REGRESSION OF RAT CARCINOSARCOMA BY PERFUSION OF TUMOUR INFILTRATING LYMPHOCYTES IN CONJUNCTION WITH HAEMATOPORPHYRIN DERIVATIVE AND HeNe LASER IRRADIATION

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Experiments were performed on six batches (Batches I ~ VI) of inbred Wistar rats with Walker-236 carcinosarcoma. The groups received, respectively, photodynamic therapy on its own (PDT, Batch I); perfusion of tumour infiltrating lymphocytes on its own (TIL, Batch II); or both of the above in conjunction therapy (PDT + TIL-A, Batch III; PDT + TIL-B, Batch IV; and PDT + TIL-AB, Batch V); and one control batch (Batch VI, which was injected with Hank's buffered salt solution) which consisted of animals with untreated Walker-256 tumours. The results were as follows: the individual treatment (PDT, TIL) gave survival rates between 28.6% ~ 49.8%, the cure rates ranging from 19.0% ~ 28.2%. The "combined" therapy in multiple doses increased significantly the survival of tumour-bearing rats (84.7%) as well as the highest incidence of complete regression (65.3%). Cell-mediated immunity test values in Batches III-V exposed to multiple PDT+TIL doses showed higher values as compared to the values noticed in Batches I-II and the control Batch VI at 14, 28 and 42 days post-treatment. Summing up, this work demonstrates that "combined" photodynamic therapy with TIL immunotherapy stimulates cell-mediated antitumoural activity, produces modifications in tumour histological structure, increases survival rate and reduces tumoural incidence in Walker-256 carcinosarcoma in the rat model.

Key words: Walker-256 carcinosarcoma, photodynamic therapy, tumour-infiltrating lymphocytes, immunotherapy, recombinant interleukin-2, concanavalin A

Introduction

Destruction of malignant tumours previously sensitized with haematoporphyrin derivative by photodynamic treatment, represents a comparatively new method of tumour therapy. In the past 15 years several thousand cancer patients have undergone PDT or dihaematoporphyrin ether (DHE) mediated PDT.⁽¹⁾ Photodynamic therapy of malignant tumours includes biological, photochemical and photophysical processes.⁽²⁾ These proc-

esses involve: (a) absorption of photosensitizing agent; (b) selective retention of the photosensitizer in tumours; and (c) irradiation of sensitized tumours by laser radiation.^(3,4) Experimental approaches and clinical investigations have shown that the association of two or more means for cancer treatment such as antitumoural agents,⁽⁵⁾ radiation, hyperthermia, bacterial extract,⁽⁶⁾ endotoxin⁽⁷⁾ and lymphokines in animal and human tumours present evident advantages and the "combined" therapeutic concept has become a frequently

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accepted treatment.^(8,9)

Animal experiments have demonstrated that tumour-infiltrating lymphocytes activated by recombinant interleukin-2 (rIL-2) have 50-100 times greater therapeutic potency than lymphokine-activated killer cells.⁽¹⁰⁾ In clinical trials, regression of tumours has been observed after the adoptive transfer of cultured TIL to patients with metastatic malignant melanoma, renal cell carcinoma, lung cancer or epithelial ovarian cancer.⁽¹¹⁻¹⁵⁾ Unlike the effects of photodynamic therapy and tumour-infiltrating lymphocytes used in various tumour models on their own, no study on the therapeutic effects of TIL in combination with photodynamic therapy has been developed so far.

In the present work, we have studied the effects of tumour-infiltrating lymphocytes in combination with photodynamic therapy upon the evolution of Walker-256 carcinosarcoma and immunological reactivity after local exposure to associated treatment.

Materials and methods

Animals and Tumour Implantation

Walker-256 carcinosarcoma was received from the Oncology Institute, Bucharest, Romania and maintained by serial transplantation in inbred Wistar rats. Large tumours were dissected under sterile conditions; macroscopically viable tumour tissue was minced with scissors and forced through sterile needles of decreasing dimensions. Tumoural suspension (0.2 ml) was subcutaneously injected in the dorsal region of syngeneic animals. Tumours were visible in 7-8 days after transplant and had a volume of between 85-120 mm³. At the start of the study, the animals were 8-10 weeks old, weighing 140-150 g. The animals were from the Cantacuzino Institute farms and were kept in subdiet and water *ad libitum*.

