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## Antitumor efficacy of the combination of photodynamic therapy and chemotherapy in murine tumors

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### Abstract

Photodynamic therapy (PDT) is based on the administration of tumor-localizing photosensitizers followed by light exposure of the tumor mass. The photocytotoxic effects are mainly caused by the generation of singlet oxygen. Recently, PDT has been proposed for use in combination with anticancer chemotherapy with a view to exploiting any additive antitumor effect. We investigated the effect of PDT with photoactivated aluminum disulfonated phthalocyanine (AlS<sub>2</sub>Pc) combined with the antitumor drugs Adriamycin (ADR) and cisplatin (CDDP) on murine tumors. Mice bearing L1210 leukemia and P388 lymphoma were treated with ADR or CDDP and subsequently treated with PDT. Low chemotherapy doses were ineffective, but the combination of antitumor drugs + PDT had a significantly additive antitumor effect. In conclusion, with this combined therapy we were able to greatly reduce the effective doses of antitumor drugs, thus lowering their toxic effects on normal host tissues. © 1998 Elsevier Science Ireland Ltd.

*Keywords:* Photodynamic therapy; Antitumor drugs; Combination therapy

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### 1. Introduction

Photodynamic therapy (PDT) with porphyrin (Photofrin<sup>®</sup>) is a cancer treatment modality currently undergoing clinical trials [1,2]. PDT is based on the administration of a tumor-localizing sensitizer followed by light exposure of the tumor [3]. The light-activated photosensitizer triggers a series of chemical

reactions that lead to the destruction of malignant tissues [4]. The most commonly used sensitizer in PDT was the hematoporphyrin derivative (HpD), but is now the commercially available semi-purified preparation called Photofrin<sup>®</sup>. However, both compounds have many features that make them less than ideal photosensitizers [5]. Phthalocyanines are second generation photosensitizers with promising properties for use in PDT. These dyes have several advantages over the porphyrin derivatives now in clinical use, i.e. they have stronger absorption in the red part of the spectrum and weaker absorption in the wavelength region of the strongest solar radiation (400–600 nm) where

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light penetrates tissues optimally [6]. They also cause less sun-induced skin phototoxicity [7]. In our laboratory we found that the aluminum disulfonated phthalocyanine (AlS<sub>2</sub>Pc) activated by laser light shows marked potential for use in PDT [8]. A logical way of reinforcing cancer therapy would be to consider the use of PDT in combination with other chemotherapeutic agents to enhance effective regimens and to permit some sparing of cytotoxic drugs so as to lessen their side-effects. Although PDT has been found to act synergistically with chemotherapy *in vitro* [9], its efficacy when combined with cytotoxic chemotherapy has not been extensively evaluated *in vivo* [10,11], except for the combination of PDT and bioreductive agents such as mitomycin C and related analogues [12].

In the present study, we investigated whether the combination of PDT, with AlS<sub>2</sub>Pc and laser light, and chemotherapy, with the antitumor drugs Adriamycin (ADR) and cisplatin (CDDP), produced an additive or synergistic therapeutic effect on murine tumors.

## 2. Materials and methods

### 2.1. Animals and tumor models

All experiments were carried out in accordance with protocols approved by the local experimental animal welfare committee and conformed to national regulations for animal experimentation. Hybrid DBA/2 × BALB/c male mice (8–10 weeks old) obtained from Charles River (Calco, Italy) were used and are hereafter called CDF<sub>1</sub>. Each group comprised eight mice.

L1210 murine leukemia and P388 murine lymphoma were obtained from the Italian Tumor Institute (Milan, Italy) and maintained by intraperitoneal (i.p.) injection of 10<sup>6</sup> cells/mouse in CDF<sub>1</sub> male mice.

### 2.2. Chemicals

Aluminum phthalocyanine with an average degree of sulphonation of 2.1 (hereafter called AlS<sub>2</sub>Pc) was kindly provided by Dr A. McLennan (Paisley College of Technology, Paisley, UK). It was dissolved in physiological solution at a concentration of 5 mg/cc. Adriamycin (ADR; Pharmacia, Italy) and cisplatin

(CDDP; Pharmacia, Italy) were dissolved in distilled water at a concentration of 10 mg/ml.

