PHARMACOLOGY AND TOXICOLOGY

Mechanisms of ^{125}I-Omnipaque Interactions with Rat Mononuclear Phagocytes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 128, No. 10, pp. 400-402, October, 1999
Original article submitted November 23, 1998

In the absence of plasma proteins, ^{125}I-omnipaque interacts with rat isolated peritoneal macrophages due to binding to the cell surface which does not depend on temperature and duration of incubation. In the presence of serum albumin and γ-globulin the binding increases 10-fold, apparently due to phagocytosis of protein-xenobiotic complexes.

Key Words: *x-ray contrast agents; omnipaque; isolated peritoneal macrophages*

Modern x-ray contrast agents (RCA) can cause transitory side effects [2,8] due to their interactions with plasma proteins and blood cells.

We investigated the mechanisms of RCA (^{125}I-omnipaque) interactions with isolated peritoneal macrophages (IPM) — the first line of body defense [7].

MATERIALS AND METHODS

IPM from outbred albino male rats weighing 170-210 g were used. For isolating IPM, 20-30 ml of cold (4°C) Hanks' solution was injected into the abdominal cavity, after which the abdomen was massaged for 2-3 min. Peritoneal fluid was slowly aspirated with a syringe. IPM were separated from lymphocytes by adhesion Petri dishes [4]. ^{125}I-omnipaque obtained by isotope exchange [3] was used.

Accumulation of ^{125}I-omnipaque by IPM was evaluated by the difference between the total RCA concentration in the RCA-cell suspension and its concentration in precipitated cells [6]. IPM (2×10^6 cells/ml) were incubated with RCA in a final concentration of 3 mM at 37°C for 240 min

The rate of ^{125}I-omnipaque accumulation in IPM was evaluated after 1-h incubation with RCA at 4 and 37°C. The samples contained 0.4-10 mM ^{125}I-omnipaque.

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For evaluating the concentration of ^{125}I-omnipaque in the sample (total volume 5-7 ml), RCA concentration was measured in 0.4-ml aliquot IPM suspension. For estimating the concentration of RCA bound to cells this sample was centrifuged on the cold in a K-24 centrifuge for 10 min and 1500 rpm, after which 200 μl cold dibutylphthalate was layered and centrifuged for 2 min at 3000 rpm, the supernatant and oil layer were carefully discarded [7]. The content of ^{125}I-omnipaque in the samples was evaluated by precipitate radioactivity measured a Gamma-1 counter.

Each value in ^{125}I-omnipaque accumulation curve is the mean of 5-6 experiments. Standard methods of statistical analysis were used. The significance of differences was assessed by Wilcoxon—Mann—Whitney nonparametrical test.

RESULTS

The time course of accumulation of ^{125}I-omnipaque by IPM (total concentration 3 mM) in a standard Hanks' solution containing serum albumin and γ-globulin in physiological concentrations (0.62 and 0.06 mM, respectively) was studied. The viability of IPM during 4-h incubation decreased by no more than 30% (Fig. 1). The incubation was carried out at 37°C (pH 7.4). Absorption of ^{125}I-omnipaque molecules by IPM started immediately after addition of RCA into the medium.

0007-4888/99/0010-1002$22.00

Further 4-h incubation in Hanks' solution only little increased the content of labeled RCA in IPM (by no more than 17%).

It is believed that no active transport in cell membranes occurs at 4°C [1]. However accumulation of ^{125}I-omnipaque by IPM in Hanks' solution did not depend on the temperature and had no saturating component as the total concentration of RCA increased to 5 mM (Fig. 2).

Accumulation of ^{125}I-omnipaque by IPM in Hanks' solution was characterized by apparent constant of RCA diffusion by the concentration gradient K=0.71± 0.14 μM $^{-1}$ and apparent maximum diffusion rate V_{max}=3.1±0.6 μmol/h, which were calculated using the Linewcaver—Burk coordinates.

