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Laser-photosensitizer assisted immunotherapy: a novel modality for cancer treatment

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Abstract

Photosensitizer-enhanced laser treatment, where dyes are activated in situ by lasers of appropriate wavelengths, provides highly selective tissue destruction, both spatially and temporally, through photophysical reactions. Although laser-sensitizer treatment for cancer can achieve a controlled local tumor cell destruction on a large scale, total tumor eradication may not be accomplished because of the incomplete local tumor killing or the presence of tumor metastases, or both. The long-term control of cancer depends on the host immune surveillance and defense systems in which both cell-mediated and humoral responses are critical. In this study we report a novel minimally invasive cancer treatment combining the laser photophysical effects with the photobiological effects. Irradiation of a rat mammary tumor by an 805 nm diode laser, after an intratumor administration of a specific photosensitizer, indocyanine green in a glycated chitosan gel, caused immediate photothermal destruction of neoplastic cells. Concomitantly this treatment stimulated the immunological defense system against residual and metastatic tumor cells. Increases in survival rate and in the eradication of tumor burden, both primary and metastatic, were observed after this treatment. Furthermore, the resistance of successfully treated rats to tumor rechallenge demonstrated a long-lasting systemic effect of the treatment. These findings indicate that our treatment has triggered a specific humoral immune response in the tumor-bearing rats. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

The use of lasers in lesion treatment utilizing photosensitizer-enhanced reactions [1,2], particularly for the destruction of malignant tissues, is gaining widespread acceptance because of the precision of energy delivery achieved with modern instruments. When a photosensitizer of appropriate absorption peak is present in the tumor, the laser energy can be directed and deposited in the targeted tissue to cause enhanced and localized photomechanical, photochemical and photothermal reactions [3–5]. This methodology provides a non-invasive treatment modality that minimizes col-

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lateral tissue damage, both local and systemic [1,2,6–8]. However, the very circumscribed effect of this procedure may also limit its overall efficacy, since local treatment could not stop the global neoplastic proliferation that occurred prior to treatment, that evolved from the surviving cells, or both. Therefore, in order to achieve a long lasting effect, it is highly desirable for the localized laser effect to be coupled with a superimposed laser-induced systemic photobiological reaction.

A novel approach which takes advantage of the precise localization of the laser thermal tumor destruction and at the same time stimulates the host's immune defense system has been developed. This method consists of three components: (1) a laser, (2) a photosensitizer, and (3) an immunoadjuvant. The laser used was an 805 nm diode laser which can penetrate organized tissue with little energy deposition. The photosensitizer, indocyanine green (ICG), is a non-toxic dye with an absorption peak around 790 nm. It has been used in lesion detection [9–11], in enhanced laser treatment [12,13], and used for hepatic, biliary, cardiovascular and ophthalmic studies in humans [14–19]. A glycated chitosan gel (GCG) was used as the immunoadjuvant; chitosan has high biodegradability and low toxicity [20,21] and it has been shown to be an immunostimulus [22–27]. In addition, GCG also functions as a carrier of ICG and prolongs the retention of the dye at the injection site. We applied this method to treat a chemically induced, transplantable, metastatic rat mammary tumor (strain DMBA-4 [28–30]) and recorded both the immediate response and the long term impact of the treatment.

2. Materials and methods

2.1. Animals

Wistar Furth female rats (Harlan Sprague–Dawley, Inc., Indianapolis, IN), aged 6–7 weeks and weighing 100–150 g, were fed with Custom High Polyunsaturated Fat Diet (ICN Biomedicals, Aurora, Ohio) throughout the experiment to facilitate tumor growth. After laser treatment, the rats were housed in individual cages. A total of 148 rats were used in our study; 33 were tumor-bearing control rats and rest were treated by laser at various power and duration settings, in conjunction with different dosages of photosensitizer and immunoadjuvant.

2.2. Tumor transplantation

The cancer cells for the animal model used in our experiments were derived from the chemically induced, transplantable, rat mammary tumor strain DMBA-4 [28–30]. Approximately 250 000 live cells were injected subcutaneously into one or both inguinal fat pads of female Wistar Furth rats and the animals were monitored daily for tumor development.

2.3. Photosensitizer and immunoadjuvant administration

Indocyanine green (Sigma Chemical Co., St. Louis, MO) was purchased in dry powder form. Glycated chitosan gel was prepared by incubating an aqueous suspension of chitosan with a three-fold excess of galactose and subsequent stabilization by borohydride reduction of the Schiff bases. The ICG-chitosan solution was prepared by grinding in an all glass homogenizer a known weight of the dry ICG powder in sufficient glycated chitosan gel to yield a solution of desired final concentration. Volumes of 70–500 μl of 0.25–1% ICG solution in 1% glycated chitosan gel were injected into the center of tumors prior to the laser treatment.

