Neodymium-Yttrium Aluminium Garnet Laser Destruction of Nonsensitized and Hematoporphyrin Derivative-sensitized Tumors

Thierry Patrice, Marie-Françoise Le Bodic, Louis Le Bodic, Thierry Spreux, Gérard Dabouis, and Luc Hervouet


ABSTRACT

The injection of hematoporphyrin derivative (5 mg/kg i.v.) followed 24 hr later by a neodymium-yttrium aluminium garnet laser irradiation shows the destruction of CX1 tumors grafted on nude mice. This acidophilic necrosis occurred with a significantly increased frequency in tumors treated by hematoporphyrin derivative injection and irradiated with the neodymium-yttrium aluminium garnet laser as compared with noninjected but irradiated tumors or with injected tumors irradiated with sunlight.

On the basis of our data, it seems difficult to maintain the hypothesis of singlet oxygen production as the only mechanism of the phenomenon. Further studies will be necessary to explain the necrosis that we observed.

INTRODUCTION

Envisaged in the 1900s by Tappeiner et al. (13), the affinity of hematoporphyrin for neoplastic tissues has been confirmed by Figge et al. (5) and Lipson et al. (9) and was demonstrated by Rasmussen-Taxdall et al. (12), Gregorie et al. (6), and Kelly and Snell (7) using HPD.

Later, the oxidizing properties of HPD under different non-ionizing rays were demonstrated regardless of the wavelength used (mercury vapor lamps or xenon arc lamps) or whether or not it was filtered (1, 2). These studies led Dougherty et al. (3) to use helium:neon or dye lasers as sources of excitation of HPD. This led us to study the effects of photo-coagulation by the Nd:YAG laser on human colonie adenocarcinoma-type tumors grafted in the hip of the nude mouse after sensitization of the tumor tissue by HPD.

The aim of the study was to evaluate under these conditions the efficacy of the Nd-YAG laser and to study the lesions created in order to determine whether they were specific or similar to those seen in control series.

MATERIALS AND METHODS

Materials

Male albino nude mice were used. A human colonie adenocarcinoma-type tumor (C x 1) was grafted by bilateral injection of 0.2 ml of crushed tumor in solution in culture medium.

PD was prepared using the method of Gregorie et al. (6) and Lipson et al. (9). Hematoporphyrin hydrochloride (Roussel) was dissolved in acetic acid:sulfuric acid (9:1). The mixture was left at room temperature for 1 hr. The pH was adjusted at 7.2 to 7.4 with the addition of hydrochloric acid solution and made isotonic by the addition of sodium chloride. The final 5-mg/ml solution was sterilized by filtration through a Millipore filter and kept in darkness at −20° until used.

The energy was produced by a Nd:YAG laser (Cilas-Marcoussis Laboratory, 91460, France) emitting in the near IR (1.06 μm). This laser is a solid-state laser commonly used in our medical applications of laser therapy. A mechanical device held the tip of the fiber constantly at 5 mm from the target. A system combining a TRG 101 thermopile and a TRG 102 energy meter (Control Data Corp., Yellow Springs Institute, Colo.) was used to measure the power at the end of the fiber.

The visualisation beam of the helium:neon laser was cut off throughout the experiment.

Methods

All of the mice received a tumor graft on the first day of the experiment. Groups were constituted as follows.

Experimental Series (Group I; 14 Tumors). On the ninth day, the mice received an injection of HPD (5 mg/kg). On the 10th day, following skin incision to prevent secondary skin necrosis caused by laser heating which did not interfere with the deep vascular supply of the tumor, the tumors received a series of Nd-YAG laser exposures. The incision was then stitched up to avoid septic contaminations. On the 12th day, the mice were sacrificed, and the tumors were removed for histopathological examination.

Comparative Control Series (Group II) with the Following Subgroups. In Group II-A, 4 tumours were removed on the 12th day, the mice having received one tumor graft only; in Group II-B (8 tumors), on the ninth day, the mice received an injection of HPD (5 mg/kg). They were sacrificed on the 12th day for examination; in Group II-C (8 tumors), the mice received a similar injection of HPD, but on the 10th day, after skin incision, the tumors were exposed to sunlight through the window glass for 5 min. This group was formed to show that the observed effect of Group I was not linked to the surrounding light. These mice were sacrificed on the 12th day; in Group II-D (8 tumors), the mice did not receive an injection of HPD, but the tumors were treated with the laser on the 10th day and removed for examination on the 12th day.

