Effect of Oxidixed Dextrans on Oxidative and Metabolic Function of Mouse Peritoneal Macrophages *In Vitro* and *In Vivo*

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We compared the effects of dextrans with a molecular weight of 35 kDa oxidized by chemical (OD_{ch}) and radiochemical (OD_r) methods on oxidative and metabolic functions of peritoneal macrophages from BALB/c mice *in vitro* and *in vivo*. It was found that none type of dextrans exhibits chemiluminescent properties. *In vitro* study showed that OD_{ch} had a priming effect on mouse peritoneal macrophages, while OD_r did not potentiate the oxidative and metabolic response of cells to zymosan. Being injected intraperitoneally, OD_r more markedly enhanced chemiluminescent response of mouse peritoneal macrophages and reduced their viability than OD_{ch}. Thus, both dextrans are biocompatible, but in OD_{ch} this parameter is higher.

Key Words: *macrophage; oxidized dextrans; biocompatibility, chemiluminescent; active oxygen metabolites*

Functional activity of phagocytosing cells can be modulated by various substances, in particular, by dextran. Modified dextran is used as a carrier for targeted delivery of bioactive substances into target cells [6,8], primarily into phagocytes [7]. Modified dextrans change functional state of phagocytes, in particular, modulate their oxidative and metabolic function, which can have negative and positive consequences for the organism due to activation of the production of activated O_2 metabolites [2].

Oxidation of dextran is a necessary condition for its conjugation with potential drug substances. The known chemical and radiochemical methods of dextran oxidation have their advantages and drawbacks [4,6]. However, biocompatibility partially determined by the applied methods of oxidations is an important property of drug compositions with oxidized dextrans (OD).

Here we compared biocompatibility of dextrans oxidized by chemical $(OD)_{ch}$ and radiochemical $(OD)_r$ methods by evaluating their effects on oxidative and metabolic function of mouse peritoneal macrophages (PM) *in vivo* and *in vitro*.

MATERIALS AND METHODS

The study was performed on 40 male BALB/c mice aging 2 months and weighing 20-22 g (nursery of the Institute of Cytology and Genetics, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk).

We used dextrans with a mean molecular weight of 35 kDa oxidized by radiochemical [5] and che-

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mical methods differing by technological elements and the degree of purity of the end product [6].

OD_{ch} was obtained by oxidation with potassium permanganate. Aqueous solution of dextran with the mean molecular weight of 35 kDa (Fluka) was heated on a boiling water bath at 100°C. Aqueous solution of acetic acid (33%, 2 vol % of dextran solution) was added to the solution of dextran and agitated. Aqueous solution of potassium permanganate (2%, 4 vol % of dextran solution) was added. The mixture was heated to 90°C until the formation of a brown-black precipitate. The solution was filtered through a paper filter (Enderol filter No. 3, Binzer). OD_{ch} was precipitated from the solution by adding a 2-fold volume of 95% ethanol at 60°C.

For obtaining PC, the animals were killed by cervical dislocation under ether anesthesia and the peritoneal cavity was washed with medium 199. Oxidative and metabolic function of PM was evaluated by luminol-dependent chemiluminescence (CL) on a CKIF-3606M biochemiluminometer (Nauka). Yeast polysaccharide zymosan A (Sigma) in a concentration of 5 μg/ml was used for additional stimulation of the oxidative and metabolic function of cells. In CL study, the integral spontaneous and zymosan-induced CL responses of cells were determined as the number of CL-pulses produced by PM over 1 h.

The CL response of cells to OD_{ch} and OD_r was evaluated in 3 stages: 1) in Hanks medium (without cells); 2) after adding OD_{ch} and OD_r (25 μg, 0.1 ml) to PM suspension (5×10^5 cells) obtained from intact mice *in vitro*; 3) mice of 2 groups received intraperitoneal injection of 0.5 ml (125 μg) OD_{ch} and OD_r. Group 3 mice received intraperitoneal injection of 0.85% NaCl. CL response of PM was evaluated 24 h after intraperitoneal injection of dextranals; cell viability (% of viable cells) was simultaneously evaluated by trypan blue staining [1].

