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EFFECT OF RADIOGRAPHIC CONTRAST MEDIA ON THE COMPLEMENT SYSTEM IN RATS

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Modern radiographic contrast media (RCM), despite their high diagnostic value, frequently induce side effects which, in some cases, require the implementation of urgent resuscitation measures [5]. An important role in the pathogenesis of the allergic and anaphylactoid reactions which present the greatest risk of administration of RCM, is played by activation of the complement system [6, 8, 11, 12]. Siegel and coworkers [13, 14] and Lasser and coworkers [10] have shown that during radiographic contrast studies in patients sensitive to RCM, the hemolytic activity of complement falls much lower than in insensitive subjects. It is therefore a matter of urgent importance to develop a simple test of sensitivity to RCM, based on preliminary assessment of their effect on the complement system, in small volumes of blood serum in vitro. The possibility cannot be ruled out that one such test may be determination of complement activation by an alternative method [2].

The aim of this investigation was to study activation of the complement system in rats by 50% iodipamide (methylglucamine salt), 76% triombrast, and iodamide-380, by an alternative method in vitro and in vivo.

EXPERIMENTAL METHOD

Experiments were carried out on 280 Wistar rats weighing 150-200 g, kept on the standard animal house diet. The animals were divided randomly into control and experimental groups [3], each consisting of not less than 10 animals.

The experiment consisted of three series. In series I rats of the experimental groups received an injection of the RCM into the caudal vein: 50% iodipamide, 76% triombrast, and 80% iodamide-380 (from the M. V. Lomonosov Kiev Pharmaceutical Chemical Factory), in doses of 1, 2, and 15 ml/kg body weight. Animals of the control group received an intravenous injection of physiological saline (37°C). The rate of injection was 0.1 ml/sec. The animals were killed by decapitation under superficial ether anesthesia after 5-10 min, their blood was allowed to stand (at 4°C) and serum was obtained by centrifugation (400g, 8 min). In experiments of series II serum was obtained from the experimental animals after incubation of the test RMC with the rats' blood (37°C, 10 min). In the experiments of series III the test RMC were incubated with serum from the experimental animals (37°C, 10 min). The concentration of RMC in contact with the blood and serum in the experiments in vitro was $2.5 \cdot 10^{-2}$-$2.5 \cdot 10^{-4}$ M. Intact rat serum served as the control for the experiments in vitro.

The effectiveness of activation of complement by the alternative method in the animals' blood serum (0.1 ml) was determined as the degree of hemolysis of rabbit's red blood cells

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TABLE 1. Effect of RCM in Vivo and in Vitro on Hemolysis of RRBC Induced by Rat Serum (M ± m)

Preparation	Incubation of RRBC with blood serum obtained 10 min after intravenous injection of test RCM into rats				Incubation of RRBC with rat serum obtained from blood of animals after exposure to test RCM*			
	Dose, g/kg	degree of hemolysis of RRBC in serum from rats of experimental groups, % (control – 100%)	p (Ho) U	degree of hemolysis of RRBC in serum of "sensitive" rats, % (control – 100%)	dose, moles/liter	degree of hemolysis of RRBC in serum from rats of experimental groups, % (control – 100%)	p (Ho) U	degree of hemolysis of RRBC in serum of "sensitive" rats, % (control – 100%)
Iodipamide	0,67	85,95±2,57 ($n = 10$)	$p = 0{,}001$	84,45±2,33** ($n = 9$)	$2{,}5 \cdot 10^{-3}$	63,52±8,19 ($n = 10$)	$p < 0{,}005$	47,88±2,47** ($n = 7$)
	1,33	72,16±8,40 ($n = 10$)	$p < 0{,}05$	51,85±1,84** ($n = 6$)	$2{,}5 \cdot 10^{-2}$	25,23±10,92 ($n = 12$)	$p < 0{,}005$	9,17±1,46** ($n = 10$)/
Iodamide	1,13	103,27±1,25 ($n = 10$)	$p > 0{,}05$		$2{,}5 \cdot 10^{-3}$	98,89±1,75 ($n = 10$)	$p > 0{,}05$	
	2,27	80,50±5,52 ($n = 10$)	$p < 0{,}05$	67,35±2,19** ($n = 6$)	$2{,}5 \cdot 10^{-2}$	65,19±7,80 ($n = 10$)	$p < 0{,}005$	50,25±2,57** ($n = 7$)
Triombrast	1,06	96,85±1,96 ($n = 10$)	$p > 0{,}05$		$2{,}5 \cdot 10^{-4}$	104,07±2,31 ($n = 10$)	$p > 0{,}05$	
	2,11	84,99±3,77 ($n = 10$)	$p < 0{,}01$	78,34±2,41** ($n = 7$)	$2{,}5 \cdot 10^{-3}$	95,93±2,84 ($n = 10$)	$p > 0{,}05$	
	15,83	45,74±8,13 ($n = 15$)	$p < 0{,}001$	32,30±4,74** ($n = 12$)]	$2{,}5 \cdot 10^{-2}$	68,15±6,35 ($n = 10$)	$p < 0{,}005$	59,26±2,80** ($n = 8$)

Legend. *) Similar results were obtained in a study of the degree of hemolysis of RRBC in rat serum after incubation with test RCM, **) values differ statistically significantly from control with probability of $p < 0.01$ by U test. p (Ho) U) Probability of hypothesis that differences exist in distributions of values of D_{540} for animals of experimental and control groups.

