PHARMACOLOGY AND TOXICOLOGY

Changes in Blood Content of Prostaglandin $F_{2\alpha}$ and Leukotrienes C_4 and B_4 in Rats After Intravenous Injection of Contrast Agents

P. V. Sergeev, Yu. K. Napolov, E. V. Markina, E. N. Bolotova, and N. L. Shimanovskii

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It is shown that Triombrast=Hexabrix=Omnipaque≤Melitrast<Ultravist in a dose-independent manner increase the level of prostaglandin $F_{2\alpha}$ and leukotrienes C_4 and B_4 in the blood of sensitive rats (50%) 15 min after intravenous injection of the preparation, the changes in prostaglandin $F_{2\alpha}$ being maximal, while those in leukotrienes C_4 minimal. The effect of nonionic contrast agents on the blood level of prostaglandin $F_{2\alpha}$ (0.1-2.0 g I/kg, except for Omnipaque) and leukotriene B_4 (in a dose of 0.5 g I/kg) is more pronounced in comparison with the ionic preparations.

Key Words: *contrast agents; eicosanoids*

Elucidation of the mechanism of adverse reactions of contrast agents (CA) is of crucial importance for the safety of radiographic studies [3,8]. The effect of CA on various biochemical parameters (the level of histamine, serotonin, kinins, and complement components) and functioning of some cells and organs has been recently reported [3,7-10,14]. However, little is known about the effect of CA on the metabolism of arachidonic acid (AA) products, whose physiological effects [2,11] are probably responsible for clinical manifestations of side reactions to intravascular injection of CA (hypotension, bronchial constriction, edema, etc.) [12,14,15].

The aim of the present study was to determine the blood level of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and leukotrienes C_4 and B_4 (LTC_4 and LTB_4) in rats before and after intravenous injection of CA of different chemical structure.

Department of Molecular Pharmacology, Russian State Medical University, Moscow

MATERIALS AND METHODS

The study was carried out on 285 Wistar rats of both sexes weighing 150-200 g. The rats were fed a standard vivarium ration. The animals were randomly assigned to experimental and control groups [6]. Each group comprised 15 animals. Omnipaque-300 (Nycomed), Melitrast-300 (Dr. Kohler Chemie), Triombrast 76% (Farmak, Ukraine), Ultravist-300 (Schering), and Hexabrix-320 (Byk Gulden) heated to 37°C were injected in doses of 0.1, 0.5, 1.0, and 2.0 g I/kg and at a rate of 0.1 ml/sec into the caudal vein. Control rats received an injection of physiological saline (37°C). The animals were decapitated under light ether anesthesia. Blood was allowed to stay at 4°C, and serum was prepared by centrifugation at 400*g* for 8 min.

$PGF_{2\alpha}$ was measured by radioimmunoassay using a ^{3}H-Prostaglandin $F_{2\alpha}$ RIA kit (Direct), Code: HTK-3 (Hungarian Academy of Sciences); LTC_4 and LTB_4 were measured using monoclonal antibodies and

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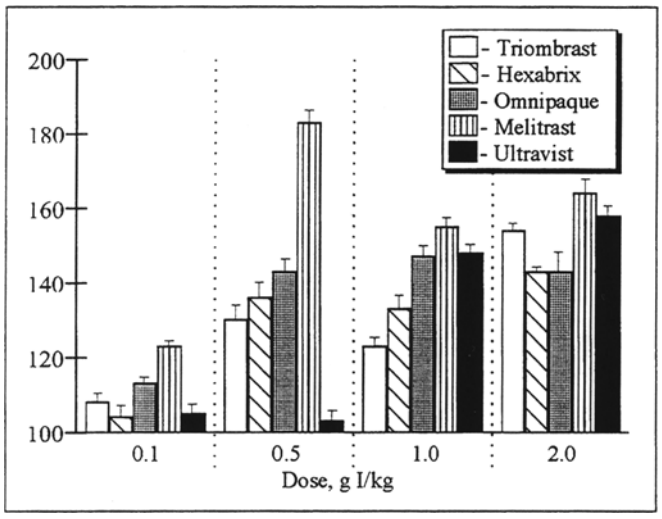

Fig. 1. Blood level of prostaglandin $F_{2\alpha}$ in sensitive Wistar rats 10 min after an intravenous injection of contrast agents (*M±m*). Differences from the control are significant at $p<0.05$ (*U* test). Ordinate: content of prostaglandin $F_{2\alpha}$, %. Control values (4.0-7.5 pg/100 μl plasma) taken as 100%.