The significance of differences between the means was evaluated using Student *t* test, the differences were significant at $p<0.05$.

RESULTS

None of the dextranals induced CL in a cell-free medium, which was seen from CL response curves that did not differ from the background (Fig. 1).

After addition of OD_{ch} and OD_r to PM suspension *in vitro*, the integral spontaneous CL increased and 2.2- and 2-fold surpassed the integral spontaneous CL in the cell-free system (Table 1). This suggests that both variants of dextranals equally enhance CL response of PM *in vitro*. However, after additional stimulation of cells with yeast polysaccharide zymosan intensively phagocytosed by macrophages similarly to dextrans [7], the CL response of PM in the presence of different forms of dextranals differed considerably. The increase in zymosan-induced CL response of PM was maximum in the presence of OD_{ch}: 2-fold surpassed that observed in the presence of OD_r.

Examination of PM obtained 24 h after intraperitoneal injection of dextranals of both types showed that their CL response considerably differed from that observed in *in vitro* system. The level of CL response was by one order of magnitude higher than in *in vitro* test (Table 1), which was probably related to the duration of PM contact with dextranals and the volume of their endocytosis. Spontaneous CL response of PM obtained 24 h after intraperitoneal injection of OD_r was by 1.5 times higher than after injection of OD_{ch} (Table 1). Moreover, after additional stimulation, the zymosan-induced CL response of PM from mice receiving OD_r and

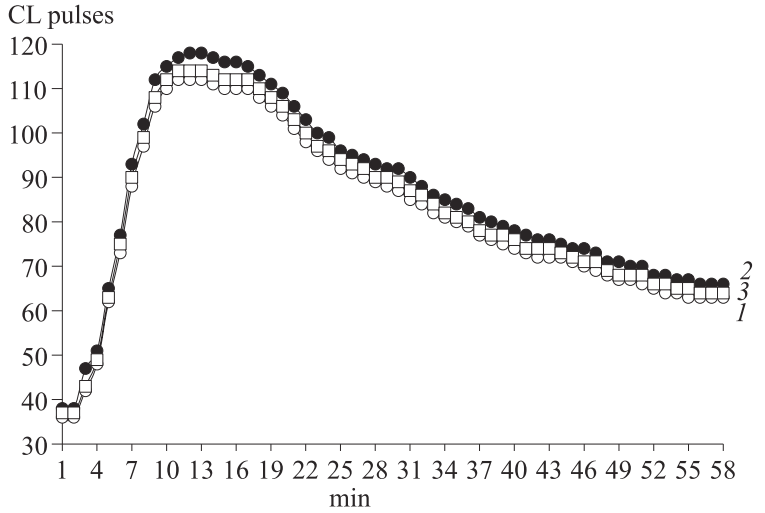

Fig. 1. CL of dextranals in cell-free medium. *1*) baseline; *2*) OD_r, *3*) OD_{ch}.

TABLE 1. Parameters of Spontaneous and Dextranal- and Zymosan-Induced CL Response of Mouse PM (*M*±*m*)

Experimental conditions	CL response (×10^3 pulses/PM/h)	
	in vitro	after 24 h
Control	2.70±0.43	3.4±0.4
OD_r	65.7±2.3*	6.90±0.79*
OD_{ch}	42.8±1.2*	7.40±0.75*
Zymosan A[1]	5.60±0.44*	5.3±0.6*
OD_r+zymosan A[1]	237.6±12.7*+	9.57±0.90*+
OD_{ch}+zymosan A[1]	124.3±7.3*+o	19.03±0.93*+o

Note. [1]Zymosan A was added to cells immediately before measurements of CL response. $p<0.05$ compared to: *control, +dextranal, oOD_r+zymosan A.

