PHOTODYNAMIC ACTION OF CONCANAVALIN A–CHLORIN CONJUGATE e_6 ON HUMAN FIBROBLASTS

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Photosensitizers and, in particular, certain porphyrin derivatives, which possess higher affinity for tumor tissues than for normal tissues and which exhibit low toxicity for the patient have been used for tumor therapy. During the action of light on the porphyrins, singlet oxygen ($^1O_2$), toxic for living cells, is formed [2]. The life span of $^1O_2$ in the aqueous phase is about 2 μsec and, consequently, its action is local. It has been suggested that the probable target for the action of porphyrins is the cell membrane in which lipid peroxidation is initiated [1].

Since normal cells can also accumulate porphyrins and are eliminated from the body very slowly, complications in the form of photoreactions may arise in patients receiving such preparations intravenously: if the skin is exposed to direct sunlight burns may occur for 1-2 months after injection of the porphyrins [1]. The problem accordingly arises of reducing the active concentration of the sensitizer. One possible way of solving this problem may be to conjugate the photosensitizer with a ligand for which specific receptors, undergoing internalization exist on tumor cells. In this way the conjugated ligand can penetrate inside the cells and be targeted to a particular compartment.

In our experiments we used concanavalin A (con A), conjugated with the photosensitizer chlorin e_6 as the ligand.

EXPERIMENTAL METHOD

Human embryonic skin fibroblasts were cultured in Eagle's growth medium with 15% heat-inactivated fetal calf serum in the presence of 5% CO_2. Chlorin e_6 was obtained from nettle leaves by Fisher's method [3]. Con A was conjugated with chlorin e_6 with the aid of cyclohexyl-3-(2-morpholinoethyl)-carbodi-imidemeto-4-toluenesulfonate [6]. The method enabled one molecule of con A to be bound with 25 molecules of chlorin e_6. The viability of the cells was determined as incorporation of labeled thymidine [4]. Fibroblasts were seeded on Petri dishes (capacity 35 ml) with ^{3}H-thymidine (10^4 Bq/ml). After formation of a monolayer the cells were incubated in growth medium with chlorin e_6 or with the Con A–chlorin e_6 conjugate at 37°C or 0°C. The concentrations of chlorin e_6 and of the Con A–chlorin e_6 conjugate were equal with respect to chlorin e_6. The cells were then washed with medium without serum (4 times) and with Hanks' solution (twice) without phenol red. The cells were allowed to stand in Hanks's solution and irradiated with a 150-W halogen incandescent lamp, with dose rate of 400 W/m^2. The maximum of the emission spectrum of the lamp was in the visible region and close to the long-wave maximum of absorption of chlorin e_6 (660 nm). After irradiation the cells were seeded in growth medium with serum and with ^{14}C-thymidine: (2.5-5) $\times$ 10^4 Bq/ml. The cells were then removed with 1% Triton, precipitated with ethyl alcohol, and the residue was transferred to flasks for determination of radioactivity. In another series of experiments, unlabeled cells after irradiation were reseeded in the presence of ^{3}H-thymidine. Next day the cells were removed for measurement of radioactivity, which was determined on a liquid scintillation counter.

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TABLE 1. Effect of Chlorin e_6 and Con A–Chlorin e_6 on Viability of Human Embryonic Skin Fibroblasts ($M \pm m$)

Experimental conditions	Incubation temperature, °C	Illumination for 2 min	^3H	Incorporation of ^{14}C (cpm)	$^{14}C/^3H \times 10^3$	p
Control	37	—	886 614±45 925	1592±305	1,59±0,29	
Chlorin e_6	37	+	975 233±21 313	1065±129	1,04±0,15	<0,1
Con A–chlorin e_6	0	+	1 030 610±109 588	1097±167	1,02±0,1	<0,005
Con A–chlorin e_6	37	+	906 063±62 910	332±65	0,34±0,09	<0,001

Legend. Duration of incubation with photosensitizer was 1 h, concentration expressed as chlorin e_6 1.57 μM.

EXPERIMENTAL RESULTS

The cells were seeded on dishes with ^3H-thymidine and then incubated with the conjugate or with chlorin e_6, irradiated, and then treated with ^{14}C-thymidine. Incorporation of ^3H-thymidine characterized the number of cells remaining adherent to the substrate after the action of the damaging agent. Incorporation of ^{14}C-thymidine demonstrated the capacity of the cells for further growth. Incorporation of ^{14}C-thymidine, normalized for the level of incorporation of ^3H-thymidine, reflected the viability of the cells remaining attached after irradiation. After exposure to light, incorporation of ^{14}C-thymidine into cells incubated with Con A–chlorin e_6 was considerably weaker than in the control cells (Table 1). The damaging action of the conjugate was stronger than that of chlorin e_6 alone. At 0°C the ligand is known to bind with receptors on the cell surface and internalization of the ligand does not take place [7]. For that reason, in the present experiments the damaging action of con A–chlorin e_6 at 0°C was weaker. If the cells were not irradiated after incubation, no damaging effect was found.

In the experiments of series II the number of surviving cells was determined after incubation with the conjugate or with chlorin e_6 (3.14 μM) followed by irradiation. After irradiation the cells were seeded on dishes with ^3H-thymidine. Ability to adhere to the substrate is a feature of undamaged cells. After incubation with the conjugate and subsequent irradiation the cells were found to die completely, whereas after chlorin e_6, incorporation was reduced by 10-20% below the control level. Only with chlorin e_6 in a concentration of 15.7 μM did all the cells die, i.e., conjugation led to a fivefold increase in the effect and, correspondingly, to an equal decrease in the active concentration of chlorin e_6.

Con A has the ability to bind with glucosides and mannosides of the surface glycoproteins of the cell [5], and then to be internalized by the cells [4]. On penetrating inside the cells con A, proceeding along endocytotic compartments, can enter lysosomes. On conjugation of con A with chlorin e_6, the damaging action of the conjugate is stronger than that of chlorin e_6 alone, for it is the intracellular compartments of the cells that are the target for the conjugate, whereas that for chlorin e_6 is mainly the cell membranes [1, 8]. For practical purposes it is therefore evident that the photosensitizer should be conjugated with a ligand whose receptors are represented mainly on tumor cells.

LITERATURE CITED

1. N. F. Gamaleya and N. A. Mikhalkin, Éksp. Onkol., No. 1, 9 (1988).
2. G. P. Gurinovich, A. N. Sevchenko, and K. N. Solov'ev, Spectroscopy of Chlorophyll and Related Compounds [in Russian], Minsk (1968).
3. G. P. Gurinovich, I. T. Zhakov, G. A. Kostenich, et al., Vest. Akad. Med. Nauk SSSR, No. 12, 24 (1987).
4. E. Ben-Hur, M. Green, A. Prager, et al., Photochem. Photobiol., **46**, 651 (1987).
5. G. M. Edelman, Science, **192**, 218 (1976).
6. H. Faultich and L. Fiume, Meth. Enzymol., **112A**, 225 (1985).
7. A. R. Robbins, R. Myerowitz, R. J. Youle, et al., J. Biol. Chem., **256**, 10618 (1981).
8. R. Santus, C. Kohen, E. Kohen, J. P. Reyftman, et al., Photochem. Photobiol., **38**, 71 (1983).