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Recombinant Tumour Necrosis Factor Alpha Administered Subcutaneously or Intramuscularly for Treatment of Advanced Malignant Disease: a Phase I Trial

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The pharmacokinetics, toxicity and biological effects of subcutaneous and intramuscular treatment of cancer patients with recombinant tumour necrosis factor alpha (rTNF-α) was investigated. 17 patients suffering from refractory malignant disease were treated with either 1.0 μg/m², 10 μg/m² or 100 μg/m² rTNF-α. Vital signs, peripheral blood cell counts, TNF and interferon (IFN) gamma serum levels, neopterin, β_2-microglobulin, C reactive protein (CRP) and cortisol levels were measured immediately before and 2, 12, 24, 48 and 168 h after the first administration of rTNF-α. Tumour response was evaluated after 4 and 12 weeks of treatment. The pharmacokinetics followed the same characteristics as those reported for other cytokines. Major toxicities were dose dependent and comprised fever, constitutional symptoms and hypotension. TNF dependent changes were observed in serum levels of IFN-α, CRP, neopterin, β_2-microglobulin, cortisol and white blood cell counts. No objective tumour response was observed. This study indicated that rTNF-α administered subcutaneously or intramuscularly results in measurable TNF serum levels, significant toxicity and biological response in absence of clinical efficacy in patients with advanced cancer.
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INTRODUCTION

TUMOUR NECROSIS factor alpha (TNF-α) was originally described as a serum factor that caused haemorrhagic necrosis of certain experimental tumours in mice [1]. This factor was found to be produced primarily by macrophages and was apparently capable of preferentially lysing tumour cells [2]. In parallel, cachectin was defined as a factor responsible for cachexia and certain metabolic effects in chronically infected rabbits. This factor was also found in supernatants of cells stimulated with endotoxin [3]. After biochemical characterisation, the identity of these two serum activities was then demonstrated by Beutler *et al.* [4]. Meanwhile TNF-α has been recognised as a molecule with pleiotropic biological activities. It plays a key role in inflammation [5]. TNF regulates the migration of blood cells to the tissues by enhancing the expression of adhesion molecules on peripheral blood cells as well as on endothelial cells [6–8]. The cytotoxic activity of various immunological effector cells, such as granulocytes, monocytes and lymphocytes, is enhanced by TNF-α [9–11]. In addition, antiproliferative and cytostatic effects on various normal and malignant cells have been shown *in vitro* and *in vivo* [12, 13].

These marked antitumour effects *in vitro* and the observation of haemorrhagic necrosis of certain experimental animal tumours observed after treatment with TNF-α elicited great expectations about the therapeutic efficacy of this agent in clinical studies. Recombinant DNA technology has provided us with sufficient amounts of rTNF-α and clinical trials have been initiated testing recombinant TNF-α (rTNF-α) for treatment of malignant disease. Recently several reports on phase I trials with intravenous and local treatment with rTNF alpha have been published (refs 14–17 and H. Gamm *et al.*). We present the data of a phase I trial on intramuscular and subcutaneous treatment of cancer patients with rTNF-α.

PATIENTS AND METHODS

Patients

The study was performed according to the guidelines of the declaration of Helsinki. The protocol was approved by the local ethics committee. All patients had given written informed consent prior to start of treatment. 17 patients suffering from refractory malignant disease were entered into the study. Ages ranged from 20 to 84 (median 59) years. 5 patients were female and 12 male. The clinical characteristics of the study patients are summarised in Table 1. Exclusion criteria included a Karnovsky index of less than 50%, major infection or other major disease.

