents a promising method for experimental and clinical therapy (DOUGHERTY et coll. 1975, 1978). The method is based on the ability of HpD to act as a photosensitizing agent and to be accumulated or retained in malignant tissues in larger amounts than in most normal tissues. Such properties have been extensively utilized for the identification and localization of several types of tumors in man, since the red fluorescence emission by porphyrins can be readily detected upon 400 nm-light excitation (GREGORIE et coll. 1968).

The cytotoxic action of photoexcited HpD is likely to affect, at least in part, the endocellular formation of an activated oxygen derivative, singlet oxygen $^1O_2$ (WEISHAUPT et coll. 1976). The consequent biochemical injury ultimately leads to cell lysis in agreement with the observed presence of high affinity receptors for porphyrins in the cytoplasmic membrane (COZZANI et coll. 1981).

The clinical phototreatment of malignant neoplasias in the presence of HpD can be performed by external irradiation with broad spectrum high-power lamps or with monochromatic light from a laser source; a combination of the light source with fiber optic allows the delivery of the activating radiation directly inside the tumor mass. HpD is constituted by a mixture of at least nine porphyrins (BONNETT et coll. 1981), whose relative phototherapeutic efficiency is not clearly defined. Hematoporphyrin (Hp) is an essential component of HpD. Hp preferentially binds to tumor cells, including AH-130 ascites hepatoma (JORI et coll. 1979) and MBL-2 leukemia cells (TOMIO et coll., unpublished results), and is an efficient photosensitizer for the killing of cultivated tumor cells (CHRISTENSEN & MOAN 1980). Therefore, the ability of Hp to photoinduce tumor regression in vivo was investigated.

Materials and Methods

All experiments were performed with a non-inbred strain of Wistar albino rats of both sexes, 20±1 days old, bearing a subcutaneous solid Yoshida hepatoma AH-130 (YOSHIDA 1949, SATO 1955). The average body weight of the rats was 60 to 80 g. Tumor cells were transplanted subcutaneously under aseptic conditions using 1 ml of Yoshida ascites cells. A solid tumor grows very fast, all the animals die after 25±5 days from inoculation. Phototherapy was initiated at day 5 after implantation when the diameter of the tumor ranged between 1.5 and 2.0 cm as measured with callipers.

Hematoporphyrin was obtained from Porphyrin Products (Logan, Utah, USA) and appeared to be about 97% pure by high pressure liquid chromatography. Hp solutions in phosphate buffer, pH 7.4, were prepared in dim light and freshly used, although they appeared to maintain their photobiologic properties for at least one week.

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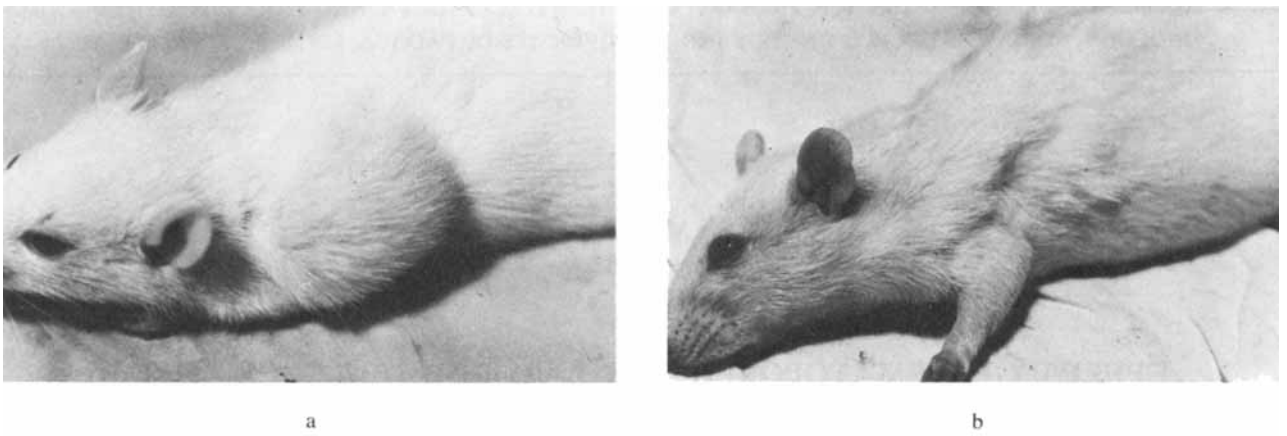

a b

Fig. 1. Yoshida hepatoma AH-130 in Wistar albino rat. a) Before treatment. b) 10 weeks after phototreatment.

