CYTOLOGY

In Vitro Effect of Oxidized Dextrans on Peritoneal Cells

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We studied the dependence of *in vitro* dextran biocompatibility on the method of oxidation of 35-kDa dextran. The biocompatibility of dextran oxidized with potassium permanganate was higher compared to that obtained by radiochemical oxidation. It was related to the formation of peroxide compounds during radiochemical oxidation.

Key Words: *oxidized dextran; biocompatibility; peritoneal cells; macrophages*

Targeted transport of drugs is an important problem of modern pharmacology. This is achieved by the use of compositions consisting of drugs and modified biopolymers. The carriers from oxidized dextrans (OD) hold much promise in this respect [6]. The chemical (treatment with periodic acid and salts) [3] and radiochemical methods of dextran oxidation (γ-irradiation) [5] differ in the technological complexity and purity of end products [4,7]. OD obtained by these methods can contain various admixtures [4], which modify dextran biocompatibility. *In vitro* comparative study of OD obtained by the chemical (OD_c) and radiochemical methods (OD_r) is important to evaluate the biocompatibility of these compounds.

Here we studied the biocompatibility of OD on the culture of peritoneal cells (PC).

MATERIALS AND METHODS

Aqueous solution (10%) 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_c was precipitated from the solution by addiing a 2-fold volume of 95% ethanol at 60°C.

Chemical purity of OD_c and OD_r was determined spectrophotometrically on a SF-2000 spectrophotometer (SPEKTR; spectrum drawing software, version 2.9.1).

OD biocompatibility was assayed on peritoneal transudate cells [2] of male BALB/c mice aging 2 months, weighing 21-22 g, and obtained from the nursery of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences, Novosibirsk). PC were obtained from animals killed by cervical dislocation under ether anesthesia. Activity of OD was estimated from the influence on adhesion properties and viability of PC (no phenotypic differentiation) [1]. Adhesion activity of peritoneal macrophages was estimated from the count of flattened macrophages (relative to the total number of cultured macrophages). OD in con-

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centrations of 250 and 500 μg/ml was added to cultured cells by the 2nd hour of culturing.

Intact culture of PC and peritoneal macrophages was used as another control group in the study of OD biocompatibility.

The state of cultured cells was *in vitro* studied 24 h after addition of OD to the culture medium. PC were cultured on coverslips (10^6 cells per 2 ml medium 199 with 10% fetal bovine serum) in glass flasks at 37°C. The percentage of living cells (viability) in cultures was evaluated by means of vital staining with trypan blue [2]. The cells were stained and photographed using an Axiostar Plus microscope (Zeiss) and Nicon Coolpix 5000 digital camera (×20). The mean number of PC of various types and number of viable cells were estimated in the same field of view (5 fields of view in each sample for the test parameters; 5 cell cultures from 5 mice for each parameter). Cultured cells were fixed in aqueous solution of formaldehyde (4% solution in phosphate buffered saline, pH 7.3-7.5) and stained with azure and eosin for 10 min. The cells were stained and photographed using an Axiostar Plus microscope (Zeiss) and Nicon Coolpix 5000 digital camera (×20, ×100). The percentage of flattened macrophages was evaluated morphometrically by means of VideoTest Morfo 3.2 software.

The significance of differences between the mean values was estimated by nonparametric White test.

RESULTS

OD_c in a dose of 250 μg/ml had no effect on viability of PC and adhesion activity of macrophages (Fig. 1). Increasing the concentration of OD_c in the cell culture to 500 μg/ml was followed by a slight decrease in the number of living cells. The percentage of flattened cells increased by 15% (relative to the count of intact cells).

OD_r in a dose of 250 μg/ml did not modulate the process of cell flattening, which reflects adhesion activity of macrophages. However, OD_r induced a decrease in PC viability (all cells of the transudate). Increasing the concentration of OD_r in the cell culture to 500 μg/ml was followed by a significant increase in the number of flattened macrophages and 50% decrease in the percentage of viable cells (more pronounced than after treatment with OD_r in a dose of 250 μg/ml).

Under the influence of OD_r in a concentration of 500 μg/ml, the majority of trypan blue-stained flattened macrophages had swollen cytoplasm. These changes reflect damage to the cell membrane. Cell membrane damage did not occur immediately after addition of OD_r to the culture medium, but was observed by the end of culturing (cell flattening). Swelling of the macrophage cytoplasm was not revealed after addition of OD_c in this dose.

