Eur J Cancer, Vol. 26, No. 5, pp. 551–553, 1990.
Printed in Great Britain

0277-5379/90$3.00 + 0.00

Fax Communication

Preactivation—a Novel Antitumour and Antiviral Approach

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Merocyanine 540 was activated by exposure to 514 nm laser light. This preactivated merocyanine 540 was then mixed (in the dark) with tumour cells, normal cells and envelope viruses to assess its antiproliferative activity. This treatment resulted in 70–90% killing of tumour cells from different cell lines while 85% of normal human peripheral blood mononuclear cells survived the treatment. However, not all types of tumour cells were affected. Preactivated merocyanine 540 was also effective in virtually completely inactivating cell-free herpes simplex and human immmunodeficiency viruses. Preactivated photoactive compounds can exert their toxic effects in the dark without further dependence on light and may have potential systemic use.
Eur J Cancer, Vol. 26, No. 5, pp. 551—553, 1990.

INTRODUCTION

PHOTOACTIVE DYES have been used as potential antitumour and antiviral agents because they are preferentially taken up by some viruses and tumour cells, which are killed after exposure to light [1–5]. One of the limitations of conventional phototherapy for systemic disease, whether it be cancer or a viral infection, is that effective illumination of the target is possible only when the target is accessible (as in the skin, in an open incision during surgery or through the use of fibreoptics). To overcome this disadvantage we use 'preactivation' by light irradiation which enables the photoactive compound to act as a target-specific cytotoxic agent without further dependence on light. We report the *in vitro* effects of preactivated merocyanine 540 on cultured tumour cells and envelope viruses.

MATERIALS AND METHODS

Merocyanine 540 was obtained from Sigma. A 1 mg/ml stock solution was prepared in 10% aqueous ethanol and stored at −20°C. The concentrations of preactivated merocyanine 540 used in this study were those of the compound before light activation. All cell lines were obtained from American Type Culture Collection (Rockville) and were maintained in recommended growth medium.

Merocyanine 540 was preactivated by exposure to a 514 nm laser light set at 4 W for 1 h or a bank of eight fluorescent lights (Phillips 20 W cool white) at a vertical distance of 10 cm (from top of the Petri dish to the bottom of the light bulb) for 18 h. There was no significant difference in the cytotoxicity by preactivated merocyanine 540 obtained by these methods for 18 h. The preactivated compound can be stored at −135°C for more than 30 days.

To study tumour cell cytotoxicity, preactived merocyanine 540 (40–120 μg/ml) was added to normal and cultured tumour cells in the dark. After overnight incubation, cell viability was assessed by trypan blue exclusion and thymidine uptake, and confirmed by staining with ethidium bromide and fluorescein diacetate [6, 7].

The antiviral activity of preactivated merocyanine 540 was investigated in cell-free herpes simplex virus 1 (HSV-1) and human immunodeficiency virus 1 (HIV-1) [8, 9]. Up to 100 μg/ml was incubated overnight at 37°C with the virus suspensions. A tenfold serial dilution of treated and untreated HSV-1 was prepared. 100 μl from each dilution was inoculated into monolayers of vero cells in quadruplicate. 90 min later the monolayer was overlaid with medium L-15 in 1.0% methylcellulose. After 3–4 days' incubation at 37°C, the overlay medium was removed, plates were stained and plaque-forming units were counted. The antiviral activity of HIV-1 was assessed by mixing 50 μl HIV-1 plus preactivated merocyanine 540 with MT-4 cells (1×10^6/ml). HIV-1 was allowed to adsorb to the cells for 2 h at 37°C. MT-4 cells were then washed three times, resuspended in fresh growth medium and cultured for 7 days at 37°C. The viability of cells was determined by trypan blue exclusion. The effect of preactivated merocyanine 540 on HIV-1 was also studied after 4 days' incubation.