2.4. Laser treatment of tumor

The Diomed 25 (Diomedics, The Woodlands, TX), a diode laser of 805 nm wavelength, was used in all experiments. Several experimental protocols with laser parameters of 2–15 W and exposure duration from 3 to 10 min were investigated. The laser energy was delivered through an optical fiber (1.2 mm in diameter) to the treatment site in a non-contact mode. The fiber tip was maintained at a distance of 4 mm from the skin surface and was slowly moved across the entire tumor.

2.5. Post-treatment observation

Post-treatment examination was made daily and the dimensions of the tumors were measured twice

weekly. The volume of tumors was calculated, assuming an ellipsoid, using $V = 4/3\pi abc$ where a is the semi-major axis, and b and c are the semi-minor axes. The average density of the tumor, measured as 1.05 g/cm^3, was utilized to calculate tumor burden.

3. Results

3.1. Effect of tumor treatment

The experimental rats, depending on the post-treatment course, fell into three discrete groups: (a) unresponsive (i.e. death at around 30 days, same as control tumor-bearing rats [8]); (b) positive response (rats survived up to 45 days, a 50% increase of the expected life span); and (c) success (tumor eradication and long-term survival up to 90 days post tumor implantation). The treatment yielded an average of 14% positive response and 8% success (see Table 1). The response to the treatment was clearly affected by both the level of laser energy and the length of laser exposure. It was evident from our experimental results that the treatment with lower laser powers (below 5 W) and longer exposure duration (above 2 min) was more effective. The two most recent experimental groups (16 rats), when treated with 2 W for 10 min using 200 μl 0.25% ICG in 1% GCG, yielded 50% positive responses and 25% long-term survivals. One experimental group (six rats) even yielded a 50% success rate. The treatment of early stage tumors also appeared to be most effective, since the photothermal destruction of smaller tumors tended to be more complete. Likewise, earlier initiation of the immune response may prevent metastatic seeding to remote sites.

3.2. Positive impacts: short- and long-term

The immediate effect of the laser-photosensitizer treatment was the destruction of tumor cells due to the photothermal interaction of the laser and ICG. All the rats with positive responses had smaller tumor burdens, about half the volume of the control tumor-bearing rats, at the time of death. Among all the long-survival rats, the tumor profile, both treated primary tumor and untreated metastasis, was unique: after treatment, the tumor growth continued but at a slower rate, and at a certain point (range 4–6 weeks), the mass began a gradual reduction. Fig. 1A–D shows the onset and the magnitude of such responses, using tumor profiles of three treated rats with and without metastases.

3.3. Tumor re-challenge

Five tumor-bearing rats, cured following laser-ICG-GCG treatment, were rechallenged with three times the standard dose of tumor cells, and no tumors developed. Sixty days after the first rechallenge, three of the rats were challenged again with the increased dose of tumor cells but the rats still remained refractory to the rechallenge. Meanwhile, 12 untreated rats of the same age all died within 30 days of tumor transplantation.

4. Discussion

The DMBA-4 tumor cell line used in these experiments is an aggressive strain in female Wistar Furth rats; 99% of tumor-bearing rats died around 30 days after tumor cells were implanted, even with effective tumor cell killing through photosensitizer-enhanced laser treatment [8]. Chemotherapy has been shown only to slow tumor metastases in this tumor model, but neither positive response nor long-term survival could be achieved (unpublished data). The results obtained (Table 1 and Fig. 1) indicated that an immunological reaction played a major role in the success of our treatment. Subsequent studies showed that our protocol triggered a humoral immune response. This deduction is based on the following observations: (1) the full scale reduction of tumor burden started approximately 4 weeks after the treatment, as demonstrated in Fig. 1; (2) at this point in time there was no evidence of increased lymphocyte or macrophage presence in histological preparations; (3) the resistance to tumor rechallenge induced by the treatment; and (4) the lack of tumor-specific, cell-mediated immunity observed in the study of cell-dependent cytotoxicity using ^{51}Cr labeling technique (unpublished data). Our preliminary immunohistological results (unpublished data) also demonstrated that serum from successfully treated tumor-bearing rats contains antibodies bound strongly to tumor cells, both live and preserved. Our

laser-ICG-GCG treatment appeared to induce and enhance a stronger immune response that led to total eradication of primary and metastatic tumors and to a long-lasting resistance to subsequent tumor challenge. Because of the short life-span of the tumor-bearing rats (approximately 30 days), the humoral response often may not be established early enough to effectively combat the tumor cell proliferation and metastases. Less aggressive animal tumor models, and particularly tumors in humans, should be more sus-