(RRBC) [7]. The serum was diluted with medinal buffer (4°C), 0.1 ml of washed RRBC ($5 \cdot 10^9$ cells/ml) was added, and the suspension was incubated in a water thermostat (37°C, 60 min). The reaction was stopped by the addition of cold buffer (2 ml). The degree of hemolysis of the RRBC was determined as the optical density (D) of the supernatant of the samples (200g, 3 min, 4°C) on an SF-26 spectrophotometer (LOMO, USSR), at a wavelength of 540 nm (D_{540}).

The value of D_{540} of the experimental samples was calculated relative to the optical density of the supernatant of RRBC, obtained after centrifugation of the red cells in cold medinal buffer.

To determine the suitability of the method, the modifying action of the RCM or RRBC was studied. The red cells were incubated with the RCM preparations, diluted with medinal buffer, under the same conditions but without serum.

The experimental data were analyzed by Student's t test, by the Wilcoxon–Mann-Whitney U inversions test, and the sequential r test (Wald and Wolfowitz) [1].

The results were processed on the EMG 666/B computer (Hungary).

EXPERIMENTAL RESULTS

The RCM in all doses, except iodamide and triombrast in a dose of 1 ml/kg, in the experiments in vivo (Table 1) reduced the ability of the rat serum to induce hemolysis of RRBC. Since the addition of RCM to the RRBC suspension in a concentration of 10^{-2}-10^{-4} M did not increase the values of D_{540} of their supernatant by more than 5-7% ($p < 0.001$ according to the U test) it can be concluded that iodipamide in all the doses tested and triombrast and iodamide in a dose of 2 ml/kg induced activation of the complement system (ACS) by an alternative method.

The forms of distribution of values of D_{540} in animals of the experimental groups, which showed a statistically significant decrease in the degree of hemolysis of RRBC compared with the control, were bimodal, whereas in the control groups they were monomodal. Accoridng to the r test differences in the shape of the distributions between these experimental and control groups were significant with a probability of $p \leq 0.025$. Values of D_{540} in the experimental groups must therefore be separated into two independent distributions.

These groups were separated in accordance with the following rule. Values of D_{540} were chosen from the ranked variables of the experimental groups and pooled until the mean value ($\bar{x}$) of this sample was below the value of $\bar{x}$ of values of D_{540} of the control animals (the last value was discarded). Animals with a value of D_{540} of this group constituted the "sensitive" sample, whereas animals with a value of $\bar{x}$ (D_{540}) which was not less than $\bar{x}$ of the control group constituted the "tolerant" sample. To prove the heterogeneity of the distributions in the experimental animals the U test was used. All the "sensitive" samples were found to differ significantly ($p < 0.01$) with respect to the distribution of D_{540} in the control group. Meanwhile the tolerant samples had no significant differences in the distribution of their D_{540} values compared with the control group (Table 1). It can accordingly be accepted that "sensitive" and "tolerant" animals relative to RCM do in fact exist with respect to their effect on complement system.

Complement is an important parameter of toxicity of RCM, as will be clear from the results obtained by injection of a sublethal dose of triombrast (15.83 g/kg). In some animals of the "sensitive" sample, which died immediately after injection of the medium, complement in the serum was activated more strongly than in other animals of the "sensitive" sample ($p = 0.05$ by Student's t test). Experiments with rat blood and serum in vitro also showed that RCM can induce ACS by the alternative method (Table 1) in vitro also, but in some cases the RCM was not always effective, in agreement with the conclusion regarding the existence of animals "tolerant" and "sensitive" to RCM. On the basis of the results obtained in vivo and in vitro, it can be tentatively suggested that small volumes of blood serum (0.3 ml) can be used to determine the sensitivity of animals to this effect of RCM.

It will be clear from Table 1 that the number of "sensitive" animals covers a wide range – from 30 to 90%. Simon and coworkers [15] observed activation of complement previously in 63% of patients after undergoing intravenous urography. Bonsette and coworkers [6] observed a change in the activity of complement after injection of RCM in 8-42% of patients. According to our results, activation of complement through the action of RCM is dose-dependent in character. With an increase in the dose of RCM there is an increase in the degree of ACS. This is a fact of definite clinical importance. We know that an increase in the dose of radiographic contrast media increases the number of side effects and their severity. The preparations which we studied can be arranged in the following order of their activating effect on ACS: iodipamide > iodamide > triombrast. Dependence of the effect on the complement system on the type of RCM was demonstrated by Land et al. [9], Siegle et al. [14], and Freyria et al. [4]. It is interesting to note that correlation exists between the effectiveness of action of RCM on the complement system and their lipid solubility (the partition coefficient in octanol/butanol versus water) [6].

It can be concluded from these results that RCM can activate the complement system by an alternative method. By means of the method of determination of ACS suggested above it is possible to determine the sensitivity of an individual to RCM in an aliquot of blood. The next problem to be tackled is therefore to carry out clinical trials of this test.

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