Trial substance

rTNF-α originally produced by Genentech Inc (San Francisco) was provided by Bender Inc. (Vienna). rTNF-α is

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Table 1. *Clinical characteristics*

Patients no.	Sex	Age	Diagnosis	Pretreatment
1 μg/m²				
1	F	80	Colorectal carcinoma	None
2	M	84	Hepatocellular carcinoma	None
3	M	65	Hepatocellular carcinoma	None
4	M	34	Malignant melanoma	CT
5	F	48	Colorectal carcinoma	CT
10 μg/m²				
6	M	66	Pharyngeal carcinoma	CT, RT
7	M	64	Malignant melanoma	CT
8	F	47	Soft tissue sarcoma	CT
9	M	61	Hepatocellular carcinoma	None
10	M	59	Hepatocellular carcinoma	None
11	F	55	Hepatocellular carcinoma	None
100 μg/m²				
12	M	51	Hepatocellular carcinoma	None
13	M	55	Colorectal carcinoma	None
14	M	63	Renal cell carcinoma	None
15	M	73	Apudoma of the pancreas	CT, IFN-α
16	M	48	Colorectal carcinoma	None
17	F	20	Soft tissue sarcoma	CT

CT = chemotherapy, RT = radiotherapy.

produced by *Escherichia coli* containing cDNA coding for human TNF-α. Purity was checked by high performance liquid chromatography (HPLC) and consistently exceeded 99%. The specific activity of this preparation is 40 MU/mg protein.

Treatment schedule

Patients were treated with either 1.0 μg/m², 10 μg/m² or 100 μg/m² rTNF-α. The patients were divided into two groups by numerical order. The first group was treated subcutaneously and the second group intramuscularly once weekly for 2 weeks. Thereafter treatment was continued with the same dose twice weekly for 3 months. Median treatment duration was 50 days (7–383) and the patients received from 1 to 83 injections (median 13) of rTNF-α.

Vital signs were assessed immediately before and every 2 hours after the administration of rTNF-α to evaluate toxic effects. Haematological parameters and serum samples for evaluation of biological response were taken before and 2, 12, 24, 48 and 168 h after the first and second rTNF-α treatment. Routine laboratory tests including liver and kidney functional parameters, electrolytes and clotting tests were performed twice weekly for the first 2 weeks and once weekly thereafter.

Side-effects of rTNF-α were evaluated and graded according to WHO toxicity criteria [18]. rTNF-α dose was reduced by 50% in case of grade II toxicity persisting for more than 4 weeks or in case of grade III toxicity. Treatment was stopped immediately in the case of grade IV toxicities. Patients treated for more than 4 weeks were evaluated for tumour response 4 and 12 weeks after the commencement of treatment. Patients who had responded or those with stable disease after 3 months were treated until disease progression.

Methods

Blood counts and differentials were performed using standard methods. Neopterin (Henning, Berlin) β_2-microglobulin (Pharmacia, Uppsala) TNF-α (Medgenix, Bruxelles) and interferon gamma (IFN-γ) (Centocor, Malvern, USA) were measured using commercially available radioimmunoassays. C reactive protein (CRP) (nephelometric assay, QM 300, Kallestad, Austin, Texas) and cortisol (radioimmunassay Kallestad, Austin) levels were measured using commercially available assays. Details of the data on the biological response were published elsewhere (ref. 19 and W.E.A. *et al.*).

RESULTS

TNF serum levels after administration of rTFN-α

Serum TNF levels were determined before, 2, 12, 24, 48 and 168 h after each administration of rTNF-α. 6 of the 17 patients had detectable serum levels of TNF prior to start of treatment ranging from 5 to 29 ng/l (mean 15 ng/l). After injection of rTNF-α, a dose dependent increase of TNF serum levels was observed (Fig. 1). Treatment with 1.0 μg/m² and 10 μg/m² rTNF-α resulted in only minor changes of TNF serum levels. Subsequent to administration of 100 μg/m² rTNF-α, serum levels increased to a mean value of 130 ng/l after 24 h and declined rapidly to pretreatment values within 48 h. Kinetics as well as peak levels of serum TNF were influenced by the route of administration: intramuscular injection resulted in higher peak levels than subcutaneous treatment (Fig. 1). Peak levels were reached 12 h after intramuscular injection, whereas with subcutaneous treatment the highest TNF serum levels were seen after 24 h.