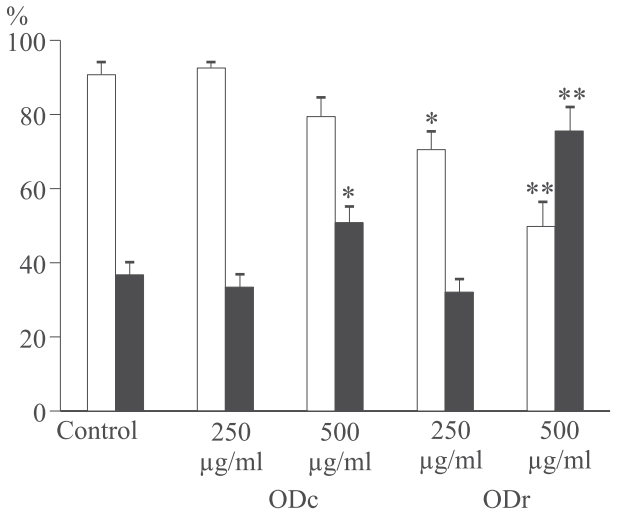

Fig. 1. Effect of OD on viability of PC (light bars) and adhesion activity of peritoneal macrophages (dark bars) in the primary culture of PC (24 h after addition of dextrans to the culture medium). $^*p<0.05$ and $^{**}p<0.01$ compared to the control.

Hence, the biocompatibility of OD_r is slightly lower than that of OD_c. OD_r probably contains microadmixtures of peroxide compounds that are formed during radiolysis of water and dextran [4]. A correlation was found between high biocompatibility of OD_c in cultured cells and UV absorption spectrum. (Fig. 2). Light absorption of OD_c was nearly undetected in long-wave UV light (above 240 nm). These data reflect the absence of peroxide admixtures and compounds with conjugated carbon-carbon double bonds, which can be formed during degradation of peroxide groups in dextran.

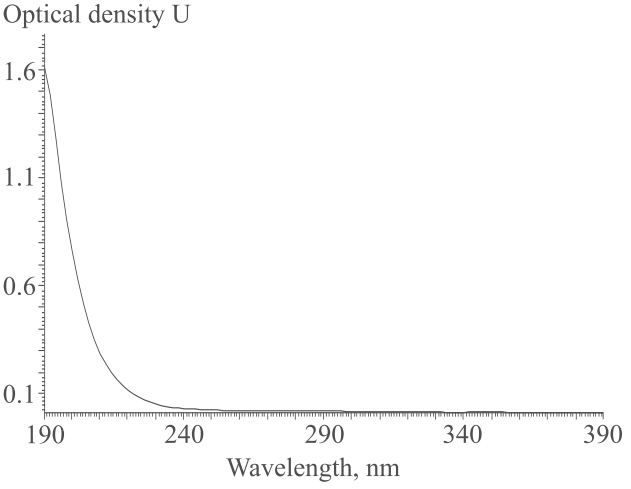

Fig. 2. UV absorption spectra of 1% aqueous solution of OD_c.

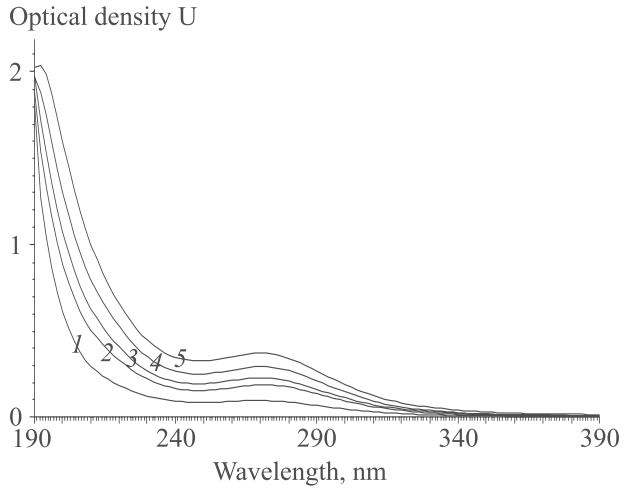

Fig. 3. Dependence of UV absorption spectra of 1% aqueous solution of OD_r on the ionizing radiation dose. 10 (*1*), 20 (*2*), 30 (*3*), 40 (*4*), and 50 kGy (*5*).

By contrast, they are present after radiochemical oxidation of dextran. This conclusion was derived from increasing optical density of aqueous dextran solutions with the increase in the radiation absorbed dose from 10 to 50 kGy at 240-290 nm (Fig. 3). The appearance of peroxide groups in irradiated dextran and their degradation by the free radical mechanism were described previously [4].

Our results indicate that OD_c has high biocompatibility and can be used as the matrix for targeted transport of biologically active substances into various target cells.

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