The energy level of the laser has been controlled for each tumor of Groups I and II-D. For every shot, the power was 45 watts, and time exposure was 1.5 sec (Table 1).

The same experimental conditions used to obtain sample material, anesthetic techniques, and darkness were applied to all of the different groups. All of the mice were sacrificed on the 12th day for histopathological examination of the tumor. The latter was fixed in 12% formol for 24 hr, cut along its long axis, and mounted in its entirety in several paraplast blocks before being studied by numerous serial sections stained with haematoxylin:eosin:safran.

RESULTS

Group I (cf. Fig. 1). The 14 tumors which photocoaagulated after sensitization with HPD showed necrosis affecting approximately 90% of the total tumor mass. In 10 of them, there was
total acidophilic necrosis leaving no detectable tissue or cellular structure. In 3 tumors, there was necrosis although it was not acidophilic, with loss of glandular arrangement, disappearance of cell borders, and retraction of the nuclei when they could still be identified.

At the extreme periphery of the tumor, and in particular at depth, in 12 of 14 cases, rare neoplastic glandular tubes persisted, representing approximately 10% of the total tumor mass. On the surface and laterally, at the level of the cutaneous tissue, edema, congestion, and moderate inflammatory infiltrate were observed.

In depth and beyond the tumor, the muscular layer was affected in more than one-half of all cases.

Group II-A (cf. Fig. 2). Upon histological examination, these 4 control tumors, varying from 7 to 10 mm in diameter, were found to be weakly mucosecreting adenocarcinomas. Tumor spread developed in the s.c. tissue. The arrangement was frankly glandular and was sometimes polyadenoid with neoplastic tubules lined by an epithelium rich in cytological and nuclear abnormalities and with a high mitotic index. There were few fibroblastic stroma. There was neither localized abscess formation nor necrosis apart from one case where there was a very limited area of necrosis.

Group II-B (cf. Fig. 3). In these mice, which had received HPD, the nonphotocoagulated tumors were the site of rare areas of scanty punctiform necrosis with persistence of >90% of the total tumor mass. However, in 2 cases, there was an area of complete acidophilic necrosis.

Group II-C (cf. Fig. 4). These mice received an injection of HPD. These tumors were exposed to sunlight 24 hr later. Five tumors were the site of areas of punctiform necrosis slightly larger than those in Group II. Approximately 85% of the tumor tissue persisted.

By contrast, 3 tumors showed areas of acidophilic necrosis. There were no notable congestive phenomena, but the tumor was surrounded by an inflammatory infiltrate consisting of histiocytes, plasmocytes, and a few rare polymorphonuclear cells.

Group II-D (cf. Fig. 5). These 8 tumors received no HPD but were photocoagulated. They never showed areas of total acidophilic necrosis. The tumor tissue was nevertheless destroyed, 95% necrotic, with isolated cellular elements with retracted nuclei in the course of lysis. In one half of the cases, no neoplastic glandular tubules remained in the periphery. In the other half, there were a few glandular islets which persisted laterally.
In 7 of 8 cases, the necrotic tumor was limited at its extremities by a congested zone. The deep muscular layer was affected in all cases.

**DISCUSSION**

Analysis of the results of this experimental study leads to the following remarks.

(a) The comparison by the \( \chi^2 \) test of Group I with Groups II-B, II-C, and II-D combined shows a significant difference between these 2 groups (\( \chi^2 = 22.8; 1 \text{ d.f.} \)).

(b) The comparison of Group I with Groups II-B, II-C, and II-D individually also has been done with the \( \chi^2 \) test. After the correcting Yates test, a significant difference between Group I and each Group II is shown (\( \chi^2 = 9.62; 1 \text{ d.f.} \)).

(b) In the nontreated tumors, virtually no necrosis was seen on the 12th day. In the mice receiving HPD only, very few changes were seen within the tumors even when exposed to light on the 10th day. By contrast, in the 2 series subjected to either the action of the laser alone or the action of the laser after injection of HPD, there was virtually total destruction of tumor tissue.