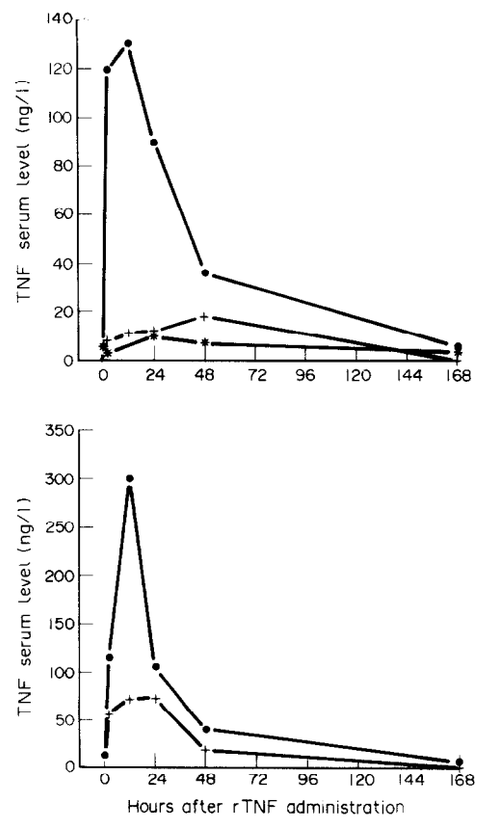

Fig. 1. Serum levels of TNF after administration of rTFN-α. Upper: mean serum levels of all patients after treatment with 1.0 μg/m² (*), 10 μg/m² (+) and 100 μg/m² (•) are shown. Lower: - mean serum levels of TNF-α after subcutaneous (+) and intramuscular (•) treatment with 100 μg/m² rTNF-α.

Table 2. Toxicity and adverse events

Symptoms	Grade (WHO)	1 μg/m²	10 μg/m²	100 μg/m²	Total
Fever	1–2	–	5/6	3/6	8/17
	3–4	–	–	2/6	2/17
Nausea	1–2	–	–	2/6	2/17
	3–4	–	–	–	–
Local reaction	1–2	2/5	1/6	–	3/17
	3–4	–	–	1/6	1/17
Pulmonal	1	–	1/6	–	1/17
	> 1	–	–	–	–
Hypotension	1–2	–	–	–	–
	3–4	–	–	1/6	1/17

Toxicity of rTNF-α

Fever and constitutional symptoms were the most frequent side-effects of rTNF-α observed in this study (Table 2). 5 out of 6 patients receiving 10 μg/m² rTNF-α had moderate febrile reactions. After injection of 100 μg/m² rTNF-α 5 out of 6 patients showed an increase in body temperature, reaching grade 3 of WHO toxicity. Only 1 patient remained afebrile after this dose. This patient experienced severe hypotension (see below). The kinetics of rTNF-α-induced febrile reactions are shown in Fig. 2. The body temperature peaked 2–10 h after injection and