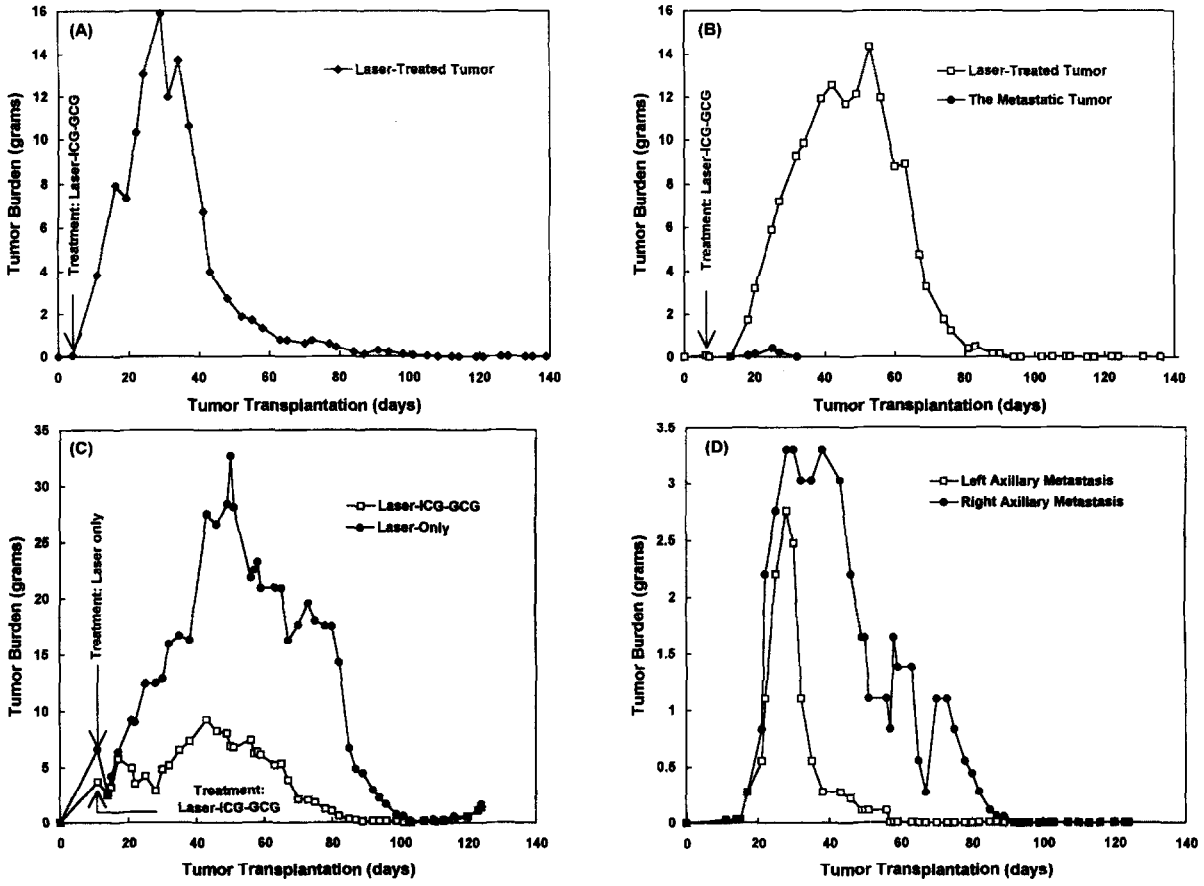

Fig. 1. The tumor burden profiles of laser-ICG-GCG-treated long-term survival rats (>90 days). (A) Rat without metastasis. The tumor cells were inoculated only in the right inguinal fat pad. A single laser treatment (arrow), 2 W for 10 min, was applied after intratumor administration of ICG-GCG solution (200 μl of a 0.25% solution of ICG in 1% glycated chitosan injected 4 h prior to laser treatment). The primary tumor continued to grow, reaching its maximum volume around 35 days after treatment and then began to recede. The tumor completely disappeared around day 100. No tumor recurrence was observed. (B) Rat with a short-lived metastasis. The tumor cells were inoculated only in the left inguinal fat pad and a single laser treatment of 2 W for 10 min (arrow) was applied. Two hundred μl of a 0.25% solution of ICG in 1% glycated chitosan was injected 1.5 h prior to laser treatment. The treated primary tumor (open squares) underwent a course similar to that shown in (A), reaching its maximum 47 days after the treatment and disappearing around day 90. The small tumor metastasis to the right inguinal area was observed around day 18 and it disappeared in less than 2 weeks (solid circles). No tumor recurrence was observed. (C,D) Long-term surviving rat with metastases and recurrence. The tumor cells were inoculated to both left and right inguinal fat pads before the treatment. The left primary tumor was treated with laser-ICG-GCG and the right tumor with laser only. The laser parameters were 5 W and 3 min. Seventy μl of 1% ICG in 1% glycated chitosan was injected into the left inguinal tumor 24 h prior to laser treatment. The primary tumors (C) underwent courses similar to that shown in (A) and (B); the laser-ICG-GCG treated tumor (open squares) grew at a much slower rate than the laser-only tumor (solid circles). The metastases to both left and right axillary areas became noticeable around day 15, peaked between day 30 and 40, and then disappeared gradually as shown in (D). Tumor recurrence was observed at the primary sites but the rat died while the recurrent tumor load was relatively small, 124 days after tumor transplantation.

Table 1

Effect of the laser-ICG-GCG treatment on tumor-bearing rats

Group (no. of rats)	Treatment time (days after inoculation[a])	ICG + chitosan administration[b]	Laser treatment[c]: power and duration	Positive response[d] number (%)	Long-term survival[e] number (%)
1 (37)	10–15	1% ICG; 1% GCG; 70–100 μl	5 W; 3–6 min	3 (8)	3 (8)
2 (3)	16	1% ICG; 1% GCG; 150 μl	15 W; 3 min	0 (0)	0 (0)
3 (27)	10–15	0.5% ICG; 1% GCG; 70–400 μl	3–5 W; 3–10 min	2 (7)	2 (7)
4 (13)	10–15	0.25% ICG; 1% GCG; 100–400 μl	5 W; 5 min	1 (8)	0 (0)