Leukotriene C_4 specific [^{3}H] assay system, Code TRK 905 and Leukotriene B_4 [^{3}H] assay system, Code TRK 940 (Amersham Int.).

By chemical structure, Omnipaque, Ultravist, and Melitrast are monomeric nonionic preparations of iohexol, iopromide, and iosarcole, respectively; Triombrast is a mixture of sodium and meglumine diatrizoate; Hexabrix is a mixture of sodium and meglumine ioxaglate.

The data were processed statistically using Wilcoxon—Mann—Whitney *U* test, Wald—Wolfowitz *r* test [1], and the method of least squares [5].

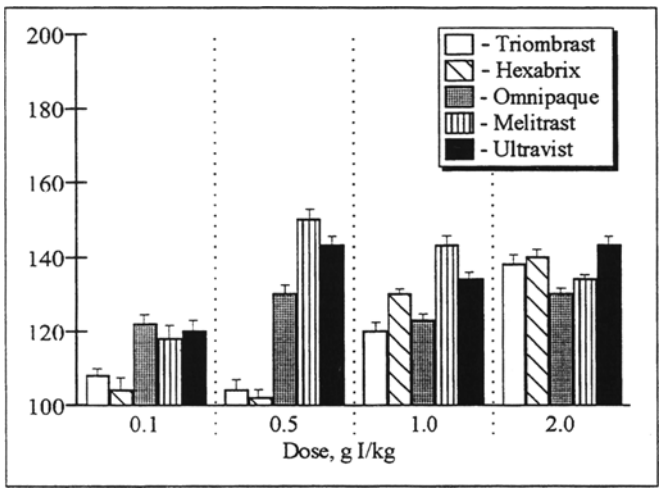

Fig. 2. Blood level of leukotriene B_4 in sensitive Wistar rats 10 min after intravenous injection of contrast agents (*M±m*). Differences from the control are significant at $p<0.05$ (*U* test). Ordinate: content of leukotriene B_4, %. Control values (16-18 pg/100 μl plasma) taken as 100%.

RESULTS

Figures 1-3 illustrate the effect of intravenous infusion of the studied CA on the blood level of $PGF_{2\alpha}$, LTC_4 and LTB_4. Ten minutes after injection of CA, blood content of $PGF_{2\alpha}$, a product of the cyclooxygenase pathway of AA metabolism, as well as those of LTC_4 and LTB_4, products of lipoxygenase enzymes increased. Thus, our experiments confirmed previous data on the modulating effect of CA on the metabolism of eicosanoids [10,13,14].

Interestingly, the rise of different AA metabolites was variously expressed: the content of LTC_4 increased by about 15%, whereas those of LTB_4 and $PGF_{2\alpha}$ rose by 30 and 50%, respectively. Thus, the induction of lipoxygenase products is less pronounced than cyclooxygenase metabolites. Different cells are known to produce primarily one type of eicosanoids. For instance, $PGF_{2\alpha}$ is synthesized in macrophages and monocytes, while neutrophils, mast cells, and lymphocytes produce no or negligible amounts of this eicosanoid [2]. LTC_4, a component of so-called slowly reacting substance of anaphylaxis (SRS-A), is produced by both mast cells and macrophages [2,11]. LTB_4 is formed primarily in mast cells, neutrophils, eosinophils, lymphocytes, but not in macrophages [2] (Fig. 4). CA have unequal effects on different cell populations and, therefore, different amounts of AA metabolites are released into the blood and other biological fluids. Thus, our findings confirm the hypothesis that leukotrienes and prostaglandins mediate side reactions of CA [10,14].

In our experiments, elevation of blood eicosanoids in response to CA varied from animal to animal. Using nonparametric *U* and *r* tests and the previously described approach [3], we proved the advantage and necessity of dividing the experimental animals by their sensitivity to CA. In sensitive rats, blood levels of $PGF_{2\alpha}$, LTC_4, and LTB_4 were significantly higher than in controls (Fig. 1-4), whereas in tolerant rats these parameters did not differ from the control. The sensitive animals constituted about 50%. Similar differences were observed in our previous experiments, in which in the sensitivity of humans and animals to CA was determined using other biochemical parameters of the blood and functional activity of some cells [3,7,8]. This implies the possibility of developing test systems for determination of potential risk of side reactions to CA before radiography.

In the sensitive rats the method of least squares revealed neither direct nor reverse linear dependence between the dose of CA and the rise of AA metabolites. All tested CA injected intravenously in doses of 0.5, 1.0 and 2.0 g I/kg practically equally increased the blood level of $PGF_{2\alpha}$, LTC_4, and LTB_4.