Photosensitizer

The compound used in the present study was Photofrin II (Photofrin Medical Inc., Cheektowage, NY). Rats were intraperitoneally injected with a drug dose of 20 mg/kg body mass. Twenty-four hours post-injection rats were photoirradiated.

Laser phototherapy

Our light source was a divergent beam from an LG HeNe laser (632.8 nm; 10 mW) whose output was checked periodically with a SpectraPhysics C power meter.

Tumour separation

Sterile surgical specimens of tumour tissue were obtained and processing was initiated within 2 h of resection. Necrotic and connective tissues were removed and

the remaining specimen was minced with scissors. The macerated fragments were suspended in an enzyme mixture of 50 mg type N collagenase; 5 mg type V hyaluronidase and 1,500 units type I DNase (Sigma Chemical Co. St. Louis, Mo, USA). The suspension was stirred for 3 h at 37°C and then harvested by centrifugation. Cell suspensions harvested after 3 h of enzyme dispersion were washed 3 times with RPMI 1640 medium.

Preparation of effector cells

Tumour-infiltrating lymphocytes (TILs) were isolated from tumour digest as previously described.^(16,17) Briefly, the washed cells were placed on Ficoll-Hypaque for single stage density gradient separation and centrifuged at 400 G for 15 min at room temperature. Dead cells, erythrocytes, granulocytes and parts of tumour cells were pelleted to the bottom of the container. The cells at the interface, consisting of lymphocytes, monocytes, macrophages and tumour cells were collected in complete medium. These procedures were found not to compromise lymphocyte function. Viable TILs and tumour cells were counted under the microscope with a trypan blue exclusion test.

The percentage of viable cells exceeded 83.5%. Lymphoid cells were discriminated from the tumour cells by their small size, roundness and lesser granularity; tumour cells were gated according to their large size and greater granularity.

Cell culture

For lymphocyte proliferation, TILs and tumour cells in a single cell suspension were washed twice and resuspended at 2.5×10^5 cells/ml in RPMI 1640 with 10% fetal calf serum (Gibco, Grand Island, N.Y.), penicillin 100 units/ml, streptomycin 50 µg/ml, gentamycin 50 µg/ml and L-glutamine (complete medium). The suspension was placed into flasks (gas permeable) with recombinant interleukin-2 (rIL-2, provided from Hoffman-LaRoche) at a final concentration of 100 U/ml. Cultures were incubated at 37°C, in 5% CO₂ and passaged weekly by harvesting and resuspending cells in fresh medium containing rIL-2. The number of lymphoid cells increased and that of tumour cells decreased until after the tenth day when nearly all of the remaining cells were lymphocytes. Approximately 65-82% fold suspensions of the original number of cells were obtained within 18 days. At 18 days of culture no tumour cells remained as assessed by cytological examination and no skin tumours were induced by injection of these cells. In preliminary investigations, before infusion in treated animals, the cytotoxic activity of the TIL effector cells were evaluated *in vitro* against fresh

Walker-256 tumour (ascitic form) and YAC-1 cells (results not included in this paper).

Experimental design

Six different treatments were evaluated in rats with Walker-256 carcinosarcoma (W-256): Photodynamic therapy (PDT) in five fractionated exposures, 60 min each (Batch I); tumour-infiltrating lymphocytes (TIL, Batch II); given peritumourally in five doses (5×10^8 cells/dose/animal) every 3 days; PDT+TIL-A, (peritumourally infused, Batch III); PDT+TIL-B (intratumourally infused, Batch IV); PDT+TIL-AD (intra- and peritumourally infused, Batch V); and injection of Hank's balanced salt solution (HBSS) (control Batch VI). TIL infusion was carried out by the method described previously by Yamaki *et al.*⁽¹⁸⁾ Fraction irradiation of animals in Batches I, III, IV and V was performed at 3 day intervals, followed by TIL infusion. The rats were regularly assessed and tumour size was measured (in mm³) along three orthogonal diameters (D₁, D₂, D₃) every third day, in the first 3 weeks and then weekly till the experiment ended (60 days). The volume (V) was calculated assuming spheroid geometry using the following formula:

$$V = \left[\frac{\pi}{6(D_1 \cdot D_2 \cdot D_3)} \right] \text{mm}^3$$

Immunologic Tests

Mitogen response of spleen cells: Splenocytes from all six batches and a further control batch consisting of 28 healthy animals (not inoculated) were fractionated on a nylon column according to the method of Julius *et al.*⁽¹⁹⁾ The resulting T cells, enriched cells, (nylon, wool non adherent) were cultured at a density of 2×10^5 cells/well in RPMI 1640 supplemented with 25 mM HEPES, 2 mM glutamine and 10% fetal calf serum with or without the addition of Con A ($5 \mu\text{g/ml}$), (Concavalin A, Pharmacia Fine Chemicals, AB, Uppsala, Sweden) in triplicate wells at 37°C in a CO₂ incubator (5% CO₂ + 95% air) for 48 h; 20 μl /aliquots of (³H)-thymidine (10 $\mu\text{Ci/ml}$; spec. act. 20 Ci/mM; IFA-Bucharest, Romania) in RPMI medium were added to the well. After 48 h cultivation, the cells were harvested on glass filter papers; washed with 5% trichloroacetic acid, methanol-dried, and introduced in vials containing 10 ml scintillation liquid using 0.4 % PPO and 0.01% POPOP solution in toluene. Radioactivity was read in a Beckman-model CPM 100 scintillation counter. Results were calculated from the uptake of ³H-TdR by the cells and were expressed as mean uptake in counts per minute (cpm) per well.

Cytotoxicity Assay: Effector cells cytotoxicity was assayed by the (³H)-uridine method described by Nishimura *et*

al.⁽²⁰⁾ at 14, 28 and 42 days post treatment. Briefly, aliquots (0.1 ml) of effector cells suspension were mixed with 0.1 ml (³H) uridine (10 $\mu\text{Ci/ml}$; spec. act. 25 Ci/mM; IFA-Bucharest), and labelled target cells (2.5×10^5 / 0.1 ml) were placed on Coster 96-well round bottom plates at 100:1 effector to target ratio and cultured for 18 hrs at 37°C. The cells were harvested and then the retained radioactivity determined by a standard scintillation technique. Target cell lysis was calculated by the following equation:

$$\text{TOX}(\%) = \frac{\text{cpmE} + \text{cpmT}}{\text{cpmT} \times 100}$$

where TOX is toxicity expressed as a percentage, cpmE and cpmT are the cpm of effector cell culture and target cell culture, respectively.

Target cells: The target cells consisted of YAC-1a Maloney murine leukemia virus T-cell lymphoma from A strain mice. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ in RPMI 1640 medium supplemented with 10% fetal calf serum.

Biodistribution of ¹³¹I-labelled TIL in normal and tumour-bearing rat tissues

To determine if there was preferential accumulation of TILs in tumour-bearing rat tissues, TILs were labeled with ¹³¹iodine (¹³¹I). Three weeks after tumour induction Batches I, III-V, Guérin tumour-bearing and healthy animals were inoculated intravenously with ¹³¹I-labeled TILs (5×10^8 cells/animal) in 0.5 ml of RPMI 1640 medium. After sacrifice (3 days later), animal tissues were removed, weighed and analyzed for radioactivity in a Beckman gamma counter. The results were expressed as a percentage of injected dose per gram of tissue.

Histopathology

Tumours were removed from animals used for immunological tests after four weeks of treatment alone, were combined, fixed in buffered formalin, sectioned and stained with haematoxylin-eosin (H-E).

Statistic analysis

Data were expressed as arithmetic mean and standard error (SE). Significance of differences between groups was calculated by the Kruskal-Willis test.

Results

Inhibition of tumour growth after local administration of combined therapy

Growth curves of tumour volumes in all the five Batches of animals are presented in Figure 1. In control rats (Batch VI) injected with HBSS (no PDT or TIL) the

Fig 1: Changes in mean tumour volume in murine Walker-265 carcinosarcoma after photodynamic therapy in conjunction with tumour-infiltrating lymphocytes.

tumour volumes increased by a factor of approximately 30 by 4 weeks post-transplantation. After 28 days of treatment, tumour volumes found in the five experimental batches were less than those of the control batch (HBSS-injected). In animals from PDT-only-treated Batch I and TIL-only-treated Batch II, a slower growth of the tumour volume was noticed compared to control Batch VI. In the rats from Batches III and IV exposed to PDT combined with TIL, we noticed a slower rate of tumoural volume growth in the first 28 days, followed by a further decrease in the following weeks. By contrast, in Batch V (animals treated with multiple doses of PDT+TIL-AB) we noticed a discrete growth

Fig 2: Therapeutic effect of tumour-infiltrating lymphocytes combined with photodynamic therapy on survival of rats with Walker-256 carcinosarcoma.

of tumoural volume only in the first 21 days, followed by a complete cessation of growth. In animals exposed to photodynamic treatment associated with TILs, the values of investigated parameters (mortality, tumour volume, progressive evolution and complete tumoural regression) were superior as compared to values recorded in the other Batches (Figures 1 and 2).

