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Hyperbaric Oxygen and Photodynamic Therapy in Tumour-bearing Nude Mice

M. Jirsa Jr, P. Poučková, J. Doležal, J. Pospíšil and M. Jirsa

THE CYTOTOXICITY of porphyrins and related substances is mediated mainly by singlet oxygen via a type II mechanism. Because hypoxic cells are less affected by porphyrins and light [1–3], hyperbaric oxygen (HBO) could increase the efficiency of photodynamic therapy (PDT). Moreover, tumour tissue has regions with various degrees of hypoxia; therefore, the oxygen tension, which controls the porphyrin-sensitised photo-oxidation rate of bilirubin and ditaurobilirubin *in vitro* [7], may be a limiting factor in tumour phototherapy [4–6]. The aim of our study was to test the influence of HBO on PDT efficiency in an experimental animal model.

27 mm^3 fragments of human rectal carcinoma I (820302) were implanted subcutaneously into the right flank of female BALB/c nude mice (n = 13, body weight 16–18 g). The mice were housed in isolators with sterilised bedding, and fed by sterile diet DOS 2b-St (VELAZ). Intake of (boiled) water was not measured. The human tumour sample characterised histologically as slightly mucoproductive tubular adenocarcinoma, was taken from the 41th passage on nude mice, latency 10 and doubling time 3 days, respectively. 10 mg/kg meso-tetra (4-sulphonatophenyl)porphine ($TPPS_4$) prepared as in our previous study [7], was injected intratumorally 1 h before irradiation.

An He–Ne laser (Metra Blansko, wavelength 632.8 nm) with a single optic fibre (type PCS, external diameter 0.2 mm) was used, and the distance between tumour surface and fibre end was 25 mm. Total light dose was 150 J for the experimental (n = 7), and 240 J for the control (n = 6) group, respectively. Irradiation time was 1 h in each group. The compression–decompression scheme for the human hyperbaric chamber (ČKD Praha) was 5 min compression in 100% oxygen; 60 min irradiation at 0.28 MPa in 100% oxygen; decompression with 5 min plateau at 0.16 MPa and 10 min plateau at 0.13 MPa, both in normal atmosphere.

The mice were monitored daily, and appearance of necrosis was observed in the first week after irradiation. Tumour volume [8], and mass were measured on the 28th day, and tumour-free animals were followed up for 2 months.

The results are shown in Table 1. PDT in HBO was more efficient in the experimental group, although the total light dose and intensity were higher in the control group and the average initial tumour volume was more than twice as high in the experimental group. Necroses appeared earlier in the experimental group (1–2 days) than in the control (2–4 days), and complete remission 2 months after PDT was more frequent in the experimental (71%) than in the control group (17%).

Table 1. PDT and HBO in tumour-bearing nude mice

Initial tumour volume (mm^3)	Relapse (14–20 days)	Final tumour: Volume (mm^3)	Final tumour: Mass (g)	Final tumour: Remission after 2 mo
Experimental group (0.28 MPa oxygen)				
220.0	no	–	–	yes
108.0	no	–	–	yes
526.5	no	–	–	yes
98.3	no	–	–	yes
196.0	no	–	–	yes
304.0	yes	864	0.60	no
270.8	yes	108	0.20	no
Control group (normal atmosphere)				
180.0	yes	425.2	0.33	no
68.7	yes	32.0	0.02	no
113.6	yes	126.0	0.15	no
137.5	no	–	–	yes
87.5	yes	13.5	0.01	no
96.0	yes	75.0	0.03	no

In our study we used the lowest possible number of animals; oxygen tension in the tumour tissue was not measured and application of $TPPS_4$ was local, in a high dose of 10 mg/kg. Notwithstanding, our results are promising.

Combining HBO and PDT could improve the efficiency of PDT by increasing depth of tumour cell damage, and/or by reducing doses of sensitisers. The use of HBO has a long tradition in clinical medicine. A 1 h application of 0.3 MPa, 100% oxygen is non-toxic and the combined therapy would involve minimal technical difficulties.

Correspondence to M. Jirsa Jr.
M. Jirsa Jr is at the Department of Clinical Biochemistry, Faculty of Medicine, Charles University, Karlovo náměstí 32, 121 11 Prague; P. Poučková and J. Pospíšil are at the Institute of Biophysics and Nuclear Medicine and M. Jirsa is at the First Medical Clinic, Faculty of Medicine, Charles University; and J. Doležal is at the Institute of Aviation Medicine, Kovpakova 1, Prague, Czechoslovakia.
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