### 2.3. Laser source

Irradiation was applied with a continuous wave dye (DCM) laser (Coherent Mod. CR-599, Palo Alto, CA) pumped by an Argon laser (Coherent Mod. Cr-18, Palo Alto, CA) and tuned at 670 nm. The laser output was coupled to a 400 μm plastic-glass optical fiber (Quartz at Silice PCS600, Paris, France). The laser power was monitored at the fiber output.

### 2.4. Experimental procedure

L1210 and P388 ascitic tumors were drawn from the peritoneum of mice bearing the tumors and the cell suspension was counted under optical light microscopy. Tumor cells (10<sup>6</sup> cells/mouse) were injected intradermally (i.d.) to obtain a visible tumor mass that could easily be effectively irradiated by the laser light. Treatment started when the tumor mass measured approximately 0.5 cm in diameter.

The animals were injected intraperitoneally (i.p.) with ADR or CDDP at doses of 1 or 2 mg/kg followed 24 h later by 5 mg/kg of AlS<sub>2</sub>Pc. After another 24 h, they were irradiated with a single dose of light (100 mW/cm<sup>2</sup> for 10 min of exposure; energy density 60 J/cm<sup>2</sup>).

### 2.5. Statistical analysis

The Mann–Whitney *U*-test was utilized to compare the survival times of the different groups [13].

## 3. Results and discussion

The antitumor activity of the combination of ADR + PDT was evaluated in mice bearing L1210 leukemia (Table 1). Three days after tumor transplantation, five groups of animals were treated with very low non-therapeutic doses (1 or 2 mg/kg) of ADR. Three of these groups were treated 24 h later with 5 mg/kg of AlS<sub>2</sub>Pc and after another 24 h the tumor masses were exposed to laser light (100 mW/cm<sup>2</sup> for 10 min of exposure). These drug doses and light exposure were adopted in agreement with the optimal pro-

Table 1

Antitumor activity of the combination of ADR + PDT (groups 6–8) on L1210 leukemia<sup>a</sup>

Group	Treatment					MST (days)	D/T
	Day 0 (tumor L1210)	Day + 3 (ADR) (mg/kg)	Day + 4 (AlS <sub>2</sub> Pc) (mg/kg)	Day + 5 (laser light <sup>b</sup> ) (mW/cm <sup>2</sup> )	Day + 6 (ADR) (mg/kg)		
1	10 <sup>6</sup>	–	–	–	–	10 (8–12)	8/8
2	10 <sup>6</sup>	1	–	–	–	11 (10–13)	8/8
3	10 <sup>6</sup>	2	–	–	–	12 (10–14)	8/8
4	10 <sup>6</sup>	–	5	100	–	14 (13–17)	8/8
5	10 <sup>6</sup>	2	–	100	–	12 (11–13)	8/8
6	10 <sup>6</sup>	1	5	100	–	24 (20–25)*	8/8
7	10 <sup>6</sup>	2	5	100	–	27 (25–30)*	8/8
8	10 <sup>6</sup>	–	5	100	2	26 (23–31)*	8/8
9	10 <sup>6</sup>	–	–	100	2	12 (10–13)	8/8

ADR, Adriamycin; MST, median survival time; D/T, dead animals/total; AlS<sub>2</sub>Pc, aluminum disulfonated phthalocyanine (5 mg/kg i.p.).<sup>a</sup>CDF<sub>1</sub> mice challenged i.d. with 10<sup>6</sup> cells of L1210 leukemia.<sup>b</sup>100 mW/cm<sup>2</sup> for 10 min of exposure (energy density 60 J/cm<sup>2</sup>).\**P* ≤ 0.001 by the Mann–Whitney *U*-test.

tocol obtained previously in our laboratory for other tumor models [8]. One group was treated only with 2 mg/kg and subsequently the tumor masses were exposed to laser light at the same dose of power. Another group was treated only with PDT, i.e. 5 mg/kg AlS<sub>2</sub>Pc, followed 24 h later by laser light. The combined treatment showed significant activity whereas chemotherapy alone was ineffective as expected at these two very low doses; PDT alone also had a weak effect on this ascitic tumor (Table 1). The MST of the combination of ADR + PDT was 24 and 27 days (groups 6 and 7, respectively)

compared to 11 and 12 days for mice treated with ADR alone (groups 2 and 3, respectively) and 14 days for PDT alone. The combined treatment was also effective if PDT was performed 1 day before the ADR treatment at the highest dose (group 8). The combination of ADR and laser light only in different sequence (groups 5 and 9) was also ineffective.