5 (19)	7–8	0.5% ICG; 1% GCG; 100–500 μl	3–5 W; 3–10 min	2 (11)	0 (0)
6 (10)	4	0.25% ICG; 0.5% GCG; 200 μl	2 W; 10 min	4 (40)	1 (10)
7 (6)	6	0.25% ICG; 0.5% GCG; 200 μl	2 W; 10 min	4 (67)	3 (50)
8 (33)	No treatment[f]	–	–	0 (0)	0 (0)

[a]Tumor transplantation: 250 000 cells injected into the inguinal fat pad, in most cases either left or right and in some cases both.
[b]The ICG solution in glycated chitosan gel was injected directly into the center of the tumor between 0 h and 24 h prior to laser exposure, in most cases to either left or right inguinal tumor and in some cases both.
[c]The energy of an 805 nm solid state laser was directed to the treatment sites through a 1.2 mm fiber which remained in a non-contact mode (4 mm distance from the skin surface); in most cases either left or right and in some cases both tumors were treated.
[d]The positive response is defined as the survival time longer than 45 days after tumor transplantation, which is a 50% increase in survival. This group also included the rats that continued on to be long-term survivors.
[e]The long-term survival is defined as the survival time longer than 90 days after tumor transplantation.
[f]Survival time (± SD) 31.5 ± 3.7 days.

ceptible to this treatment, since they would allow this laser-sensitizer-assisted immunotherapy to achieve a maximum response before the hosts reach the moribund stage.

In summary, our treatment protocol constitutes a novel approach among laser-based modalities for the treatment of malignant tumors. We have shown that the sensitizer-enhanced photothermal interaction, as well as possible photochemical interactions as yet to be specified, destroys targeted tumor cells on a large scale and in a circumscribed fashion. The glycated chitosan then elicits an immune reaction against the remaining population of tumor cells, by combining with cellular antigens released from disrupted tumor cells to form an in situ autovaccine. This hypothesized tandem effect is consistent with the local and systemic response observed after laser-ICG-GCG treatment. We suggest that the successful eradication of the tumors and subsequent resistance to tumor challenge were the result of a significant response by the immune system, primarily the humoral arm, as evidenced by our post-treatment observations. It is possible that other lasers, immunomodulators and sensitizers could be employed using the same principles. Further investigation is currently in progress involving large groups of animals and applying different treatment parameters in order to determine the efficacy of our treatment modality. Immunological studies are also in progress in order to understand the working mechanism of this novel method.

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