SHORT COMMUNICATION

Verapamil and hematoporphyrin derivative for tumour destruction by photodynamic therapy

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In recent years, photodynamic therapy (PDT) has shown much promise for the local and selective destruction of malignant tumours. Although tumour destruction is believed to be mediated through the production of highly reactive intermediate singlet oxygen by photocatalytically activated hematoporphyrins (Weishaupt et al., 1986), considerable evidence has accumulated to suggest that the primary site of photodynamic damage is the small vessels and capillaries of the tumours (Nelson et al., 1988; Berenbaum et al., 1986). Some studies have shown vascular effects occurring with PDT such as the fall of tumour-blood flow (Selman et al., 1984; Wieman et al., 1988) and the shutdown of tumour vessels (Henderson et al., 1985). In one report a complete cessation of tumour blood flow was described in rat tumours after PDT (Star et al., 1986). In solid tumours the drug uptake is limited by the tissue perfusion rate, the membrane permeability and the transport across the vessel wall (Gerlowksi et al., 1986). Therefore, it seemed plausible that vasoactive drugs might influence tumour destruction by PDT. In particular the calcium channel blockers have generated much interest in cancer research since it has been demonstrated that verapamil, the prototype calcium channel blocker, increases the cytostatic effects of Adriamycin and vincristine (Tsuroo et al., 1983) and has a reversible anti-proliferative effect itself (Schmidt et al., 1988). Although the precise mechanism of action is not known, some studies indicate that verapamil inhibits the P-glycoprotein pump which drug-resistant tumour cells use to pump out anticancer agents (Ince et al., 1986; Garman et al., 1983).

Recently an enhanced photodynamic destruction of tumours was described when verapamil was concurrently administered with the photosensitiser, or similarly when verapamil was injected after PDT, a delay of the regrowth of tumours was implicated (Cowled & Forbes, 1989). In contrast to these authors, who administered high doses of hematoporphyrin derivative (HPD, 30–50 mg kg⁻¹ body weight), we injected doses of hematoporphyrin derivative enriched with dihematoporphyrin-ether (DHE, 1.5 or 9 mg kg⁻¹ body weight) according to previous experiments (Sroka et al., 1989a). In our experiments we used two different tumour models to examine the effects of verapamil on the photodynamic destruction of tumours.

Our first tumour model, the isogenic fibrosarcoma SSK-2 was implanted into the flank of female inbred C3H-mice. This fibrosarcoma grows with a doubling time of approximately 1.5 days. The tumour size was measured with calibration masks, gauged to the weight of tumours (Kummermehr & Trott, 1982). The photodynamic efficiency was quantified by means of the tumour regrowth delay time, i.e. the time a tumour needs to regain a defined weight (Begg, 1980). When the tumours reached a weight of 60 mg, the photosensitiser and verapamil, both diluted with saline solution, were injected intraperitoneally at a dose of 9 mg kg⁻¹ body weight according to previous experiments (Stocker, 1986). The values of the individual regrowth delays were plotted and, assuming their Gaussian statistical distribution, approximated in a least square fit procedure by an exponential curve. Mean values and standard deviations of the regrowth delay have been calculated for a clearer presentation. In addition, the extent of tumour necrosis after PDT was examined histologically in a second group of tumour bearing mice in order to compare the regrowth delay with the tumour necrosis.

Our second in vivo model was a human adenocarcinoma of the colon, heterotransplanted with the standard technique (Sroka et al., 1989b) into nude mice. When the tumours reached a 1 cm diameter, the drugs were administered and the tumours were irradiated.

Five days after PDT the mice were sacrificed and the tumours were resected. The percentage of the tumour necrosis was evaluated histologically by three independent examiners. Mean values and standard deviations of the tumour necroses were calculated (SAS users guide: Basics and statistics, 1985).

In both murine tumour models and animals were anaesthetised (Inhalation narcosis with Enfluran: Ethane, Abbot GmbH, FRG) during the time of irradiation. The mice were divided into six groups:

| Group | Treatment |
|-------|-----------|
| A     | No drugs, no light, typical growth/spontaneous necrosis. |
| B     | Only photosensitiser administered (DHE). |
| C     | Only verapamil administered. |
| D     | Only light without drugs. |
| E     | Photosensitiser and light administered (PDT). |
| F     | Photosensitiser + verapamil and light administered. |

In each group, ten animals were treated per experiment. All experiments were repeated twice so that a total of 30 mice were treated in each group.