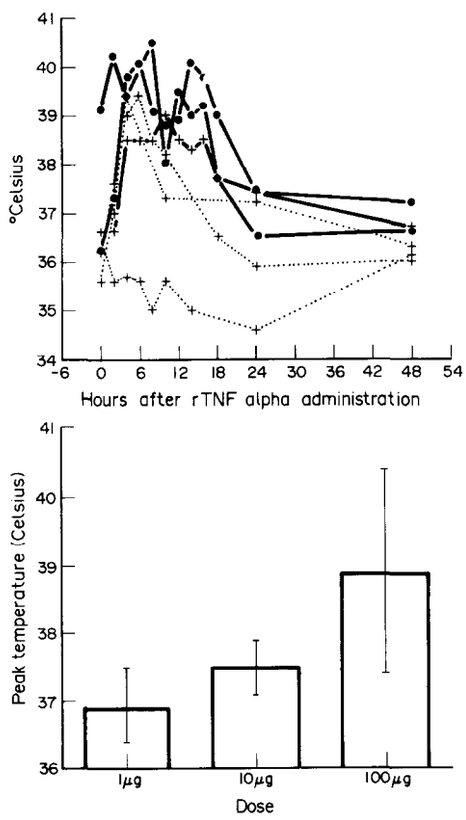

Fig. 2. Body temperature after rTNF-α treatment. Upper: kinetics after administration of 100 μg/m² rTNF-α. Lines represent data of individual patients. Dotted lines represent the patients treated intramuscularly and solid lines those treated subcutaneously. Lower: dose dependency of fever after rTNF-α. Values shown are means (S.E.) of peak levels after the first administration.

Table 3. Duration of treatment, dose, route of administration and clinical response

Patient	Dose (μg/m²)	Route	No. of applications	Duration of treatment	Response
1	1	i.m.	20	146	PRO
2	1	i.m.	5	24	n.e.
3	1	s.c.	2	7	n.e.
4	1	s.c.	4	18	n.e.
5	1	s.c.	23	146	PRO
6	10	i.m.	5	24	n.e.
7	10	i.m.	22	88	PRO
8	10	i.m.	27	102	PRO
9	10	s.c.	3	15	n.e.
10	10	s.c.	2	9	n.e.
11	10	s.c.	5	19	n.e.
12	100	i.m.	22	78	SD
13	100	i.m.	37	222	PRO
14	100	i.m.	1	7	n.e.
15	100	s.c.	83	383	PRO
16	100	s.c.	21	106	PRO
17	100	s.c.	13	50	PRO

i.m. = intramuscular, s.c. = subcutaneous, PRO = progression, n.e. = not evaluable, SD = stable disease.

remained elevated for up to 20 h. The pyrogenic effect of rTFN-α was clearly dose related (Fig. 2). Repeated application of the same dose did not lead to tachyphylaxis in our patients (data not shown). Constitutional symptoms, similar to the influenza-like syndrome seen after IFN treatment, were also frequently observed in patients treated with rTNF-α. Major complaints were malaise, fatigue, myalgia and arthralgia. Severe constitutional symptoms leading to transient bed rest were observed in 3 of the 6 patients treated with 100 μg/m² rTNF-α and represented the dose limiting toxicity in 2 of them. Local reactions were observed only in patients treated subcutaneously. They were severe (induration more than 5 cm) and dose limiting in 1 patient treated with 100 μg/m² rTNF-α. Other side-effects such as nausea in 2 and a bronchospasm in 1 patient were only moderate and did not require intervention. No significant long-term effects of rTNF on blood counts, renal and liver function tests were observed (data not shown).

The only serious adverse event related to rTNF-α therapy was hypotension and subsequent acute renal failure in 1 patient after the first intramuscular administration of 100 μg/m² rTNF-α. As this patient showed lymphoma surrounding the renal vessels, we propose that vascular insufficiency contributed to renal failure in this patient. Hypotension resolved after fluid replacement and kidney function improved spontaneously within 2 weeks.

Side-effects during long term treatment with rTNF-α twice weekly were essentially identical as those after single dose treatment. In particular, neither tachyphylaxis nor additional adverse events were observed in patients treated for more than 4 weeks.

Dose reduction was required in 4 of the 6 patients treated with 100 μg/m² rTNF-α. Dose-limiting toxicities were fever in 2, hypotension in 1 and a severe local reaction after subcutaneous treatment in a further patient.

Clinical response to rTNF-α

9 patients treated for more than 4 weeks were evaluated for clinical response (Table 3). None of the patients showed

Table 4. Acute biological response to rTNF-α

	Response 100 μg/m²		Kinetics peak/nadir	Duration
White blood cell counts	Mean (%) of pre-treatment value			
Monocytes	5%		2 h	< 24 h
Lymphocytes	31%		2 h	< 24 h
Band forms	216%		2 h	< 24 h
Body temperature	increase		10 h	< 24 h
	pre	post		
Serum markers	mean value			
Neopterin (nmol/l)	7.8	23.8	48 h	< 7 days
β_2-microglobulin (mg/l)	1.7	2.2	48 h	< 7 days
CRP (mg/dl)	22	69	24 h	< 5 days
IFN-γ (pg/ml)	11	52	24–48 h	< 5 days
Hormones				
Cortisol (μg/dl)	13.8	18.0	2 h	< 24 h

measurable improvement of their disease. 3 patients receiving the highest dose level remained stable for the first 3 months of treatment and 2 of them were maintained on rTNF-α treatment. Evidence for disease progression was noted in these patients after treatment for 6 and 12 months, respectively.