RESULTS

There was a significant reduction in the survival of certain tumour cells treated with preactivated merocyanine 540; 85%

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Table 1. Inhibition (%) of [^3H]thymidine uptake by normal cells and tumour cells treated with merocyanine 540

Cell type*	Conc. (μg/ml)	Not activated	Preactivated	*P*
Mononuclear	40	8.4 (5.6)	6.8 (2.54)	>0.1
cells	80	12 (4.8)	8.3 (3.21)	<0.1
	120	—	15.0 (5.80)	
Daudi	40	12.6 (3.18)	51.8 (5.07)	<0.005
	80	40.1 (1.83)	88.8 (12.11)	<0.01
	120	58.2 (7.99)	90.0 (15.51)	<0.05
HL-60	40	22.3 (5.51)	53.9 (4.52)	<0.005
	80	31.7 (3.02)	65.7 (10.34)	<0.025
	120	64.0 (6.67)	87.9 (4.87)	<0.01
H-69	40	10.6 (4.80)	45.1 (9.28)	<0.025
	80	25.6 (5.94)	57.4 (6.50)	<0.01
	120	48.0 (5.94)	88.6 (3.56)	<0.005
ARH-77	80	25.3 (7.02)	5.3 (5.03)	
	120	35.3 (6.03)	11.0 (3.60)	>0.1
G361	80	18.3 (8.50)	8.3 (3.51)	
	120	25.0 (5.57)	10.7 (4.04)	>0.1
A549	80	—	—	
	120	21.7 (4.72)	8.3 (5.69)	>0.1

Mean (S.D., $n = 3$).

* Mononuclear cells were from normal human peripheral blood; Daudi cells were from human Burkitt's lymphoma, HL-60 from human leukaemia, H-69 from human small cell lung carcinoma, ARH-77 from multiple myeloma, G361 from malignant melanoma and A549 from lung adenocarcinoma.

of normal cells survived the treatment (Table 1). However, preactivated merocyanine 540 was ineffective against ARH77, G361 and A549 cell lines.

The concentration of preactivated merocyanine 540 that inactivated 50% and 90%, respectively, of cell-free virus was 10 and 17.5 μg/ml for HSV-1 and 60 and 90 μg/ml for HIV-1 (Fig.1). The effect of the preactivated compound on T-cells (HUT-78) infected with HIV-1 was also examined. HIV-1-infected HUT-78 cells were treated with 200 μg/ml and long-term cultures were maintained. All untreated HIV-1 infected HUT-78 cells were killed by the virus on day 9, while exposure to preactivated merocyanine 540 resulted in 72 and 45% survival of these cells on days 7 and 9, respectively (Table 2). These results suggest that preactivated merocyanine 540 may have a protective

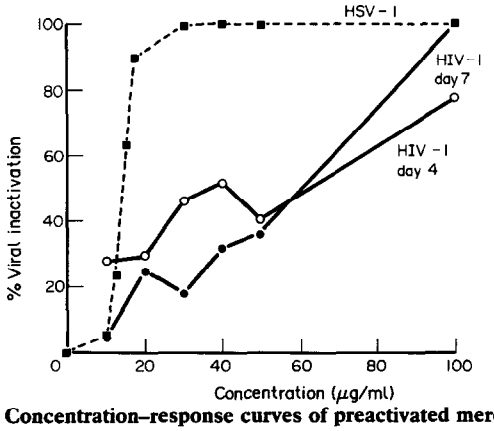

Fig.1. Concentration–response curves of preactivated merocyanine 540 on HSV-1 and HIV-1. Mean of triplicate experiments.

Table 2. Effect of preactivated merocyanine 540 on HIV-1 infected HUT-78 T cells

Day	*% viability*	
	HUT-78 untreated	*HUT-78 treated**
1	100.0	100.0
2	74.1	76.5
5	51.7	60.0
7	25.9	71.7
9	0	44.7

* HUT-78 cells were treated with 200 μg/ml. After overnight incubation, cells were washed, resuspended in fresh medium and maintained in culture (5×10^5 cells per ml) at 37°C.

effect on cells infected with HIV-1, probably by destroying the virus.

