Effect of Contrast Media on Free Calcium Concentration in Rat Blood *in Vivo*

Yu. K. Napolov, E. N. Bolotova, E. V. Zalevskaya, V. M. Sal'nikova, N. K. Sviridov, and N. L. Shimanovskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 126, No. 11, pp. 542-545, November, 1998
Original article submitted March 21, 1997

The X-ray contrast media omnipaque=melitrast=ultravist <peritrast=hexabrix<triombrast< bilignost decrease the concentration of free calcium ions in blood serum of "sensitive" Wistar rats *in vivo* by 15-30%. The number of "sensitive" rats (33-67%) did not depend on the dose and type of the agents. The magnetic resonance imaging agent magnevist decreased serum calcium by 10% in all rats.

Key Words: *contrast diagnostic media; serum free Ca^{2+}*

Free calcium ions (Ca^{2+}) in blood regulate a number of vital physiological and biochemical processes [5]. Specifically, exocytosis of histamine and the arachidonic acid metabolism and secretion of its products from intracellular depots are regulated by the Ca^{2+}-calmodulin complex and require extracellular Ca^{2+} [2,3,5,7]. An increase in the cytosolic free Ca^{2+} due to its entry from peripheral blood leads to platelet aggregation [2,4] and lymphocyte proliferation [1]. Certain concentration of serum Ca^{2+} is necessary for the synthesis of globular proteins of the complement system via the classical pathway [13] and for production of thrombin and fibrin during hemo coagulation [1,4,5,9]. Ca^{2+} deficiency in the blood decreases vascular tone and motor activity [2]; it inhibits the secretion from the parathyroids' and thyroid c-cells [5]. The organism is characterized by a very low tolerance to large deviations of blood Ca^{2+} from the normal level, which is maintained within a very narrow range by the known mechanisms, and do not vary by more than 3% [5].

There is evidence that X-ray contrast media (XRCM) decrease blood Ca^{2+}. For example, 90 sec after intravenous injection of 60 ml ioxitalamic, diatrizoic, and amidotrizoic acids, blood contente of free Ca^{2+} decreased by 6-10% and restored after 30 min [11]. The reasons for a decrease in blood Ca^{2+} are not clear, therefore the possibility of using the variations of serum Ca^{2+} concentration as an indicator of the anaphylactoid side effects of XRCM has not been assessed. In addition, the application of new diagnostic agents requires comparing their effects on blood Ca^{2+} with these of conventional XRCM.

Our aim was to compare the effects of various contrast media (CM) on the concentration of free Ca^{2+} in rat blood *in vivo*.

MATERIALS AND METHODS

The study was carried out on Wistar rats of both sexes (150 g) maintained on the standard diet. The rats were randomized into control and experimental groups ($n \geq 7$). The experimental rats were injected intravenously with warmed (37°C) CM in the doses corresponding to those used in clinics. The controls were injected with isoosmotic physiological saline under the same conditions. The infusion rate was 0.1 ml/sec. After 15 min the rats were decapitated under weak ether anesthesia. Serum was obtained by sedimentation of the blood at 4°C followed by a 8-min centrifugation (400*g*, 4°C). Serum Ca^{2+} concentration was determined spectrophotometrically

Department of Molecular Pharmacology and Radiobiology, Russian State Medical University, Moscow

0007-4888/98/0011-1124$20.00 ©1999 Kluwer Academic/Plenum Publishers

with a Calcium standard kit (Sigma). The results were statistically analyzed using Wald-Wolfovitz's *r* serial test and Wilcoxon-Mann-Whitney's inversion *U* test.

The following CM were examined: the iodine-containing XRCM 50% bilignost-300 (Farmak, Ukraine), hexabrix-320 (Byk Gulden), melitrast-300, and 80% peritrast-400 (Dr. Kohler Chemic), omnipack-300 (Nycomed), 76% triombrast-380 (Farmak), ultravist-370 (Schering), and the resonance imaging agent magnevist (Schering).

RESULTS

In some rats intravenous CMs did not change the level of serum Ca^{2+} and decreased it in others. Previously, we demonstrated both necessity and feasibility of dividing animals into a "sensitive" group a CM-induced decrease in serum Ca^{2+}, and a "tolerant" group without variations in serum Ca^{2+} under the same conditions [8]. Table 1 shows the data on "sensitive" rats. Nonionic XRCM (omnipaque, ultravist, and melitrast) produced the same decrease in serum Ca^{2+} (by 15%) only if they were applied in the maximum dose of 2 g I/kg. Peritrast and hexabrix produced 20% effect in doses of 1 an 2 g I/kg, respectively. These doses of triombrast decreased serum Ca^{2+} by 25%. Bilignost was equally effective in all tested doses (0.5, 1, and 2 g I/kg), and decreased serum Ca^{2+} by 30%. In doses 0.1, 0.2, and 0.3 mmol/kg magnevist lowered serum Ca^{2+} by 10%.