Biological response of rTFN-α

Although no clinical responses were observed in this study, clear evidence of biological response to rTNF-α was observed (Table 4). Treatment with rTNF-α influenced trafficking of peripheral blood cells leading to transient lymphopenia and monocytopenia: lymphocyte counts decreased to 31 (5)% [mean (S.E.)] of pretreatment values 2 hours after treatment with 100 μg/m² rTNF-α and remained low for at least 48 h. Monocytes disappeared almost completely from the peripheral blood. 2 h after administration of 100 μg/m² rTNF-α counts were reduced to 5 (3)% of pretreatment values. Whereas marked acute effects of single doses of rTNF-α on blood counts where observed, no significant changes of haematological parameters occurred upon long term treatment (Fig. 3).

Several serum markers also indicated biological response to rTNF-α: neopterin levels increased from 7.8 (0.9) nmol/l before to 23.8 (2.6) nmol/l 48 h after injection of 100 μg/m². Serum levels of β_2-microglobulin followed the same kinetics and increased from 1.7 (0.2) to 2.2 (0.2) mg/l after two days. In addition, production of IFN-γ was induced by treatment with rTNF-α. The median IFN-γ level after treatment was 52 (19) pg/ml and significantly higher than pretreatment levels (mean 11 (11) pg/ml). Synthesis of CRP, an acute phase reactant, was also enhanced upon injection of rTNF-α (Fig. 4). As shown, serum levels increased from 22 (20) mg/l before to 69 (19) mg/l 24 h after administration of the highest dose levels of rTNF-α.

In addition, treatment with rTNF-α caused the release of cortisol, a compound known to counterbalance TNF action [20]. Patients treated with 1.0 μg/m² rTFN-α showed a decrease of cortisol levels during the day similar to the circadian rhythm observed in normals (Fig. 5). In contrast, 100 μg/m² rTNF-α led to an increase in cortisol levels at the same time points in all patients tested.

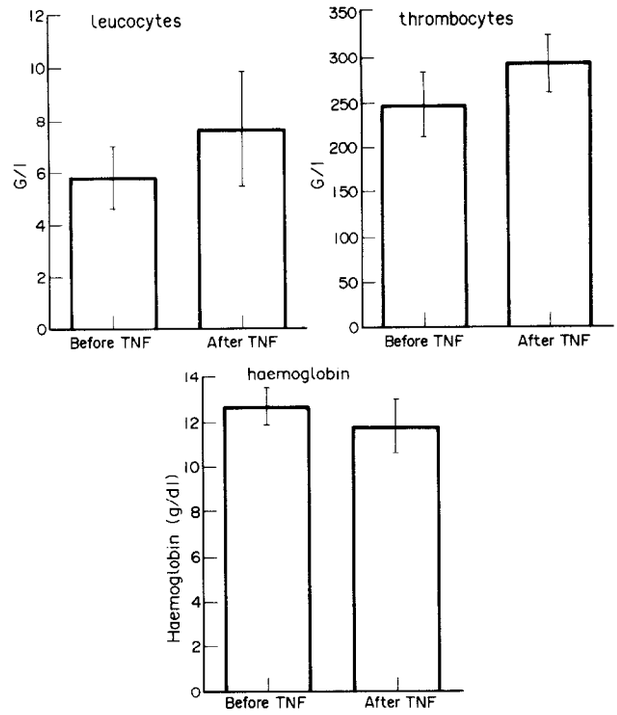

Fig. 3. Effects of long-term treatment with 100 μg/m² twice weekly on haematological parameters. Shown are the mean values before start of treatment and at cessation of therapy of those patients, who were treated with this dose for more than 4 weeks.