DISCUSSION

Merocyanine 540 is one of the most studied photoactive compounds and is selectively taken up by leukaemia, lymphoma, neuroblastoma, multiple myeloma, lung carcinoma and certain classes of immature blood cells [4, 5, 10, 11]. This property of tumour cell selectivity remained intact after preactivation of the dye because selective killing of certain tumour cell types was observed. Why some cell lines are refractory to preactivated merocyanine 540 is not clear. However, it has been reported that A549 and G361 cell lines are highly resistant to merocyanine 540 mediated phototherapy, probably due to poor uptake or retention of dye by these cell types [12]. This dye binds to the cholesterol-free regions of plasma membrane phospholipids [13, 14]. Also, the preferential uptake of merocyanine 540 by neoplastic plasma membrane may be a function of its electrical property [15]. Some of these properties of the parent compound may have been retained in the preactivated form. It is also conceivable that different cell types differ with regard to their intrinsic sensitivity to the toxic products in preactivated merocyanine 540.

The mechanism of cellular and viral toxicity of the preactivated dye remains to be elucidated. However, singlet oxygen produced by direct and sensitized photooxidation rapidly attacks most cyanines, particularly those with low oxidation potential [16]. Reactive oxygen species play a major role in conventional photodynamic therapy [17]. The reactive oxygen species produced by excited states are very short-lived (10^{-9}–10^{-6} s) in aqueous solutions. Because the preactivated compounds can be stored for more than a month, it appears unlikely that the observed toxicity is due to reactive oxygen species produced via the triplet excited state. Our preliminary data indicate that at least one cytotoxic species may be a peroxide. The significant killing of tumour cells and HIV-1 and increased survival of HIV-1 infected cells by preactivated compounds in the dark with minimal cytotoxicity to normal cells warrants further investigation.

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Acknowledgements—We thank Mrs Helen Skiles and Sylvia Trevino for technical assistance. This work was supported in part by grants from the Strategic Defense Initiative MFEL Program, the Leukemia Association of North Central Texas, and the cell biology fund of the Baylor Research Foundation.

Eur J Cancer, Vol. 26, No. 5, pp. 553–555, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

Papers

The Limited Value of Routine Chest X-Ray in the Follow-up of Stage II Breast Cancer

Vibeke B. Løgager, Aage Vestergaard, Jørn Herrstedt, Henrik S. Thomsen, Karin Zedeler and Per Dombernowsky

In 280 patients with stage II breast cancer, chest X-ray was performed at 6 and 12 months and yearly thereafter to the 6th year or until recurrence, another cancer was detected, the patient refused further follow-up or died. Among 1289 scheduled chest X-rays, malignant changes were found in 20 patients, of which only 3 had pulmonary symptoms. In a further 14 patients malignant changes were suspected, but follow-up examinations could not prove malignancy. 26 patients presented within 12 months after the last scheduled X-ray with pulmonary symptoms and a work-up chest X-ray revealed malignant changes. Thus, in only 1.3% of the scheduled X-rays were unsuspected malignant changes diagnosed. Median survival of patients with malignant chest X-rays found at scheduled controls versus between scheduled controls did not differ significantly ($P = 0.26$). It is concluded that routine chest X-ray is not indicated in patients with stage II breast cancer.
Eur J Cancer, Vol. 26, No. 5, pp. 553—555, 1990.

INTRODUCTION

CHEST X-rays are part of follow-up programmes of breast cancer patients in order to provide an early diagnosis of intrathoracic spread. The examination is easily performed, cheap and considered reasonably sensitive. We recently reported that yearly chest X-rays in patients with stage I breast cancer who were otherwise apparently free of disease have a low cost/benefit ratio [1], as only 0.3% of the scheduled chest X-rays revealed unsuspected malignant changes. Patients with stage II breast cancer have a higher recurrence rate than stage I [2], suggesting a higher cost/benefit ratio. The present study was therefore undertaken in order to examine retrospectively whether repeated X-rays performed at fixed intervals after mastectomy had any value in patients with primary operable breast cancer, Danish Breast Cancer Cooperative Group (DBCG) stage II [2, 3].