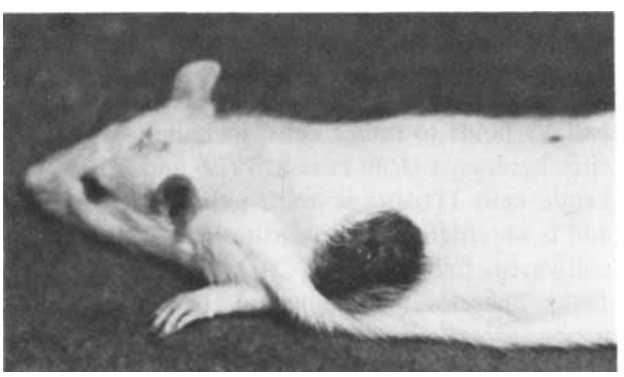

Fig. 2. Yoshida hepatoma AH-130 in Wistar albino rat 4 days after phototreatment with 10 mg/kg hematoporphyrin and red light.

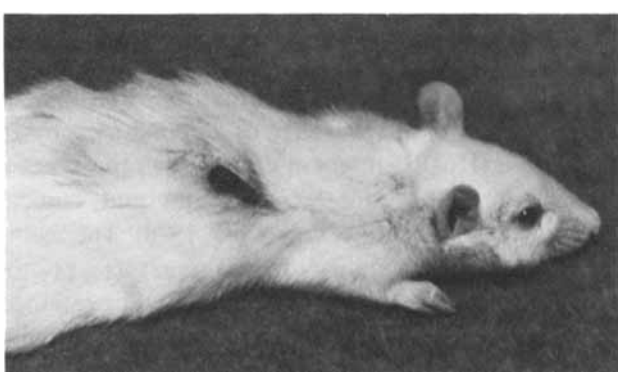

Fig. 3. A rat 4 weeks after phototreatment.

Hp was injected intraperitoneally into rats at a dose of 5 to 10 mg/kg body weight 24 h before exposure of the nodules to light. The light source was an Osram 4000 W Xenon arc-lamp endowed with continuous emission in the UV and visible spectral region. A chemical filter and a heat-reflecting glass filter were inserted in the light beam to eliminate infrared radiation and wavelengths below 590 nm. The beam was focused by a parabolic reflector placed behind the lamp and a condensing lens placed between the filter and the animal. Under these conditions, the fluence at the level of the rat skin was 20 mW/cm^2 as estimated with a radiometer. During irradiation, the rats were restrained within plastic holders specifically shaped to fit the animal body; suitable holes were cut in correspondence with the tumor. In all cases, rats were exposed to light for one hour.

Control animals included one group with tumor and no Hp either kept in the dark or irradiated, and another one with tumor and Hp kept in the dark. After phototreatment the rats were kept under daily control during the initial 10 days and biweekly control for the next 2 months. Occasionally, treated and untreated rats were killed and the tumor was fixed with formalin. Sections stained with hematoxylin and eosin were examined by one of us (F. C.), who ignored the treatment given.

Results

Macroscopic observations. All of the 30 animals treated with Hp and red light were cured. Cure was defined as the absence of any palpable tumor at least 2 months after treatment. In all cases, the tumors responded rapidly to phototreatment, no tu-

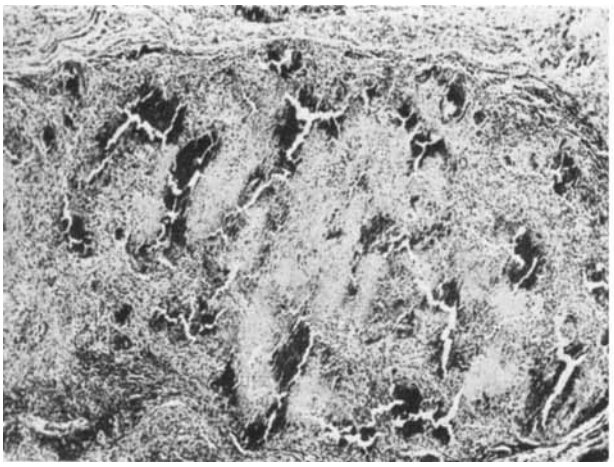

Fig. 4. Massive coagulation necrosis of the tumor 24 h after phototreatment (×40).

