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Effects of photosensitizing and ionizing irradiation on the activity of antioxidant and phase II enzymes in Ehrlich ascites cells

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Hematoporphyrin dimethyl ester (Hpde)-sensitized photoirradiation of Ehrlich ascites cells or their ionizing irradiation (2 Gy) in the presence or absence of Hpde insignificantly influenced the activity of the antioxidant enzymes catalase, superoxide dismutase and glutathione reductase determined 2 h after irradiation. The above procedures as well as Hpde in the dark decreased the activities of the phase II enzymes glutathione-S-transferase and DT-diaphorase. The possible significance of these phenomena in combined tumour therapy is discussed.

Key words: photosensitization, ionizing irradiation, catalase, superoxide dismutase, glutathione reductase, glutathione-S-transferase, DT-diaphorase

INTRODUCTION

Currently the combined use of radio-, chemo- and photodynamic tumour therapy attracts considerable interest, since it significantly increases the therapeutic outcome and decreases the side effects [1]. In order to develop the new protocols in combined therapy and to understand its mechanisms, it is necessary to evaluate the cytotoxic consequences of each treatment step. The antioxidant enzymes catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD, EC 1.15.1.1), and glutathione reductase (GRD, EC 1.6.4.2) protect the cell against the damage by the reactive oxygen species and, together with the phase II xenobiotics metabolizing enzyme gluta-thione-S-transferase (GST, EC 2.5.1.18), confer the cell resistance against prooxidant and alkylating antitumour agents [2, 3]. An increase or a decrease in the activities of CAT, SOD or GST following photosensitizing or ionizing irradiation was observed depending on the dose and the time after irradiation [4–7]. However, the overall impact of irradiation on the cell defence enzymatic system remains unclear, since the antioxidant and phase II enzymes were assessed in separate studies under different doses and conditions of irradiation. Besides, data on the possible changes in GRD activitiy are absent. The aim of this work was to characterize the parallel changes in the activities of CAT, SOD, GRD and GST following the photodynamic treatment and ionizing irradiation of Ehrlich ascites cells. Since the expression of GST occurs in parallel with another phase II enzyme, DT-diaphorase (DTD, NAD(P)H: quinone reductase, EC 1.6.99.2) [3], the activity of DT-diaphorase was also verified.

MATERIALS AND METHODS

Murine Ehrlich ascites tumour (EAT) was inoculated intraperitoneally (i.p.) to healthy female mice aged 6–7 weeks as described [8]. On the 7th day after inoculation, a 2.5 mM hematoporphyrin dimethyl ester (Hpde) solution in physiological saline was injected i.p. at 60 mg/kg body weight. After 3 h, EAT cells were prepared *ex vivo* in the dark as 3.7×10^6/ml suspension in the physiological saline. During all the experiments, the mice were under general anesthesia. The cell suspension

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was irradiated (370 nm< λ >680 nm, 50 mW/cm^2, total light dose 4.5 J/cm^2) in a 2 mm cuvette with a 500 W tungsten lamp equiped with a light focusing system and optical filter. The ionizing irradiation of EAT cells was performed using a ^{60}CO source at 1.0–1.6 Gy/min (total dose, 2 Gy) in a 1 mm cuvette as described [8]. After treatment, the cells were kept for 2 h at room temperature, then frozen at –80 °C. The protein content was determided according to the Lowry method. The activity of catalase, superoxide dismutase, glutathione reductase and DT-diaphorase was determined spectrophotometrically using a Hitachi-557 spectrophotometer, in a 0.1 M K-phosphate buffer (pH 7.0), containing 1 mM EDTA and 0.16 mg/ml digitonin, at 25 °C, as described previously [9]. The activity of glutathione-S-transferase was determined according to the rate of formation of conjugate ($\Delta\varepsilon_{340}$ = = 9.0 mM^{-1} cm^{-1}) between 0.3 mM 2,4-dinitrochlorobenzene and 5.0 mM reduced glutathione [10]. Pig liver NADPH:cytochrome P-450 reductase prepared as described [11] was a generous gift of Dr. H. Nivinskas. The enzyme activity in the reduction of 75–10 μM Hpde was determined spectrophotometrically according to the rate of oxidation of 100 μM NADPH in the absence of digitonin.