To confirm these positive results, we carried out experiments on a different murine tumor. Mice bearing P388 lymphoma were treated 3 days after tumor transplantation with ADR (1 or 2 mg/kg), 4 days later with AlS<sub>2</sub>Pc (5 mg/kg) and 5 days later with laser light

Table 2

Antitumor activity of the combination of ADR + PDT (groups 6 and 7) on P388 lymphoma<sup>a</sup>

Group	Treatment				MST (days)	D/T
	Day 0 (tumor P388)	Day + 3 (ADR) (mg/kg)	Day + 4 (AlS <sub>2</sub> Pc) (mg/kg)	Day + 5 (laser light <sup>b</sup> ) (mW/cm <sup>2</sup> )		
1	10 <sup>6</sup>	–	–	–	12 (9–13)	8/8
2	10 <sup>6</sup>	1	–	–	14 (12–16)	8/8
3	10 <sup>6</sup>	2	–	–	14 (13–17)	8/8
4	10 <sup>6</sup>	–	5	100	15 (13–17)	8/8
5	10 <sup>6</sup>	2	–	100	13 (12–15)	8/8
6	10 <sup>6</sup>	1	5	100	22 (20–25)*	8/8
7	10 <sup>6</sup>	2	5	100	23 (21–28)*	8/8

ADR, Adriamycin; MST, median survival time; D/T, dead animals/total; AlS<sub>2</sub>Pc, aluminum disulfonated phthalocyanine (5 mg/kg i.p.).<sup>a</sup>CDF<sub>1</sub> mice challenged i.d. with 10<sup>6</sup> cells of P388 lymphoma.<sup>b</sup>100 mW/cm<sup>2</sup> for 10 min of exposure (energy density 60 J/cm<sup>2</sup>).\**P* ≤ 0.001 by the Mann–Whitney *U*-test.

Table 3

Antitumor activity of the combination of CDDP + PDT (groups 6–8) on L1210 leukemia<sup>a</sup>

Group	Treatment					MST (days)	D/T
	Day 0 (tumor L1210)	Day + 3 (CDDP) (mg/kg)	Day + 4 (AlS <sub>2</sub> Pc) (mg/kg)	Day + 5 (laser light <sup>b</sup> ) (mW/cm <sup>2</sup> )	Day + 6 (CDDP) (mg/kg)		
1	10 <sup>6</sup>	–	–	–	–	11 (8–12)	8/8
2	10 <sup>6</sup>	1	–	–	–	12 (10–13)	8/8
3	10 <sup>6</sup>	2	–	–	–	12 (10–14)	8/8
4	10 <sup>6</sup>	–	5	100	–	15 (13–18)	8/8
5	10 <sup>6</sup>	2	–	100	–	13 (12–15)	8/8
6	10 <sup>6</sup>	1	5	100	–	24 (20–26)*	8/8
7	10 <sup>6</sup>	2	5	100	–	25 (23–27)*	8/8
8	10 <sup>6</sup>	–	5	100	2	25 (21–28)*	8/8
9	10 <sup>6</sup>	–	–	100	2	12 (10–15)	8/8

ADR, Adriamycin; MST, median survival time; D/T, dead animals/total; AlS<sub>2</sub>Pc, aluminum disulfonated phthalocyanine (5 mg/kg i.p.).<sup>a</sup>CDF, mice challenged i.d. with 10<sup>6</sup> cells of L1210 leukemia.<sup>b</sup>100 mW/cm<sup>2</sup> for 10 min of exposure (energy density 60 J/cm<sup>2</sup>).\**P* ≤ 0.001 by the Mann–Whitney *U*-test.

(100 mW/cm<sup>2</sup> for 10 min of exposure), following the same protocol as before (Table 2). The combined action of ADR + PDT significantly prolonged the survival compared to the single ADR or PDT treatments, which were ineffective or weakly effective. As for L1210, the combination of ADR + laser light had no effect.

In another set of experiments we investigated the efficacy of combination therapy with PDT and another antitumor compound with a different origin and mechanism of action, cisplatin (CDDP), to see whether this therapeutic approach could be applied with different antitumor drugs used in clinical oncology. We again selected two very low non-therapeutic doses of CDDP (1 and 2 mg/kg) and the same PDT protocol as before. Mice bearing L1210 leukemia (Table 3) and P388 lymphoma (Table 4) were treated 3 days after tumor transplantation with CDDP (groups 2, 3 and 5–7), 4 days later with AlS<sub>2</sub>Pc and 5 days later the tumor masses were exposed to laser light (groups 4, 6 and 7) following the same protocol as before. Animals in group 5 were given one dose of CDDP and laser light 48 h later.