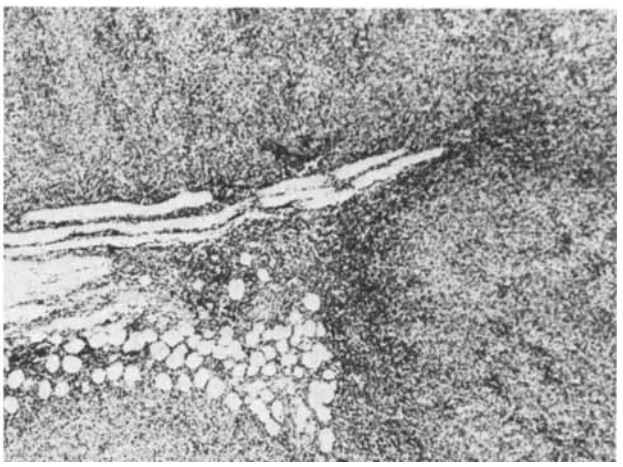

Fig. 5. Control rat. No evidence of necrosis (×40).

mor could be palpated a few days after exposure of the rats to light. It should be emphasized that up to 20 to 30 per cent of the tumors used may undergo spontaneous regression. However, this fact could not contribute to the regression observed in rats exposed to Hp and red light as only in these animals did a severe necrosis become apparent within 24 h after irradiation. On day 3 or 4 after the end of the phototreatment, a brown crust formed in the tumor area with a hard consistency, sharply defined from the surrounding tissue. The continuous tumor regression is depicted in Figs 1 to 3. The skin healed completely and regrowth of the hair occurred.

Tumors in the control animals did not respond detectably to treatment with only Hp or light, and exhibited a behaviour identical with that of untreated animals.

Microscopic observations. A massive coagulation necrosis of the tumor bulk was produced only by the combined effect of red light and Hp (Fig. 4). The process was evident already at 24 h after treatment. The cells in the damaged tissue showed pyknosis and karyorrhexis with eosinophilic cytoplasm or complete nuclear dissolution. On the other hand, none of the control nodules or untreated nodules were injured (Fig. 5).

Discussion

Hp is an efficient photosensitizer of biologic systems to the action of visible light (SPIKES 1975). Hp-induced photoinjury was reported for cultured cells (CHRISTENSEN & MOAN), animals (SMETANA 1938), and humans (ZALAR et coll. 1977). In particular, GRANELLI et coll. (1975) obtained photoregression of gliomas transplanted subcutaneously in rats which had been injected with 20 mg/kg of Hp. Subsequent reports (DOUGHERTY et coll. 1977) stated that HpD is endowed with better tumor-localizing properties than Hp, thus causing faster and more complete therapeutic effects.

Clearly, the present data agree with previous observations by GRANELLI et coll. Moreover, efficient killing in vivo of neoplastic cells can be obtained in the presence of Hp also if the phototreatment is performed at relatively long times after the porphyrin administration. Actually, relatively high amounts of Hp remain in the tumor area of rats affected by Yoshida hepatoma AH-130 up to 5 days after Hp administration (JORI et coll., TOMIO et coll. 1982). A high tumor to tissue ratio of Hp concentration was also observed in animals by RASMUSSEN-TAXDAL et coll. (1955). Thus, at least with the kind of neoplasms used in the present experiments, Hp can be used as an efficient phototherapeutic agent.

The results show that, when the necrosis of the tumor is complete, a fast and long-lasting regression takes place. No recurrence was detected in most rats within 2 months; in 2 cases, the irradiation had to be repeated, possibly because the large volume of the tumor prevented complete necrosis during the first exposure to light.

The microscopic observations on the phototreated tumor tissue indicate that cell injury mainly arises from the photosensitizing action of Hp on subcellular structures. However, the occurrence of

a concurrent injury to blood vessels also contributes to the overall photoprocess, as reported also by KELLY et coll. (1975) and DOUGHERTY et coll. (1979). No transient or permanent undesired effects on normal tissues were observed. This fact makes possible a repetition of phototreatment in the case of recurrence. Positive responses of the Yoshida hepatoma AH-130 were obtained in preliminary experiments with Hp-injected rats which were exposed to monochromatic 632.8 nm-radiation from an He/Ne laser source supplied by Valfivre (Firenze, Italy). The laser source was equipped with fiber optic directly implanted into the tumor. The use of the latter irradiation modality should enhance the effectiveness of phototherapy (DOUGHERTY et coll. 1981).

SUMMARY

Thirty Wistar albino rats with a subcutaneous Yoshida hepatoma AH-130 were exposed to 590 to 690 nm light from a high pressure Xenon arc-lamp 24 h after intraperitoneal injection of hematoporphyrin, 5 to 10 mg/kg. In all cases, the tumor decreased rapidly in size, due to necrosis caused both by direct action of the photoactivated porphyrin on the tumor cells and by secondary effects on blood vessels. No injury of normal tissues was detected. At 2 months after phototreatment, no recurrence had occurred and the skin over the tumor area was healed. Favourable therapeutic response was obtained in preliminary experiments by irradiating the Hp-injected tumor-bearing rats with 632.8 nm-light from an He/Ne laser source.

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