Therefore, all examined CM decreased serum Ca^{2+} in a dose-dependent manner, although to various degrees. Their effect increased in the following order (statistical significance $p(H_0)_r \leq 0.025$): omnipaque=melitrast=ultravist<peritrast=hexabrix<triombrast<bilignost. It is noteworthy that the percentage of "sensitive" rats (33-67%) did not depend on dose and type of XRCM. For example, 44% rats were "sensitive" to the ionic XRCM bilignost (0.5 and 2 g I/kg) and to nonionic CM melitrast (2 g I/kg). However, all the rats were sensitive to magnevist.

Based upon studies where XRCM were incubated in aqueous solutions of known Ca^{2+} concentrations [12,15], some researchers have concluded that Ca^{2+} decrease results from Ca^{2+} binding to the iodized aromatic structure of XRCM and does not depend on Ca-chelate adjuvant agents added to commercial XRCM. The chelate effect of XRCM towards serum free Ca^{2+} should be observed in humans and animals. For example, it affects anticoagulant activity of XRCM [8]. However, our results and literature data indicate that strong coordinate binding of Ca^{2+} by an XRCM molecule cannot be responsible for a decrease in serum free Ca^{2+}.

The same doses of XRCM applied under similar conditions did not decrease Ca^{2+} in all rats. Calcium binding was different *in vivo* and *in vitro*. For example, cheletropic effects of hexabrix and triombrast were similar when they were incubated in Ca^{2+} water saline solution [12]. Intravenous injection of triombrast induced a greater decrease in serum Ca^{2+} ($p(H_0)_r=0.025$ for doses 1 and 2 g I/kg). Clinical studies of diatrizoate salts (triombrast) as XRCM revealed a decrease in serum Ca^{2+} from 90 to 75

TABLE 1. Serum Concentration of Free Ca^{2+} in Rats after Intravenous Injection of CM (*M±m*)

CM, dose	Serum concentration of CM in sensitive rats, %	Sensitive rats, %
Bilignost, g I/kg		
0.5	71±2*	44
1	68±6*	67
2	72±4*	44
Hexabrix, g I/kg		
0.5	—	0
1	82±3*	37.5
2	80±4*	50
Melitrast, g I/kg		
0.5	—	0
1	—	0
2	85±4*	44
Omnipaque, g I/kg		
0.5	—	0
1	—	0
2	86±6*	33
Peritrast, g I/kg		
0.5	—	0
1	79±2*	50
2	80±4*	56
Triombrast, g I/kg		
0.5	—	0
1	73±3*	57
2	76±4*	62.5
Ultravist, g I/kg		
0.5	—	0
1	—	0
2	86±4*	75
Magnevist, mmol/kg		
0.1	90±3*	100
0.2	92±2*	100
0.3	91±1*	100

Note. Serum Ca^{2+} concentration in control rats was 3.0-5.6 mg/dl. *$p(H_0)_r \leq 0.025$) compared with control. Dash: no effect.

mg/liter on the 9th sec, while the anions of ioxaglate (hexabrix) did not change the total Ca^{2+} blood level [11]. The absolute value of Ca^{2+}-binding effect of XRCM in the *in vitro* experiments did not correspond to the decrease in free Ca^{2+} in rats and humans caused by intravenous XRCM. Thus, an ionic monomeric XRCM in the maximum concentration of 500 mmol/liter decreased the initial Ca^{2+} concentration of 1 mmol/liter in an aqueous solution (which is about the lower limit of 1.05 mmol/liter for normal human blood) by 75% within 0.5 sec [12]. The maximum dose of XRCM was 10-fold lower (50 mmol/liter), while the maximum effect produced by bilignost was only 2.5-fold weaker (decrease in free Ca^{2+} by 30%, Table 1). Ca^{2+} binding by XRCM in aqueous-saline solutions was dose-dependent [12], while the decrease in rat serum Ca^{2+} produced by intravenous CM did not depend on the dose. The contrast media produced the same effect in various doses. For example, bilignost decreased serum Ca^{2+} in doses of 0.5, 1, and 2 g I/kg, but the maximum difference in this effect was only 5.5% (Table 1).