Photosan 3 (Seehof Laboratory, FRG), a hematoporphyrin derivative enriched with dihematoporphyrinether (DHE), was administered intraperitoneally to the animals at a concentration of 1.5 mg kg⁻¹ (human adenocarcinoma and fibrosarcoma SSK-2) and 9 mg kg⁻¹ (fibrosarcoma SSK-2) body weight.

Verapamil (Isoptin, Knoll AG, FRG), formulated for clinical use, was injected concomitantly with the photosensitiser at a dose of 2 mg kg⁻¹ body weight. Twenty-four hours after application of the drugs, the tumours were irradiated with laser light.

Tumours were treated with laser light tuned to the wavelength of 630 nm. The radiation was delivered from an Argon-ion laser-pumped dye laser (model 171 and 375 B, Spectraphysics Inc., USA; Dye: Kiton red). A tube, covering the tumour, was fed by a flexible quartz fibre (core diameter: 600 μm) and guaranteed nearly homogenous irradiation due...
to multiple inner reflection (Sroka et al., 1989b). The total energy density was 150 J cm\(^{-2}\) at a power density of 
400 mW cm\(^{-2}\). The tube was cooled by a flow of N\(_2\) gas in order to avoid hyperthermic effects at this high power 
density. With gas cooling, a maximum temperature of 38°C was 
recorded. The temperature was measured subcutaneously 
between skin and tumour.

In the fibrosarcoma SSK-2 tumour model the photosensitiser 
and verapamil were concurrently administered and tumours 
were irradiated 24 h later. Both, regrowth delay time and 
extent of tissue necrosis of the treated tumours were 
examined. The results are shown in Table I and II. These 
data show that verapamil did not enhance photodynamic 
destruction of tumours. This drug did not markedly affect 
the regrowth delay time measured in the fibrosarcoma. The 
comparison of tumours treated with PDT plus verapamil 
group D) and PDT alone (group E) showed no effective 
inhibition of tumour regrowth. Verapamil alone (group C) 
and DHE without irradiation (group B) did not affect the 
regrowth delay time.

With respect to the percentage of necrosis measured (Table 
II) the tumours demonstrated a similar behaviour in each 
group. A concentration of 9 mg kg\(^{-1}\) body weight Photosan 
3 showed a subtotal destruction of the tumour by PDT 
alone. Therefore, the photosensitiser dosage was reduced to 
1.5 mg kg\(^{-1}\) body weight. At this concentration tumour 
control was less effective: There was a significant reduction of 
tumour necrosis to 18%. Verapamil plus PDT did not in-
crease the amount of tumour tissue necrosis in both cases. 
Verapamil alone (group C) did not affect the tumours macro-
or microscopically.

Our second in vivo model involves tumours of human 
adenocarcinoma of the colon which were transplanted into 
nude mice. In this model we examined the effects of 
verapamil and PDT on tumour destruction alone (Table III). 
Under the conditions tested, verapamil did not enhance the 
photodynamic destruction of the human colon carcinoma. 
Verapamil plus PDT had no effect on the degree of tumour 
tissue necrosis when compared to PDT alone. The extent of 
tumour necrosis was not influenced by verapamil alone 
(group C), DHE alone (group B) or light without drugs 
(group D) compared to controls (group A).

The process in which tumour damage is caused by 
photodynamic therapy is complex dependent on many 
different factors. Experimental studies have shown that the 
most important parameters are the applied energy density, 
the concentration of the administered photosensitiser in the 
tissue and the time interval between irradiation and admini-
stratation of the photosensitiser (Barr et al., 1989; Potter et al., 
1987). The photosensitiser uptake and thus the concentration 
in tissue are thought to be affected by the tissue perfusion 
rate. Therefore, the influence of vasoactive drugs such as 
verapamil on PDT was examined in recent studies. Cowled 
and Forbes described an increased photodynamic destruction 
of tumours with verapamil by using a transplantable tumour 
model in mice. In contrast to doses of 30–50 mg kg\(^{-1}\) HPD 
as used by Cowled and Forbes, low photosensitiser doses 
were applied according to previous experiments which proved 
to be sufficient to cause a subtotal tumour destruction (Goss-
nier et al., 1991). Higher drug doses did not enhance the 
amount of tumour destruction, only the danger of adverse 
phototoxic side-effects could possibly increase. Even if these 
dosage schedules cannot easily be transferred to clinical 
application, it seems to be clear that the lowest possible 
photosensitiser concentration should be applied to avoid 
phototoxic side-effects of the skin (Wooton et al., 1988).