The type of necrosis seen was not the same in Group I (laser plus administration of HPD) and in Group II-D (laser only). When the tumor had been previously sensitized by the administration of HPD, there was total massive acidophilic necrosis with disappearance of all tissue or cell structures. This acidophilic necrosis appeared to be different from the coagulation obtained by the laser alone. Furthermore, the distribution of the few residual glandular tubules is different in the 2 cases. After use of the laser alone, the glandular tubules which were found only in one-half of the cases studied were invariably distributed on either side of the coagulation lesion and never at depth. The underlying muscular layer was affected. By contrast, when the tumor had been previously sensitized with HPD (Group I), residual glands were found in 3 of 4 cases and were distributed in a wreath-like arrangement laterally as well as in depth. The underlying muscular layer was affected less frequently than that in Group II-D. It appeared as if the destructive effect of the laser was potentiated by HPD with regard to the tumor itself but limited with regard to the depth of the effects. In the absence of the administration of HPD, congestive phenomena seen at the periphery of the tumor tissue were more frequent and more marked.

It should be noted that HPD alone, in the absence of photo-coagulation, had an effect on the tumor cells. Islets of punctiform necrosis were seen in Group II-B. This was already reported in vitro by Malik and Djaldetti (10) in 1980 when following the administration of HPD scanning electron microscopy revealed destruction of plasmic membranes and of the coat of Burkitt-type tumor cells with inhibition of RNA and DNA synthesis. This effect was increased with exposure to light (Group II-C), and necrotic areas then were more extensive. Moan et al. (11) have emphasized the fact that the action of rays appears to be more marked when HPD is intracellular.

Using tumor cell cultures, Kinsey et al. (8) estimated that the mass of cells destroyed was greater when the wavelength of the light source was shorter. All of this did not seem to be corroborated by our own study.

While there was no evidence to show that the laser lesions were specific, there was nevertheless no similarity whatsoever between the few areas of necrosis seen in the control series and the total necrosis of tumors subject to photo-coagulation.

(c) Similar findings were reported by Dougherty et al. (4) who used a helium-neon or dye laser pumped by an argon laser to destroy breast tumors. However, they did not make any study of the histopathological lesions. Similarly, Thomson (14) obtained tumor destruction (using an argon laser) of tumors sensitized with acridine orange instead of HPD.

In summary, in answer to the question raised at the beginning of this report, the following conclusions may be drawn: (a) The Nd-YAG laser destroyed colonic adenocarcinoma-type tumors grafted in the mouse when these tumors were previously sensitized with hematoporphyrin derivative; (b) The coagulation necrosis observed after the use of the laser alone was replaced here by acidophilic necrosis; (c) This lesion was in no way specific. The same type of lesion was seen in nontreated lesions. Nevertheless, the laser increased necrosis considerably, while this increase did not occur when tumors sensitized by hematoporphyrin were irradiated with ordinary light.

A doubt remains as far as the mechanism of action is concerned. From this point of view, this paper raises more questions than it gives answers. Single oxygen production as mentioned by Weishaupt et al. (15) could be one of the mechanisms; however, that would require an absorption of HPD at 1.06 \( \mu \)m. Although we believe it could exist, technical problems prevent us from asserting it clearly and drawing a comparison with the 0.632-\( \mu \)m absorption. A direct alteration of HPD into a toxic compound under the heating effect could be another mechanism. Further studies are being conducted by our department to try to provide an answer.

**REFERENCES**

Fig. 3. Group II-B, rare areas of scanty punctiform necrosis after administration of HPD. Hematoxylin, eosin, and safran; A, x 2.2; B, x 250.

Fig. 4. Group II-C, areas of punctiform necrosis after administration of HPD followed by local exposures to light. Hematoxylin, eosin, and safran; A, x 2.7; B, x 250.

Fig. 5. Group II-D, necrosis of tumor photoagulated by Nd-YAG laser. Hematoxylin, eosin, and safran; A, x 2.8; B, x 250.
Neodymium-Yttrium Aluminium Garnet Laser Destruction of Nonsensitized and Hematoporphyrin Derivative-sensitized Tumors

Thierry Patrice, Marie-Françoise Le Bodic, Louis Le Bodic, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/43/6/2876

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.