RESULTS AND DISCUSSION

The insignificant activity changes of catalase and superoxide dismutase following the Hpde-sensitized irradiation of Ehrlich cells (Table) are consistent with the previous data [4–7]. Indeed, after a 1.5 J/cm^2 photosensitization of mouse mammary carcinoma cells by hypericin, the activity of SOD and CAT transiently increased 2–2.5 times and subsequently declined to control levels after 1–1.5 h [6]. In contrast, high light doses (15–20 J/cm^2) caused phthalocy-anine-sensitized inactivation of CAT in K562 leukemic cells [12]. Following 2–6 Gy irradiation of mice, SOD and CAT activity increased 2–3 times after 0.5 h [4], but decreased after 2 h [7]. Probably an increased SOD activity following ionizing irradi-ation of Hpde-treated cells (Table) reflects a slower decline in the SOD activity after its transient increase. Taken together with the unchanged GRD activity (Table), our data show that the photosensitizing and ionizing irradiation of Ehrlich cells under given conditions may not significantly affect the cell antioxidant capacitance. However, a significant decrease in the activity of phase II enzymes GST and DTD was observed (Table). Since the induction of GST and DTD is mediated by the common transcriptional enhancer called the antioxidant response element [3], one may suppose that the photosensitizing or ionizing radiation may affect their synthesis. However, we have found that the 9 J/cm^2 irradiation of frozen, thawed and digitonin-permeabilized Ehrlich cells in the presence of 25 μM Hpde decreased the GST activity by 50%. Thus, photosensitized inactivation of GST may take place as well. In view of the low activity of DTD in Ehrlich cells (Table), we were unable to assess the kinetics of its photoinactivation in lysed cells. Nevertheless, our data show that the photosensitizing and ionizing irradiation of Ehrlich cells under given conditions may increase their sensitivity to alkylating agents.

The mechanism(s) of decrease in the GST and DTD activity after cell treatment in the dark by

Table. **Changes in the activity of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GRD), glutathione-S-transferase (GST), and DT-diaphorase (DTD) following different treatment of Ehrlich ascites cells and determined 2 h after irradiation (U/mg).**

The statistically significant differences *versus* the control values are indicated if available. The unit of CAT activity is expressed as μmol H_2O_2/min × mg, the unit of SOD activity is expressed as the amount of protein inhibiting the xanthine/xanthine oxidase-mediated reduction of nitrotetrazolium blue by 50%. The units of GR, GST and DTD activity are expressed as nmol substrate/min × mg, n is the number of animals.

Enzyme	Control	Hpde, dark	Hpde, photoirradiation	Ionizing radiation	Hpde, ionizing radiation
CAT	1.57 ± 0.28 (n = 5)	2.07 ± 0.34 (n = 5)	2.23 ± 0.22 (n = 3)	1.58 ± 0.55 (n = 3)	1.48 ± 0.19 (n = 3)
SOD	5.84 ± 0.55 (n = 5)	6.9 ± 1.1 (n = 3)	7.16 ± 2.0 (n = 3)	5.9 ± 0.49 (n = 3)	11 ± 1.2 (n = 3, p < 0.005)
GRD	31.7 ± 3.0 (n = 6)	31.8 ± 5.6 (n = 3)	32.8 ± 2.2 (n = 3)	29.1 ± 5.6 (n = 3)	27.7 ± 3.6 (n = 3)
GST	76.5 ± 10.8 (n = 5)	44.3 ± 7.5 (n = 4, p < 0.05)	15 ± 2.0 (n = 4, p < 0.002)	44.8 ± 4.4 (n = 4, p < 0.05)	32.2 ± 3.0 (n = 3, p <0.02)
DTD	2.5 ± 0.3 (n = 4)	1.8 ± 0.5 (n = 3)	<1.0 (n = 3)	<1.0 (n = 3)	<1.0 (n = 3)

Hpde (Table) are currently unclear. Since Hpde potentiated the effects of ionizing irradiation (Table), one of the possible mechanisms of its action in the dark may be the generation of free radicals in enzymatic reactions or under ionizing irradiation. Single-electron reduction of photofrin II by rat hepatic microsomes is described [13]. We found that Hpde may be reduced by microsomal NADPH:cytochrome P-450 reductase with $k_{cat} > 1\ s^{-1}$ and $k_{cat}/K_m = 1.4 \cdot 10^4\ M^{-1}s^{-1}$. Although this is a relatively slow process, the k_{cat}/K_m of Hpde is four times higher than the reduction rate constant of the well-known radiosensitizer metronidazole, determined under identical conditions.

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FOTOSENSIBILIZUOTO IR JONIZUOJANÈIO ÐVITINIMO POVEIKIS ANTIOKSIDACINIØ IR II FAZËS FERMENTØ AKTYVUMUI ERLICHO ASCITO LÀSTELËSE

S a n t r a u k a

Hematoporfirino dimetilo esteriu (Hpde) sensibilizuotas Erlicho ascito làsteliø apðvitinimas matomàja ðviesa arba jø jonizacinis apðvitinimas (2 Gy) esant arba nesant Hpde nedaug paveikë antioksidaciniø fermentø katalazës, superoksido dismutazës ir gliutationo reduktazës aktyvumà, iðmatuotà po 2 val., taèiau sumaþino II fazës fermentø gliutationo S transferazës ir DT diaforazës aktyvumà. Veikdamas tamsoje ðiø fermentø aktyvumà sumaþino ir Hpde. Aptarta galima ðiø efektø reikðmë kombinuotoje navikø terapijoje.