In view of the results found in our two different tumour 
models, we conclude that verapamil does not increase 
photodynamic damage concurrently administered with low 
doses of DHE in our in vivo models. It could be demon-
strated that for a low photosensitiser concentration neither a 
regrowth delay nor an increased extent of tumour tissue 
necrosis is achieved. However, in other studies intracellular 
concentrations of cytotoxic agents such as Adriamycin and 
vincristine were increased, suggesting that verapamil im-
paired uptake and induced transport of drugs through the 
cell membrane (Tsuruo et al., 1983). Thus the supposed 
pharmacological mechanism is the existence of a drug 
elimination pathway in the plasma membrane of cancer cells. 
A possible explanation could be the concept that verapamil 
blocks the P-glycoprotein pump which cell membranes use 
to transport anticancer drugs out of the cell (Ince et al., 1986). 
But a certain intracellular concentration of the applied drug 
has to be reached to activate the P-glycoprotein mechanism.

It is known that the photosensitiser concentration ratio 
between tumour and normal tissue is only 2.5:1 (Barr et al., 
1989). In accordance with the P-glycoprotein mechanism 
hypothesis, this photosensitiser concentration could be too 
low to trigger this drug elimination pathway and might be 
the reason why we did not find an enhanced destruction of

### Table I

| Animals (n) | Growth time (d) Mean | s.d. | Regrowth delay (d) Mean | s.d. |
|-------------|---------------------|------|------------------------|------|
| A Control   | 30                  | 6.0  | 1.5                    | 0    |
| B DHE alone | 30                  | 6.6  | 0.9                    | 0    |
| C Verapamil alone | 30      | 6.5  | 1.5                    | 0    |
| D Light alone (without drugs) | 30      | 5.6  | 1.1                    | 0    |
| E DHE + light (PDT) | 30    | 16.5 | 3.6                    | 10.5 |
| F PDT + verapamil | 30    | 17.4 | 3.8                    | 11.4 |

Differences between A through D and E, F are significant (P < 0.05).

### Table II

| Animals (n) | Tumour necrosis (%) Mean | s.d. |
|-------------|-------------------------|------|
| A Control   | 30                      | 4.2  | 4.0                     |
| B DHE alone | 30                      | 5.0  | 3.4                     |
| C Verapamil alone | 30      | 2.8  | 1.0                     |
| D Light alone (without drugs) | 30      | 4.8  | 3.2                     |
| E DHE + light (PDT) | 30    | 95.3 | 1.1                     |
| F PDT + verapamil | 30    | 18.0 | 12.0                    |

Differences between A through D and E, F are significant (P < 0.05).

*With a photosensitiser concentration of 1.5 mg kg\(^{-1}\) body weight.*
malignant tissue by verapamil.

Cowled and Forbes used a different tumour model with different drug concentrations. Therefore, the question remains to be solved whether the lower photosensitiser concentration or the type of tumour tested is the reason why we did not find an enhanced tumour destruction in combination with verapamil. From our results it seems to be clear that there is no generality in the phenomenon described by Cowled and Forbes.

In spite of our negative experiments, the possible enhancement of photodynamic destruction of tumours by vasoactive drugs deserves further investigations. In a recent study, nonverapamil, a major metabolite of verapamil with no systemic side effects, has proved to be as effective as verapamil (Merry et al., 1989) offering new possibilities in testing vasoactive drugs and photodynamic therapy.

For low dose administration of DHE, our current experimental strategies comprise different potential modifiers such as the application of glucose (Thomas & Girotti, 1989) or improved targeting with liposomes (Jori et al., 1986) or monoclonal antibodies (Mew et al., 1983).

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