It should be noted that the dose-effect dependence is not a universal characteristic of the influence of CA on the organism. For instance, degranulation of mast cells increases along with the dose of CA [3,8], whereas the concentration and the size of circulating immune complexes do not depend on the dose of CA [4]. This confirms the concept that CA variously affects different reactive systems of the organism and indicate different role of these systems in the pathogenesis of side reactions.

When comparing the effects of nonionic agents (Melitrast, Ultravist, and Omnipaque) and ionic (Triombrast and Hexabrix) on the blood level of the three AA metabolites, we found that the nonionic agents (except for Omnipaque) induce a more pronounced rise of $PGF_{2\alpha}$ than ionic preparations, especially in doses of 0.5 and 1.0 g I/kg. The ionic CA in doses of 0.1 and 0.5 g I/kg practically did not change the blood level of LTB_4, whereas Melitrast, Ultravist, and Omnipaque had a pronounced effect on this parameter. However, in doses of 1.0 and 2.0 g I/kg, both ionic and nonionic CA produced the same effects on the LTB_4 level (Fig. 2). A slight increase in the level of LTC_4 was noted for Ultravist and Triombrast in a dose of 1.0 g I/kg and for Ultravist, Triombrast, and Hexabrix in a dose of 2.0 g I/kg. The effects of ionic CA on this parameter did not differ from that of nonionic preparations (Fig. 3). The effect of CA on the blood level of

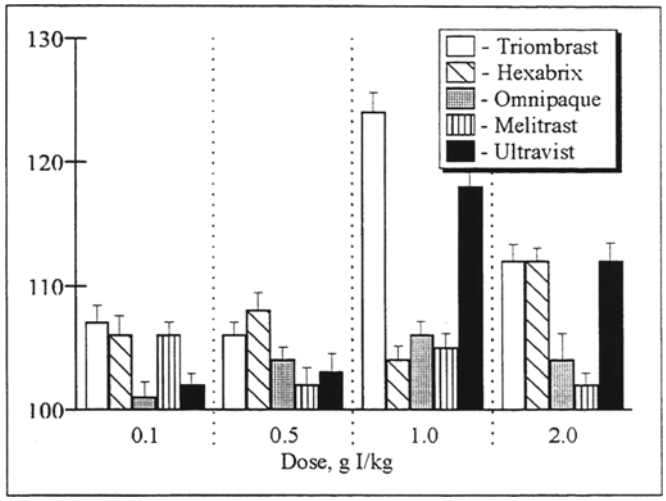

Fig. 3. Blood level of leukotriene C_4 in sensitive Wistar rats 10 min after an intravenous injection of contrast agents (*M±m*). Differences from the control are significant at $p<0.05$ (*U* test). Ordinate: content of leukotriene C_4, %. Control values (6.3±1.1 pg/100 μl plasma) taken as 100%.

$PGF_{2\alpha}$, LTC_4, and LTB_4 increases in the following sequence: Triombrast=Hexabrix=Omnipaque<Melitrast≤Ultravist. There are contradictory data on the effect of ionic and nonionic CA on the metabolism of AA. For instance, ionic CA containing ioxaglate and diatrizoate anions, but not the nonionic preparation iopamidol, suppressed *in vitro* the ADP-induced aggregation of human platelets [13]. These