Sixty days post-transplantation the examination of tumour volume curves suggested two aspects: (a) isolated (i.e. PDT or TIL alone) treatment induced a partial delay of tumour growth and (2) PDT in conjunction with TILs led to a significant decrease in tumour volume. These facts demonstrate the efficacy of PDT treatment in conjunction with tumour-infiltrating-lymphocytes in experimental Walker-256 carcinosarcoma.

Table 1: Results of lymphoproliferation assay in Walker-256 carcinosarcoma in rats. Data show mean value of cpm after ConA stimulation.

Treatment Batch	Treatment	Days After Treatment		
		14	28	42
I	PDT	37,966 ± 1,682	41,235 ± 1,522	48,195 ± 2,105
II	TIL	44,316 ± 1,215	48,544 ± 2,107	53,062 ± 1,572
III	PDT+TIL-A	55,137 ± 1,734	62,314 ± 1,851	69,558 ± 2,251
IV	PDT+TIL-B	52,958 ± 1,576	67,749 ± 2,142	73,812 ± 1,923
V	PDT+TIL-AB	54,437 ± 2,520	74,593 ± 1,652	86,467 ± 2,149
VI	CONTROL (HBSS)	37,853 ± 1,638	35,144 ± 1,297	—

Healthy animals; 43,552 ± 1,689 cpm/min

cpm = counts per minute; Con A = Concavalin A; HBSS = Hank's balanced salt solution

Changes in mitogenic response of the host splenic cells

The counts-per-minute (cpm) mean values after stimulation of lymphocytes with Con A at 4, 28 and 42 days after treatment are presented in Table 1. Examination of these data demonstrates that after 14 days, exposure to multiple doses of combined treatment produced a marked increase in incorporation of the radioactive precursor ^3H -TdR into Con A stimulated lymphocytes, when splenic cells were cultured at 2.5×10^5 cells/well density; in contrast, after exposure to PDT or TIL on their own, we noticed a moderate mitogen response to Con A.

Twenty-eight and 42 days after starting the treatment the cpm values found in Con A stimulated lymphocytes were significantly superior to those found after 14 days, especially in Batches III-V, as compared to Batches I, II and control Batch VI (Table 1).

Cell-mediated cytotoxicity of spleen cells from tumour-bearing rats treated with PDT in conjunction with TIL

The experiments presented in Table 2 were designed to determine the capacity of "combined" therapy to stimulate cytotoxic activity of splenic lymphocytes and to lyse

target YAC 1 cells 14, 28 and 42 days post treatment. Percentage values found for anti-YAC I activity of spleen cells from the treated batches of animal were superior to the control values (Batch VI, HBSS). Fourteen days post-treatment, maximal cytolysis of YAC 1 cells was noticed in splenic lymphocytes from multiple PDT-TIL doses treated animals in Batches III-V. In contrast, animals in Batches I and II (PDT and TIL only, respectively) were not capable of demonstrating significantly increased cytotoxic activity of effector cells. Four and six weeks post-treatment, in all the batches studied, a higher splenic cell cytotoxicity was noticed against YAC target cells, in comparison with the values determined at 14 days post-treatment. Anti-YAC cytotoxic activity in the irradiated batch and in those where adoptive immunotherapy (TIL-A, TIL-B, TIL-AB) was administered in conjunction with PDT were superior to the other batches (Table 2).

The results suggest two aspects: (a) local treatment increases cytotoxic activity against target cells and (b) these activities take place in a dose-dependent manner (data not shown). Spontaneous release of the isotope from target cells was less than 10.5% during the test.

Table 2: Cytotoxicity assays using splenic effector cells from Walker-256 carcinosarcoma injected rats. Data show the percentage of cytotoxicity against the target YAC cells.