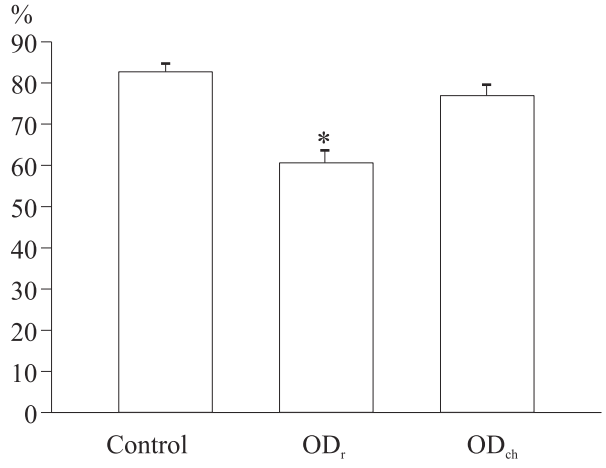

Fig. 2. Number of viable PM after intraperitoneal injections of dextranals. *$p<0.05$ compared to the control.

OD_{ch} surpassed the spontaneous response by 3.5 and 2.8 times, respectively. Thus, zymosan-induced CL response of PM from mice receiving OD_r was by 1.9 times higher than in animals receiving OD_{ch} ($p<0.05$). It should be noted that the number of viable PM engulfing OD_r was below the control value, while in mice receiving OD_{ch} this parameter only tended to decrease (Fig. 2).

The results show that OD_r and OD_{ch} exhibit similar capacity of induce CL response of PM under *in vitro* conditions. However, in the presence of OD_{ch} zymosan more markedly increased the CL response of PM than in the presence of OD_r. This fact probably suggests that OD_{ch} can prime macrophages and they respond to the additional zymosan stimulation by more active CL surge [2]. On the other hand, CL response of PM from mice receiving intraperitoneal injections of dextranals differed from that observed in *in vitro* experiments. In this case, the zymosan-induced CL response of PM from mice receiving OD_r was significantly higher than after injection of OD_{ch}. It can be hypothesized that intraperitoneal injection of OD_r more markedly stimulates the CL response of PM (due to H_2O_2 admixtures formed during radiolysis of water [5]), but induces their death probably because of exhaustion of the antioxidant system in macrophages most actively endocytosing OD_r.

Thus, both dextranals stimulate metabolic processes in macrophages, but OD_{ch} is characterized by higher biocompatibility compared to OD_r.

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REFERENCES

1. E. D. Goldberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culture in Hematology* [in Russian], Tomsk (1992).
2. N. K. Zenkov, V. Z. Lankin, and E. B. Men'shchikova, *Oxidative Stress: Biochemical and Pathophysiological Aspects* [in Russian], Moscow (2001).
3. D. N. Mayanskii, D. D. Tsyrendorzhiev, O. P. Makarova, *et al., Diagnostic Value of Leukocytic Tests. Part 2. Evaluation of Biocydal Properties of Leukocytes (Methodical Recommendations)* [in Russian], Novosibirsk (1996).
4. S. G. Panasenko, A. B. Pupyshev, Yu. N. Kurunov, and V. A. Shkurupiy, *Method of Obtaining Isoniazid-Dextran Conjugate* [in Russian], Patent RU No. 2125451 of 27.01.1999
5. V. A. Sharpatyi, *Radiats. Biol. Radioekol*., **39**, No. 1, 156-161 (1999).
6. V. A. Shkurupiy, Yu. N. Kurunov, O. V. Grishin, *et al*., *Method of Obtaining Prolonged-Action Isoniazid*, Patent RU No. 2143900 of 10.01.2000.
7. V. A. Shkurupiy, Yu. N. Kurunov, and N. N. Yakovchenko, *Lysosomotropism: Problems of Cell Physiology and Medicine* [in Russian], Novosibirsk (1999).
8. V. A. Shkurupiy, V. A. Krasnov, E. P. Gulyaeva, *et al., Radiat. Phys. Chem*., **63**, 691-695 (2002).