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Comparative *In Vitro* Study of the Effect of Contrast Media on Complement Activity and Eicosanoid Content in Rat Blood

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The study compares *in vitro* effect of different contrast media on complement activity and eicosanoid content. Ionic agents (Bilignost>Iodamide>Triombrast>Hexabrix) exert pronounced complement-activating effect, while nonionic agents markedly increase blood content of arachidonic acid metabolites. The complement-activating effect of contrast media did not correlate with their ability to elevate blood content of prostaglandin $F_{2\alpha}$ and leukotrienes C_4 and B_4.

Key Words: *contrast media; complement system; prostaglandin $F_{2\alpha}$; leukotriens C_4 and B_4*

We have previously demonstrated that the complement system (CS) and eicosanoids: prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and leukotrienes C_4 and B_4 (LTC_4 and LTB_4) contribute to the development of systemic side effects of contrast media (CM) [5,6]. Complement modulates the release of arachidonic acid metabolites through the plasma membrane receptors. On the other hand, some cell populations, in particular macrophages, secrete CS components and eicosanoids upon activation [2,3,7]. Prevention of anaphylactoid reactions should be based on the understanding of the relationships between these transmitters of side reactions during simultaneous CM-induced activation of CS and upsurge of blood eicosanoids.

In the present study we compared the effects of CM on the alternative pathway of CS activation and on the contents of $PGF_{2\alpha}$, LTC_4, and LTB_4 *in vitro*.

MATERIALS AND METHODS

Experiments were carried out on 300 Wistar rats of both sexes (body weight 100-150 g) maintained on standard vivarium diet. The rats were randomly assigned to control and experimental groups 12 animals in each. The rats were decapitated under light ether narcosis; blood was incubated (37°C, 10 min) in the presence of 3.0×10^{-5}-3.0×10^{-2} M CM or isoosmotic physiological saline. Serum was isolated by centrifugation for 8 min at 400*g* and 4°C.

Hemolytic activity of the alternative complement pathway was assayed according to T. Hidvegy *et al.* [5,6], the content of $PGF_{2\alpha}$ was measured by radioimmunoassay using ^{3}H-Prostaglandin $F_{2\alpha}$ RIA Kit (DIRECT), Code: HTK-3 (Hungarian Academy of Sciences), LTC_4 and LTB_4 using Leukotriene C_4 and B_4 specific [^{3}H] assay systems (Code TRK 905 and TRK 940, respectively, Amersham).

The data were processed statistically using nonparametric Wilcoxon—Mann—Whitney *U* test and serial Wald-Volfowitz *r* test. Mathematical approach to separating animals by their sensitivity to RA was described previously [4-6].

The following CM were used: 76% Triombrast-370 and 80% Iodamide (monomeric ionic CM, Farmak), 80% Peritrast-400 (modern ionic monomeric CM, Dr. Kohler Chemic), 50% Bilignost (dimeric ionic CM for cholecystography, Farmak), Hexabrix-

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320 (dimeric ionic CM for angiourography, Byk Gulden), and nonionic monomeric CM Omnipaque-300 (Nycomed) and Ultravist-370 (Schering).

RESULTS

Bilignost in concentrations 3×10^{-4}, 3×10^{-3}, and 3×10^{-2} M activated the alternative complement pathway CS by 31, 55, and 86%, respectively (Table 1). Iodamide and Triombrast were effective in two and other CM only in one maximum concentrations (Table 1).

Bilignost, Omnipaque, and Ultravist in all concentrations increased the content of $PGF_{2\alpha}$ (Table 2): in the presence of nonionic CM and Bilignost this parameter attained 110-130% and 120-140% of the control, respectively. The content of LTB_4 significantly increased by 20-85% in the presence of Ultravist in all concentrations and by 25-45% in the presence 3×10^{-4}, and 3×10^{-2} of Omnipaque and by about 40% in the presence of Iodamide and Bilignost in maximum concentrations. The content of LTC_4 increased only in the presence of nonionic CM. Ultravist increased the content of LTC_4 in concentrations 3×10^{-2} and 3×10^{-3} M.

These data suggest that CM produce different effects on CS activity and blood content of eicosanoids *in vitro*. The ability of CM to activate CS decreased in the following order: Bilignost>Iodamide>Triombrast>Hexabrix>Omnipaque=Ultravist> Peritrast ($p(Ho)_t \leq 0.025$). Similarly ($p(Ho)_t \leq 0.025$ or $p(Ho)_U < 0.01$), their ability to increase the content of $PGF_{2\alpha}$ and leukotrienes decreases in the following order: Bilignost>Omnipaque=Ultravist for $PGF_{2\alpha}$,

TABLE 1. Activation of Alternative Pathway of the Complement System (%) after Incubation of Blood Samples with CM (*M*±*m*)