Treatment Batch	Treatment	Days After Treatment		
		14	28	42
I	PDT	21.4 ± 0.52	26.9 ± 0.75	37.4 ± 1.10
II	TIL	23.8 ± 0.88	25.4 ± 0.92	34.1 ± 1.35
III	PDT+TIL-A	26.1 ± 1.14	35.7 ± 1.44	39.5 ± 1.27
IV	PDT+TIL-B	25.3 ± 0.79	39.2 ± 2.01	43.8 ± 2.15
V	PDT+TIL-AB	27.8 ± 1.32	42.5 ± 2.25	47.5 ± 2.19
VI	CONTROL (HBSS)	16.9 ± 0.56	17.5 ± 1.06	—

Healthy animals: 22.85 ± 0.79

HBSS = Hank's balanced salt solution

Biodistribution of ^{131}I -labeled TIL cells in rat tissues

The tumour-bearing animals exposed to photodynamic treatment associated with immunotherapy in multiple doses and intravenously inoculated with ^{131}I -labeled TIL (5×10^8 cells/animal) and sacrificed three days after injection, showed the highest labelling in the liver (from 14.1 at 16.9%) and lung (7.54 at 9.35%) followed by lower labelling in the kidney, spleen, gastrointestinal tract, skin and urine. The total radioactive values found in tissues of animals with treated and untreated Walker tumours, Guérin tumour tissues and healthy animals varied between 42.59% and 55.85%. The remaining radioactivity of the values may have been retained in other tissues and in animal carcass which was not measured. Furthermore, in this experiment, relatively significant values of ^{131}I -labeled TIL found were between 6.85% and 10.27% localized in the Walker-256 tumours (Batches III-V), in comparison with the values found in Guérin tumour cells (2.06%). These observations suggested that the increased amount of ^{131}I -labeled TIL found in Walker-256 tumours, especially in Batch V (PDT+TIL-AB) as compared with Guérin tumour, was possibly due to the effects of direct photodynamic damage to the vascular endothelial cells associated with the localization of tumour-infiltrating lymphocytes at sites of Walker-256 carcinosarcoma. However, in these studies, preferential localization of TIL at tumour sites was demonstrated.

Histopathological Findings

The following morphological changes were noticed four weeks after treatment in test and control animals with Walker-256 carcinosarcoma. In the samples obtained from control Batch VI, we noticed tumoural proliferation with small oval, rounded cells presenting nuclei with rich chromatin (hyperchromatic), reduced cytoplasm and weakly basophilic; larger cells with budding or multiple nuclei presenting characteristic atypia for tumour cells could also be observed (Figure 3); and

Fig 3: Control rat: Histopathological aspects of untreated Walker-265 carcinosarcoma (H-E staining, original magnification $\times 441$).

Fig 4: Skin fragment removed from animals with PDT-treated Walker-265 carcinosarcoma. Note the presence of fragments of tumour infiltration between two vessels with obliterating endothelia. (H-E staining, original magnification $\times 175$).

individualized haemodilated or haemorrhagic capillaries in the tumour mass could be seen. In the tumour mass, we noticed unequally zones of necrosis, leading to the conclusion that the tumour was a weakly differentiated sarcomatous type.

Samples obtained from Batch I animals with PDT treated tumours showed an integument without significant modifications and tumoural proliferation; under

Fig 5: Section through Walker-265 carcinosarcoma after PDT+TIL-A treatment. The presence of lymphocyte infiltration at the periphery of the tumour mass are apparent. (H-E staining, original magnification x 280).

Fig 6: Tumoural fragment from PDT+TIL-B treated animal. Note the presence of lymphocyte infiltration in the zones of tumour lysis. (H-E staining, original magnification x 197).

the striated muscular layer could be seen fusiform, round, oval infiltrates, separated by local oedema; cells with tumoural characteristics were isolated and rare. At various levels we noticed obliterating endovascularitic vascular modifications (Figure 4).

From Batch III (PDT+TIL-A), we obtained the section seen in Figure 5. Histological variations could be seen from one zone to another showing alternative tumour cells with vacuolar and dystrophic lesions with

a cytoplasm having a foam-like appearance surrounded by a lymphocytic crown. In other zones Walker-256 cells were isolated or in small groups near necrobiotic tumour cells with picnotic nuclei and vacuolated cytoplasm. In some zones with cellular lysis tumour absence was seen but in other vacuolar and foamy cell types the presence of lymphocytic infiltration around the tumour capsule could be observed.