Thus, our data show a decrease in serum Ca^{2+} content caused by XRCM *in vivo* due to Ca^{2+} entry into cells. Using other biochemical and cytological reactions induced by XRCM, we demonstrated individual sensitivity of humans and animals to CM, and ordered them according to Ca^{2+}-lowering activity: nonionic XRCM<ionic monomeric and dimeric XRCM with bulk side radicals, low viscosity and low osmolality<monomeric ionic XRCM<dimeric ionic XRCM. XRCM cause the release of histamine from mast cells and basophilic leukocytes, increase the titer of blood eicosanoids, induce platelet aggregation, activate the complement system, inhibit the ADP activity of cell membranes, decrease the vascular tone, and produce antithyroid and mitogenetic effects on blood monocytes [6,8,10, 13,14]. All these effects of XRCM depend on free Ca^{2+} entry from blood into the cytoplasm or on its binding by the proteins of the complement system in the vascular bed, which finally leads to a decrease in free Ca^{2+}. The nonionic agents melitrast, omnipaque, and ultravist have the large side radicals of the benzene rings that determine their biological inertness in comparison with ionic XRCM [8]. They are less effective in provoking pathological processes that involve extracellular calcium. At the same time, the existence of the bulk substituents produces the steric effect that prevents formation of coordinate bonds (specifically, of chelate type) between two ligands (the atoms of nitrogen, sulfur, oxygen, or anionic groups of XRCM molecule and Ca^{2+}) [1]. Thus, the nonionic XRCM should have a weaker Ca^{2+}-binding effect *in vitro*. By the chemical structure, magnevist is a chelate compound, so the decrease in serum Ca^{2+} may occur due to competition of Ca^{2+} and gadolinium for the chelate-forming groups of a CM molecule. For example, since the alkaline-earth metal calcium has a smaller ionic radius than lanthanide gadolinium, it has a higher affinity for the chelate group [1].

It is of particular importance for practical roentgenology that modern nonionic XRCM produce a statistically significant although not very large change in blood Ca^{2+}, so they are more safe in the respect of disturbance of blood electrolyte balance than the ionic substances. Magnevist, a CM used in magnetic resonance imaging, produces a slight analogous effect. Since intravenous injection of CM may decrease blood Ca^{2+} concentration, these agents should be applied with caution in patients with insufficiency of parathyroid glands, thyroid tumor, renal osteodystrophy, hypoproteinemia, tubulopathy, rachitis, pancreatitis, at late periods of pregnancy and in patients that have been using glucocorticoids for a long period.

REFERENCES

1. A. Albert, *Selective Toxicity: The Physico-Chemical Basis of Therapy* [Russian translation], Vol. 2, Moscow (1989).
2. M. V. Bilenko, *Ischemic and Reperfusion Organic Damage* [in Russian], Moscow (1989).
3. J. H. Levy, *Anaphylactic Reactions in Anesthesia and Intensive Care* [Russian translation], Moscow (1990).
4. D. O. Levitskii, *Calcium and Biological Membranes* [in Russian], Moscow (1990).
5. R. Marray, D. Granner, P. Mayes, and V. Rodwell, *Harper's Biochemistry* [Russian translation], Moscow (1993).
6. Yu. K. Napolov, N. L. Shimanovskii, E. V. Markina, and P. V. Sergeev, *Eksp. Klin. Farmakol.*, **57**, No. 2, 54-57 (1994).
7. P. V. Sergeev, P. A. Galenko-Yaroshevskii, and N. L. Shimanovskii, *Essays On Biochemical Pharmacology* [in Russian], Moscow (1996).
8. P. V. Sergeev, N. K. Sviridov, and N. L. Shimanovskii, *Contrast Media* [in Russian], Moscow (1993).
9. E. F. Shamrai and A. E. Pashchenko, *Clinical Biochemistry* [in Russian], Moscow (1970).
10. J. Fareed, R. Moncada, H. L. Messmore, *et al.*, *Semin. Thromb. Hemost.*, **10**, No. 4, 306-328 (1984).
11. A.-M. Freyria, A. Pinet, J. Bellevill, *et al.*, *J. Allergy Clin. Immunol.*, **69**, No. 4, 397-403 (1982).
12. W. Morris, L. G. Sahler, M. Violante, and H. W. Fischer, *Invest. Radiol.*, **18**, No. 1, 231-232 (1983).
13. S. H. Neoh, M. R. Sade, R. B. Willis, *et al.*, *Ibid.*, **16**, No. 2, 152-158 (1981).
14. Z. Parvez, R. Moncada, H. L. Messmore, and J. Fareed, *Ibid.*, **18**, No. 3, 279-284 (1983).
15. H. G. Wolpers, D. M. Hunneman, M. Stellwaad, and G. Hellige, *J. Pharm. Sci.*, **70**, 231-232 (1981).