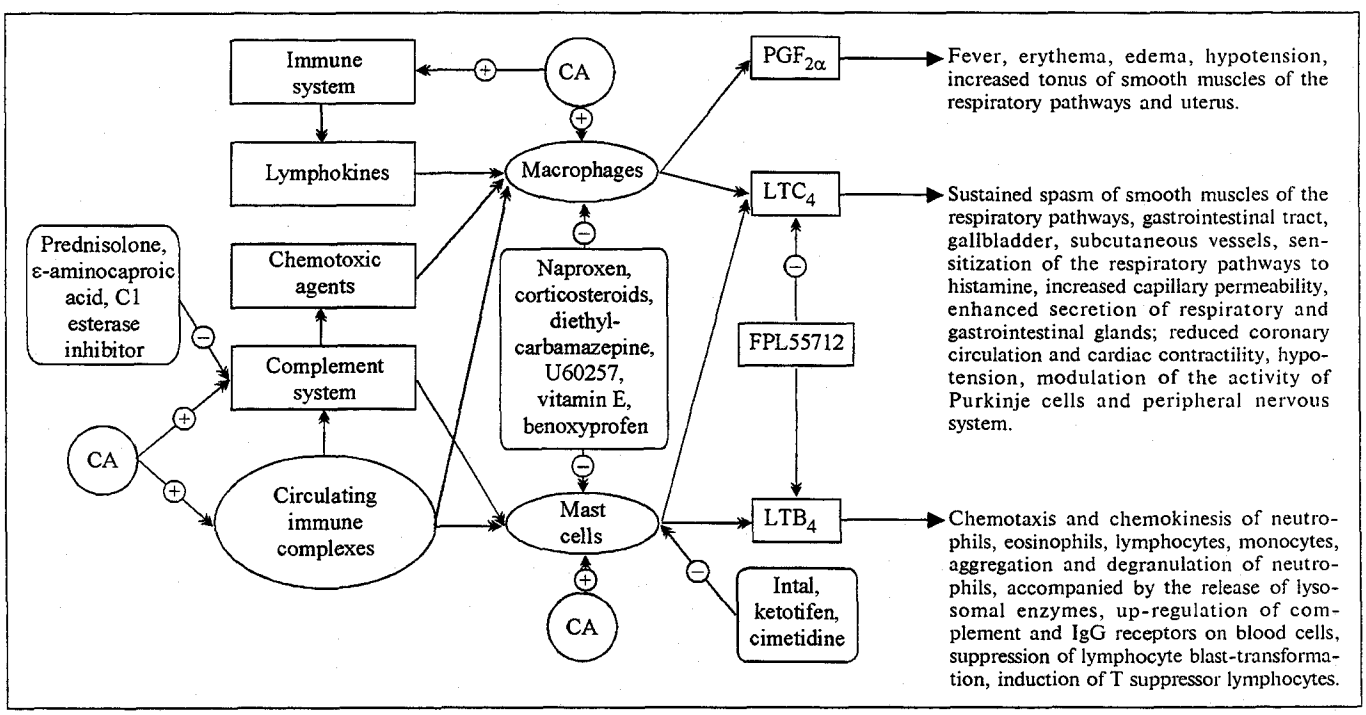

Fig. 4. Possible involvement of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and leukotrienes C_4 (LTC_4) and B_4 (LTB_4) into pathogenesis of side effects of contrast agents (CA) and their prevention.

investigators also reported that iopamidol inhibited, whereas diatrizoate stimulated, the production of all AA metabolites in isolated hamster lungs. Other researchers showed that hyperosmotic Hypaque-76 (methylglucamine-sodium diatrizoate) and hypo-osmotic Omnipaque-300 (iohexol) equally increase the blood level of prostacyclin and have no effect on thromboxane B_2 in patients after angiography of peripheral blood vessels.

Consequently, the use of new low-osmotic CA cannot guarantee against anaphylactic reactions. The role of $PGF_{2\alpha}$, LTC_4, and LTB_4 in these reactions is outlined in Fig. 4. This is also consistent with previous data [2,10,12,14]. The release of eicosanoids from mast cells and macrophages can be not only triggered by direct action of CA on these cells (chemotoxic action) but also be mediated by lymphokines (formed due to activation of the immune system), circulating immune complexes, and the complement system. We should emphasize an interplay of various biochemical systems of the organism in the formation of side reactions. This is in conformity with the concept that the adverse effects of CA cannot be attributed to a unique mechanism.

Considering the mechanism of side reactions of CA (Fig. 4), it can be suggested that the adverse effects of $PGF_{2\alpha}$, LTC_4, and LTB_4 can be effectively prevented by the following drugs: inhibitors of the complement system (prednisolone, ε-aminocaproic acid, and C1 esterase inhibitor), since chemotoxicants and anaphylotoxins formed due to activation of complement trigger the release of eicosanoids from macrophages and mast cells; calcium channel blockers intal and ketotifen and H_2-antagonist cimetidine preventing the release of leukotrienes from mast cells; FPL55712, a leukotriene (LTC_4) receptor blocker preventing the interaction of these eicosanoids with the target cells; selective: BW755C, naproxen, corticosteroids, diethylcarbamazepine, U60257, and nonselective: antioxidants vitamin E and nordihydroguaiaretic acid, anti-inflammatory drug benoxyprofen, lipoxygenase inhibitors suppressing the synthesis of prostaglandins and leukotrienes in cells [2,11,12].

Moreover, preliminary testing is of practical importance, since detection of certain prostaglandins and leukotrienes in small volumes of the blood before radiography makes it possible to pick up patients with a high risk of side effects and to provide adequate pharmacological correction.

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