DISCUSSION

This study aimed to investigate pharmacokinetics, toxicity and biological response to rTNF-α in patients with advanced cancer. The pharmacokinetics displays the same patterns as those reported for other cytokines such as α, β or γ interferons [21]. Detectable increments of TNF serum levels were observed at higher dose levels both after intramuscular and subcutaneous treatment with rTNF-α (Fig. 1). Peak serum levels were reached within the first 12 h after administration and levels remained elevated for less than 48 h. The serum levels after subcutaneous treatment were lower than those after intramuscular application

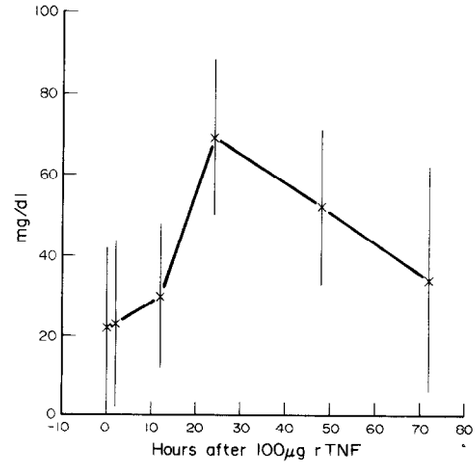

Fig. 4. Changes in the serum level of CRP after treatment with 100 μg/m² rTFN-α. Values represent the mean (S.E.) before, 2, 24, 48 and 168 h after treatment with rTNF-α.

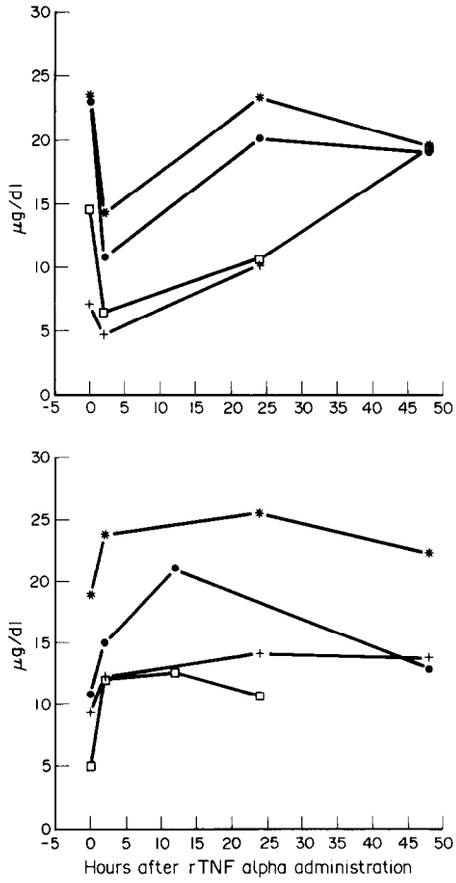

Fig. 5. Changes in cortisol levels after treatment with 1.0 μg/m² (upper) and 100 μg/m² (lower) rTNF-α. Values show the serum cortisol levels of individual patients. rTNF-α was injected between 8.30 and 9.00 a.m.

(Fig. 1). The serum levels observed after treatment suggest a lower bioavailability after subcutaneous administration of rTNF-α. However, since the biological responses to rTNF-α such as febrile reactions were comparable after both routes of administration, it seems questionable if serum levels truly reflect the biological activity of this compound (Fig. 2).