The results confirm the previous observations, the combination of CDDP + PDT showing significant activity against both tumor models, whereas the single treatments (CDDP or PDT), like the combination CDDP + laser light, had no or very weak effect. In these experiments the positive effects of the combina-

tion were also observed if PDT was performed 1 day before the CDDP treatment at the highest dose (group 8 of Table 3).

Although there are a few reports of an interaction between PDT and chemotherapeutic agents [14,15], the mechanism is unclear. In one study, ADR inhibited the photodynamic destruction of Raji or Lewis lung carcinoma cells in vitro, partly by reducing the uptake of Hpd [16]. However, in apparent contradiction, enhanced uptake of Hpd in Lewis lung tumors in vivo was described, resulting in a potentiation of photodynamic therapy [10].

In our study, treatment with two cytotoxic drugs representative of the main classes of compounds in common clinical use did not cause any reduction of the murine tumors tested. PDT at the optimal therapeutic dose used against other murine tumors [8] was otherwise inactive against the two ascitic tumors L1210 and P388. However, when drugs + PDT were combined, the antitumor effects were strong.

It is difficult to propose a satisfactory explanation for this enhancement. It may be connected to the sum of the damage induced by both modalities on the cell membranes and on the vasculature by free radical and molecular oxygen [17]. The sequence of the combination is not important because the potentiated effect was noted even when the drugs were injected after PDT (see group 8 of Tables 1 and 3). Absorption of light by ADR and a direct photochemical interaction

Table 4

Antitumor activity of the combination of CDDP + PDT (groups 6 and 7) on P388 lymphoma<sup>a</sup>

Group	Treatment				MST (days)	D/T
	Day 0 (tumor P388)	Day + 3 (CDDP) (mg/kg)	Day + 4 (AlS <sub>2</sub> Pc) (mg/kg)	Day + 5 (laser light <sup>b</sup> ) (mW/cm <sup>2</sup> )		
1	10 <sup>6</sup>	–	–	–	13 (9–14)	8/8
2	10 <sup>6</sup>	1	–	–	15 (12–16)	8/8
3	10 <sup>6</sup>	2	–	–	16 (13–18)	8/8
4	10 <sup>6</sup>	–	5	100	15 (14–18)	8/8
5	10 <sup>6</sup>	2	–	100	14 (13–16)	8/8
6	10 <sup>6</sup>	1	5	100	26 (23–27)*	8/8
7	10 <sup>6</sup>	2	5	100	26 (25–30)*	8/8

ADR, Adriamycin; MST, median survival time; D/T, dead animals/total; AlS<sub>2</sub>Pc, aluminum disulfonated phthalocyanine (5 mg/kg i.p.).<sup>a</sup>CDF<sub>1</sub> mice challenged i.d. with 10<sup>6</sup> cells of P388 lymphoma.<sup>b</sup>100 mW/cm<sup>2</sup> for 10 min of exposure (energy density 60 J/cm<sup>2</sup>).\**P* ≤ 0.001 by the Mann–Whitney *U*-test.

are possible, but the absorption peaks of ADR are in the UV region and at 500 nm, not the 670 nm used with PDT in this study [18].

Since both drugs we tested are potentiated by heat [19], it could be postulated that the hyperthermia known to occur even at very low dosages with PDT treatment is responsible for the increased activity [20]. However, the fact that activity was not increased by the combination of laser light treatment and drugs in our experiments (see group 5 in all tables and group 9 in Tables 1, and 3) casts doubt on the theory of thermal potentiation. Finally, PDT may serve as a debulking treatment leaving fewer tumor cells to be killed by cytotoxic drugs and immune effector cells (T lymphocytes and macrophages), as we already observed [21].

In conclusion, the interaction between PDT and cytotoxic drugs may have important clinical implications and merits further investigation. In cancer treatment, PDT could play a role in combinations of available therapies. It might be considered in a politherapy anticancer protocol using lower doses of cytotoxic drugs to restrict their toxic effects on normal host tissues.

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