

# EFFECT OF x-RAY CONTRAST AGENTS ON HUMAN BLOOD PLASMA INTERLEUKIN-2 LEVEL IN VITRO

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The mechanisms of the toxic effects of x-ray contrast agents (RCA), associated with anaphylactoid reactions, are being studied intensively at the present time world-wide [7, 9-11]. However, there is no information in the literature on a possible direct effect of RCA on the immune system.

The aim of this investigation was to study in vitro the effect of RCA on human blood levels of interleukin-2 (IL-2), a central mediator of the immune system, leading to clonal or polyclonal activation of T lymphocytes [8].

## EXPERIMENTAL METHOD

Heparinized blood from healthy blood donors (200 U heparin/ml) of the experimental series was incubated with 50% bilignost (iodipamide), 76% triombrast, and 80% iodamide-380 (M. V. Lomonosov Kiev Pharmaceutical Chemical Factory) in final concentrations of  $2.5 \cdot 10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$  M at 37°C for 10 min. Blood samples from healthy donors, incubated with physiological saline iso-osmotic with RCA, under the same conditions constituted the control. The blood plasma collected after incubation with RCA (3000 rpm, 10 min) was kept at -25°C for not more than 4 weeks. IL-2 in the samples of plasma was determined by radioimmunoassay, using a standard interleukin-2 (IL-2) Reagent Pack for RIA, code: 1 M. 1971, obtained from Amersham International plc. The experimental results were subjected to statistical analysis by the nonparametric inversion (V) test of Wilcoxon-Mann-Whitney [1]. The results were calculated on the EMG 666/B computer (Hungary).

## EXPERIMENTAL RESULTS

Bilignost, triombrast, and iodamide, in all concentrations tested during incubation with blood, caused an increase in the plasma IL-2 concentration in the experimental groups of donors (Table 1).

A characteristic feature of the action of RCA on the IL-2 concentration in the present investigation was that the RCA caused effects of different magnitude in different individuals. After construction of ordered series of experimental values obtained from each donor for the experimental series some of the results were comparable with the control, others differed (IL-2 was not present in the donors blood plasma before incubation with RCA). It was

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TABLE 1. Plasma IL-2 Concentration of Healthy Donors after Incubation for 10 Min with RCA ( $M \pm m$ )

Preparation	Concentration, M	Quantity of IL-2 in plasma, mg/ml		Number of donors in experimental groups	"Sensitive" individuals, %
		experimental groups of donors	"sensitive" human subjects		
Bilignost	$2.5 \cdot 10^{-2}$	$1.05 \pm 0.33^*$	$2.3 \pm 0.33^*$	12	50
	$2.5 \cdot 10^{-3}$	$1.02 \pm 0.28^*$	$1.9 \pm 0.13^*$	13	54
	$2.5 \cdot 10^{-4}$	$0.21 \pm 0.06^*$	$0.4 \pm 0.06^*$	15	53
Triombrast	$2.5 \cdot 10^{-2}$	$1.02 \pm 0.29^*$	$1.9 \pm 0.18^*$	13	54
	$2.5 \cdot 10^{-3}$	$0.45 \pm 0.14^*$	$0.9 \pm 0.15^*$	14	50
	$2.5 \cdot 10^{-4}$	$0.34 \pm 0.07^*$	$0.5 \pm 0.06^*$	16	69
Iodamide	$2.5 \cdot 10^{-2}$	$1.58 \pm 0.47^*$	$3.6 \pm 0.16^*$	16	44
	$2.5 \cdot 10^{-3}$	$1.25 \pm 0.39^*$	$2.7 \pm 0.06^*$	13	46
	$2.5 \cdot 10^{-4}$	$0.3 \pm 0.11^*$	$0.7 \pm 0.12^*$	14	43

**Legend.** Asterisk indicates that values of experimental group differ statistically differently from control with a probability  $p(H_0) < 0.01$ , where  $H_0$  is the statistical hypothesis of the existence of differences in the distribution of values obtained in experimental and control groups of donors.

thus suggested that the RCA under investigation lead to an increase in the IL-2 concentration on incubation with blood obtained only from a certain number of donors. We proved by the use of nonparametric statistical tests for calculating the results that it is useful to divide individuals into those "sensitive" and "tolerant" to the action of RCA. This enabled the magnitude of the effect induced by the RCA to be determined more accurately, and the percentage of "sensitive" individuals to be obtained (Table 1). Of the preparations studied, iodamide in a concentration of  $2.5 \cdot 10^{-2}$  M had the greatest effect, bilignost and triombrast in a concentration of  $2.5 \cdot 10^{-4}$  M had the least effect. The intraspecific sensitivity was characteristic of the response of man and animals to injection of RCA. Humans and Wistar rats differ in their individual sensitivity toward the histamine-releasing and complement-activating action of RCA, but do not, however, react by a change in circulating immune complexes [2-6, 9]. The percentage of sensitive human subjects, moreover, was the same (about 50).

The increase in the plasma IL-2 concentration during incubation with the test RCA is dependent on and directly proportional to dose. An increase in concentration of RCA leads to increased release of IL-2 from the competent blood cells, obtained from "sensitive" donors (Table 1). The dose-dependent effect characteristic of RCA must be taken into account if RCA are to be used repeatedly, for changes in the biochemical and physiological characteristics of the patient arise most frequently during repeated administration of the contrast agent. It was iodamide and not bilignost or triombrast that had the greatest effect on the plasma IL-2 level. Depending on the effect of RCA on degranulation of mast cells, on activation of complement, and on a change in concentration and size of immune complexes [2-6, 9], they can be arranged in the following order: bilignost > iodamide > triombrast. These results are not contradictory, for they confirm our previous conclusion that the action of RCA on the body is highly selective and depends on the chemical structure and/or their physicochemical properties [4, 10].

Elevation of the IL-2 level 10 min after addition of RCA cannot be due to stimulation of IL-2 synthesis de novo, for this process lasts 2 h [8], but it is most likely to be connected with the direct action of RCA molecules on T lymphocytes.

The discovery of the stimulating action of RCA on lymphocytes indicates the need for additional research, which must help to reveal the mechanism of the effect of RCA on immune reactions and its role for the patient. In our opinion there is an urgent need for a more detailed study of the effect of RCA on other mediators of the immune system, and for the degree of reaction of immunocompetent cells to be determined under the influence of RCA in human subjects and experimental animals.

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