The toxic effects of rTNF-α observed in our patients were essentially identical to those observed in previously published TNF studies using other routes of adminstration and similar to those observed in studies with IFN-α and IFN-γ [14–17, 22, 23]. Most frequent side-effects were fever and constitutional symptoms. After subcutaneous treatment, local reactions represented a major problem. The maximum tolerated dose for a single dose, defined as the dose causing grade 3–4 toxicities in more than 50% of the patients, was 100 μg/m². Upon long-term treatment with this dose, more than 50% of the patients required dose reduction because of severe toxicities. Moderate doses up to 50 μg/m² were well tolerated for long-term treatment and not associated with additional or unexpected toxicities.

We conclude that administration of rTNF-α subcutaneously or intramuscularly once or twice weekly induces (i) dose dependent toxicity which was significant at the 10 μg/m² and 100 μg/m² dose level; and (ii) dose dependent and significant biological responses involving various subsets of white blood cell, serum levels of acute phase proteins, β_2-microglobulin and neopterin, and the activation of the cytokine network. In addition, rTNF-α induces one of its inhibitors by increasing serum cortisol levels.

Despite the fact that many biological TNF activities known from *in vitro* studies could be demonstrated in these patients, no tumour response was seen among our patients. This result is in agreement with other studies where tumour responses were also rare events [14–17]. This raises the question whether the antiproliferative effects seen in laboratory systems and animal models are achieved at dose levels where unacceptable toxicities are observed in humans, or whether the diseases selected for this study were refractory to the antitumour activity of rTNF-α. A third possible explanation for the lack of clinical response is that *in vivo* antitumour activity of TNF-α is limited by neutralising mechanisms induced by the compound itself. To answer these questions basic research efforts are needed to identify conditions and diseases where patients might benefit from treatment with rTNF-α. This is essential to justify the use of this biologically highly active compound in further clinical studies.

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Photodynamic Immunopotentiation: *in vitro* Activation of Macrophages by Treatment of Mouse Peritoneal Cells with Haematoporphyrin Derivative and Light

Nobuto Yamamoto, Sadamu Homma, Theodore W. Sery, Larry A. Donoso and J. Kenneth Hoober

Peritoneal macrophages treated *in vivo* with haematoporphyrin derivative (HPD) exhibited significant enhancement of Fc receptor mediated ingestion activity. To examine this process more rigorously, we studied photodynamic activation of macrophages by exposure *in vitro* of mouse peritoneal cell cultures (containing macrophages and B and T-lymphocytes) to HPD and red fluorescent light. A short (10 s) exposure of peritoneal cells in medium containing 0.03 ng HPD/ml produced the maximal level of ingestion activity of macrophages. A singlet oxygen quencher, DABCO, inhibited the effect of HPD. Photodynamic treatment of macrophages alone did not activate the cells and activation was only observed when macrophages were mixed with photodynamically treated non-adherent cells (B and T-lymphocytes). These results imply that activation of macrophage is a consequence of peroxidation of lymphocyte membrane lipids by photodynamically generated singlet oxygen.
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INTRODUCTION

RECENT STUDIES of porphyrin photosensitisation have focussed on the mechanism of action of haematoporphyrin derivative (HPD) in cancer photodynamic therapy (PDT). Kessel [1, 2] and Kessel and Chou [3], using murine leukaemia L1210 cells in culture, showed that phototoxicity of HPD depends on cellular uptake of the most hydrophobic components. Lesions in various organelles (e.g. mitochondrial, lysosomal, nuclear and endoplasmic reticulum) generated by PDT have been described [4]. It is now generally accepted that cell membranes are the principal sites of HPD-induced photodamage [4, 5]. For example, membrane rupture of chinese hamster ovary cells exposed to PDT correlates directly with survivability, as measured by colony formation [6]. The principal target moieties in the cell membranes seem to be phospholipids. Their photodynamic modification appears to be peroxidation of unsaturated fatty acyl groups [7, 8] and ultimately results in membrane rupture and cell death.

The aim of HPD-PDT is to obtain selective destruction of cancer cells. HPD-PDT has been used on many patients with encouraging results [9, 10]. Cure requires total elimination of the cancer cells in the targeted tissues as well as in metastasised cancer cells. It would be advantageous if the metastasised cancer

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