Therefore, in the absence of proteins ^{125}I-omnipaque poorly penetrated into IPM. Presumably this was common adsorption on IPM surface followed by slow penetration of RCA molecules into the cells. These processes little depend on the time and temperature of incubation. In the presence of serum albumin IPM absorption of ^{125}I-omnipaque monotonously increased and significantly (p<0.05) differed from that in Hanks' solution starting from the 20th min of incubation, 10-fold surpassing the control by the 240th min. This accumulation was most probably due to phagocytosis of serum albumin and associated RCA molecules by IPM. Similar increase in IPM-associated RCA is observed in γ-globulin solution (no significant differences from the values recorded during incubation of IPM with serum albumin and ^{125}I-omnipaque). Since the concentration of γ-globulin is by an order of magnitude lower than albumin concentration, it is correct to presume that phagocytosis of γ-globulin-bound ^{125}I-omnipaque by IPM is additionally enhanced due to the presence of immunoglobulin receptors on IPM membrane.

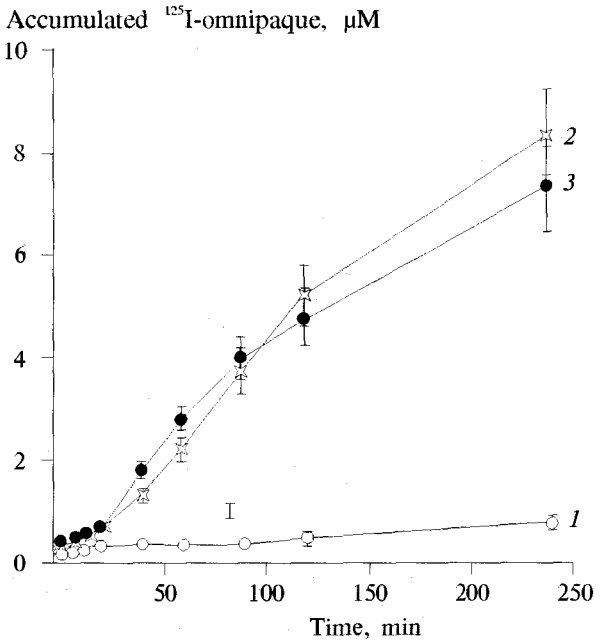

Fig. 1. Time course of accumulation of ^{125}I-omnipaque (3 mM) by isolated peritoneal macrophages (2×10^6 cells/ml) in Hanks' solution (*1*) containing 0.62 mM serum albumin (*2*), or 0.06 mM γ-globulin (*3*). *p<0.05 compared to *1*.

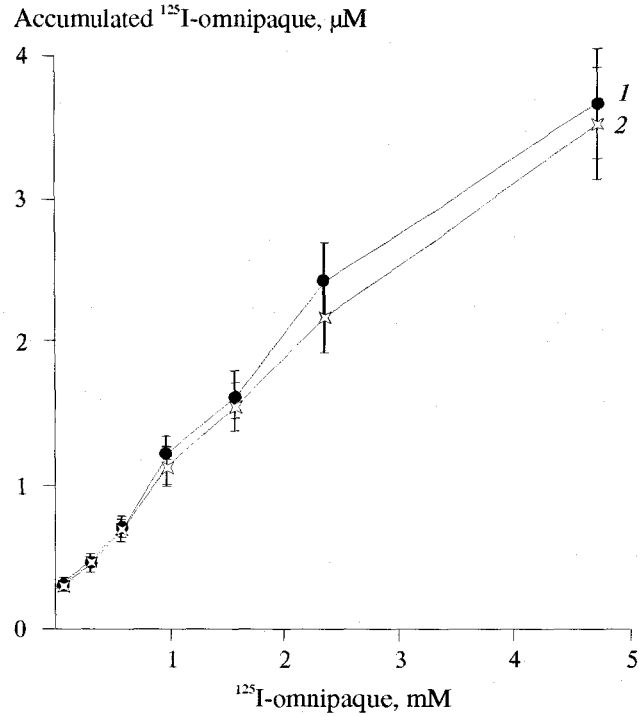

Fig. 2. Relationship between accumulation of ^{125}I-omnipaque by isolated peritoneal macrophages (2×10^6 cells/ml) and its concentration in Hanks' solution after 60-min incubation at 37°C (*1*) and 4°C (*2*) (pH 7.4).

Hence, RCA interacts with IPM either via simple adsorption on IPM the membrane followed by penetration into the cell, or via phagocytosis of protein-RCA complexes. It can be hypothesized that adsorption of RCA on IPM surface and, still more so, their penetration into the cell can stimulate secretory activity of IPM and their participation in the immune response. Products synthesized by IPM (plasminogen activator, kallikrein, anaphylatoxins, *etc.*) promote histamine release, activation of the complement system [5,10], and can mediate side allergic reactions to RCA.

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