In samples obtained from treated Batches IV (PDT+TIL-B) and V (PDT+TIL-AB), we noticed few isolated tumour cells which were sometimes difficult to recognize by size and their intralymphocytic distribution; we also noticed the presence of profuse neovascularization emphasized by the presence of erythrocytes and predominance of lymphocytic infiltration from the tumour periphery to the central area in small or larger groups. These changes may be due to the proliferation of intra- and/or peritumourally infused lymphocytes. Arterioles and venules with endovascular processes showing an obliterating tendency were also present. No tumor proliferation was detected (Figures 6-8).

Discussion

In this paper we perceived that photodynamic therapy given in conjunction with adoptive immunotherapy significantly reduced mortality, tumour volume and incidence in inbred rats infected with Walker-256 carcinosarcoma. The incidence of tumour cell death decreased (both as progression and regression) and was associated with exposure to photodynamic therapy but additionally to the presence of tumour-infiltrating lymphocytes,

thereby increasing the cell-mediated immune response. The values of the studied experimental parameters, in the course of the 60 days from the initiation of treatment, revealed variations of the immune response among the different batches dependent upon the administered treatment. The results showed that at 14, 28 and 42 days after initiation of treatment T-splenic lymphocytes significantly reacted to Con A stimulation,

Fig 7: Tumour fragment from PDT+TIL-AB treated rat. Tumour lysis and the presence of lymphocyte infiltration are clearly seen. (H-E staining, original magnification $\times 175$).

Fig 8: Detail of tumour fragment from PDT+TIL-AB treated rat. Large atypical tumour cells and lymphocytic infiltration can be seen. (H-E staining, original magnification $\times 441$).

in comparison with healthy control animals. The cells giving the best response were from the rats in those groups that received "combined" treatment and splenocytes from these groups demonstrated an increase anti-YAC activity, compared to HBSS injected controls and uninjected healthy animals at 14, 28 and 42 days post-treatment.

This apparent increased cytotoxic activity could be assigned to three factors: (a) stimulation with tumoural antigens; (b) photomodulated cytokine production following HeNe laser irradiation;⁽²¹⁾ and (c) the proliferation of intra- and/or peritumourally infused lymphocytes.

One of the most important question about TIL cell therapy is regarding TIL affinities to both tissues and organs. In our studies we induced the biodistribution of ^{131}I -labelled TIL cells in both untreated rats and rats with infected with Walker-256 carcinosarcoma. The results pointed to a predominant accumulation of ^{131}I labelled TIL cells in liver and lung and lower amounts in other organs and tissues three days post-intravenous perfusion. The accumulation of radioactive TIL cells at tumour site was demonstrated comparatively with Guérin tumour cells. These results suggest a selective localization of labelled TIL cells in Walker-256 tumour tissue. Similar investigations for preferential localization of ^{131}I -labelled TIL cells have been reported by other researchers.^(9,22,23)

The morphological changes noticed in these experiments, after exposure to PDT, are in full agreement

with previous observations of other authors with reference to mechanisms and pathways which are most probably involved in photodynamic therapy. Among the most important PDT mechanisms we have emphasized are: (a) changes in mitochondrial structure and/or function;^(24,25) (b) damage to DNA with single-stranded breaks and sister chromatid exchanges;⁽²⁶⁻²⁸⁾ (c) direct effects on malignant cells induced by oxygen presence as singlet oxygen or other members of reactive oxygen species (ROS);⁽²⁹⁾ (d) superoxide production;⁽³⁰⁾ (e) ischaemia resulting from vascular lesions which could be the main cause of tumour destruction during photodynamic therapy;⁽³¹⁾ and (f) potentiation of cell-mediated antitumoural immune response after photo-exposure to multiple doses of PDT.⁽⁶⁾

In addition to the PDT mechanisms mentioned above we could add the following antitumoural effects of tumour-infiltrating lymphocytes such as: (a) cytotoxic activity of T-cells enhanced by perforin-mediated induction of membrane lysis and fas-induced DNA fragmentation and apoptosis;⁽³²⁻³⁴⁾ (b) the inhibition of suppressor T-cell activity by accelerated production of lymphokines; and (c) induction of the so-called "cytokine cascade" which is responsible for immune response amplification, involving various kinds of soluble factors produced by a variety of immunocompetent and immuno-associated cells.^(18,35)

Conclusions

Data presented in this work demonstrate that exposure to multiple doses of local photodynamic treatment in conjunction with infiltration of tumour-infiltrating lymphocytes stimulates cell-mediated antitumoural activity, produces modification in tumour histological structure, increases survival rates and reduces tumoural incidence of Walker-256 carcinosarcoma in the rat model.

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