Agent	Concentration, M	Sensitive rats	Tolerant rats
Bilignost	3×10^{-2}	14.3±0.7*	95.3±6.1
	3×10^{-3}	45.6±2.0*	109.4±12.8
	3×10^{-4}	68.5±8.7*	100.6±9.1
	3×10^{-5}	—	107.8±6.2
Hexabrix	3×10^{-2}	60.1±4.2*	91.4±6.9
	3×10^{-3}	—	109.1±3.8
	3×10^{-4}	—	114.6±9.9
	3×10^{-5}	—	101.4±4.3
Iodamide	3×10^{-2}	47.1±11.2*	96.6±2.5
	3×10^{-3}	79.6±2.2*	95.1±18.0
	3×10^{-4}	—	109.3±3.4
	3×10^{-5}	—	108.4±6.3
Omnipaque	3×10^{-2}	74.3±6.7*	92.4±4.0
	3×10^{-3}	—	90.6±14.9
	3×10^{-4}	—	123.2±11.0
	3×10^{-5}	—	109.5±5.0
Ultravist	3×10^{-2}	79.0±1.3*	103.7±0.4
	3×10^{-3}	—	112.2±8.2
	3×10^{-4}	—	89.6±19.9
	3×10^{-5}	—	94.2±6.9
Peritrast	3×10^{-2}	88.6±2.2*	100.1±17.1
	3×10^{-3}	—	109.2±0.1
	3×10^{-4}	—	91.6±17.5
	3×10^{-5}	-	107.5±8.6
Triombrast	$3*10^{-2}$	58.6±5.9*	114.5±9.6
	$3*10^{-3}$	90.7±0.4*	105.4±18.7
	$3*10^{-4}$	—	88.0±10.3
	$3*10^{-5}$	—	101.4±9.6

Note. Control is taken as 100%. *$p<0.01$ in comparison with the control (*U* test).

TABLE 2. Content of $PGF_{2\alpha}$, LTB_4, and LTC_4 (%) in the Serum of Sensitive Rats after Incubation with CM (*M±m*)

Agent	Concentration, M	$PGF_{2\alpha}$	LTB_4	LTC_4
Bilignost	3×10^{-2}	234.7±11.3*	137.4±6.2*	109.6±8.4
	3×10^{-3}	174.8±1.4*	105.0±6.9	100.7±3.9
	3×10^{-4}	144.7±6.1	105.9±5.1	89.9±7.4
	3×10^{-5}	123.4±6.5*	98.1±8.9	102.5±4.9
Iodomide	3×10^{-2}	109.6±5.8	144.3±7.1*	92.3±3.8
	3×10^{-3}	101.4±11.6	103.9±8.2	99.7±11.4
	3×10^{-4}	123.6±11.8	90.6±6.2	98.3±6.5
	3×10^{-5}	90.4±7.2	105.5±4.2	96.6±7.5
Omnipaque	3×10^{-2}	127.7±8.0*	126.7±3.9*	369.3±3.8*
	3×10^{-3}	115.2±8.1*	127.3±3.9*	285.1±2.7*
	3×10^{-4}	130.8±5.5*	145.4±6.1*	142.9±6.0*
	3×10^{-5}	114.9±7.4*	107.4±3.9	94.1±4.2
Ultravist	3×10^{-2}	121.4±7.1*	153.6±4.0*	205.7±3.9*
	3×10^{-3}	119.2±4.0*	171.2±4.9*	164.9±6.9*
	3×10^{-4}	119.8±5.0*	185.7±7.3*	113.2±5.3
	3×10^{-5}	113.3±0.9*	120.5±5.0*	103.7±1.4

Note. Serum content of $PGF_{2\alpha}$ in experimental rats varied from 9.4 to 16.3 ng/100 μl, LTB_4 from 1.9 to 3.0 ng/100 μl, and LTC_4 from 10.8 to 23.7 ng/100 μl. *$p<0.025$ compared with the control (100%, *r* test).

Ultravist>Omnipaque>Bilignost=Iodamide for LTB_4, and Omnipaque>Ultravist for LTC_4. Triombrast, Peritrast, and Hexabrix had no effect on blood eicosanoid content. Moreover, the content of $PGF_{2\alpha}$ in the presence of Iodamide did not differ from the control. Neither Iodamide, nor Bilignost affected the content of LTC_4. Thus, none CM had simultaneous effect on the CS and eicosanoid content. These findings suggest that stimulation of eicosanoid secretion did not depend on the CS and resulted from direct effect of CM on cells, while activation of the CS did not depend on blood cells and resulted from direct interaction between CM and CS protein components. Contrast media have selective effect on blood cells: in our experiments some CM selectively increased the content of a certain eicosanoid subtype. Hence, the rise in the content of LTB_4 but not LTC_4 and $PGF_{2\alpha}$ in the presence of Iodamide can be attributed to the fact that it predominantly activates LTB_4-producing eosinophils, polymorphonuclear leukocytes, and lymphocytes and has no effect on monocytes and macrophages synthesizing primarily LTC_4 and $PGF_{2\alpha}$ [2].

These findings suggest that CM-induced activation of the CS and the enhanced release of eicosanoids are independent processes. They are not successive stages in the pathogenesis of CM-related adverse effects and can simultaneously occur in the organism. Complement is activated by ionic rather than nonionic CM. The content of cyclooxygenase arachidonic acid metabolites ($PGF_{2\alpha}$) increases primarily in the presence of ionic dimeric high osmolar CM (Bilignost) nonionic agents (Omnipaque, Ultravist). The content of LTC_4 and LTB_4, the products of lipoxygenase enzymes, increased only in the presence of nonionic CM. It can be hypothesized that anaphylactoid reactions to ionic CM are primarily mediated via activation of the CS, while side reactions to nonionic CM are predominantly associated with eicosanoid release from tissue stores. Therefore, in patients with high risk of adverse effects to CM premedication with inhibitors of CS activation, such as blood esterase blockers (ε-aminocaproic acid) and synthetic glucocorticoids (prednisolone) is indicated before injection of ionic CM, while inhibitors of the lipoxygenase and cycloxygenase pathways of arachidonic acid metabolism (vitamin E and naproxen) should be administered prior to radiography with nonionic CM.

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