Induction of Apoptosis in Bone Marrow Cells is Mediated via Purinergic Receptors

Yu. A. Uspenskaya, S. V. Mikhutkina*, E. I. Taksanova*, N. N. Popova*, R. Ya. Olovyannikova*, and A. B. Salmina*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 8, pp. 135-138, August, 2004
Original article submitted December 30, 2003

ATP activity in mouse bone marrow cells was *in vitro* estimated by expression of phosphatidylserine on the outer membrane surface using FITC-labeled annexin. ATP induced apoptosis in bone marrow cells. Purinergic receptor antagonists PPADS and suramin modulated the apoptotic effect of ATP on hemopoietic cells. Acute and subacute administration of doxorubicin, an inductor of oxidative burst, decreased cell sensitivity to ATP and abolished its apoptotic effect.

Key Words: *bone marrow; apoptosis; ATP; doxorubicin*

The appearance of ATP acting as a signal molecule in the extracellular space can be induced by hypoxia, acidosis, mechanical deformation, stimulation of receptors, and membrane depolarization [6]. The amount of accumulated ATP is sufficient for activation of cells by the paracrine and autocrine mechanisms [6]. ATP acting via purinergic receptors modulates functional activity of cells in various tissues, cardiovascular activity, contraction of skeletal and smooth muscles, endo- and exocrine secretion, adhesion of leukocytes, and immune, inflammatory, and platelet reactions [3,9]. Stimulation of blood cells with ATP is followed by changes in proliferation and differentiation of hemopoietic cells, modulation of chemotaxis, release of cytokines or lysosomal enzymes, and generation of reactive oxygen species or nitrogen [10]. P^7_{2Y} and P_{2X}/P_{2Z} purinergic receptors were found in mammalian bone marrow cells (BMC) [20].

Modulation of apoptosis is an important component in the total biological effect of ATP. ATP in high concentrations produces apoptotic and total cytotoxic effects on various cells, which is mediated by changes in intracellular calcium homeostasis. Among a variety of BMC, hemopoietic precursors are most sensitive to the cytotoxic effect of ATP [10].

The major mechanisms of cytostatic-produced injury in cells include damage to nucleic acids, changes in biomembranes, impairment of structural and functional characteristics in cell proteins, induction of mitochondrial dysfunction and cell death, and modulation of receptor-regulated signal pathways in cells [4,8].

Here we evaluated the role of purinergic receptors in apoptotic activity of ATP and studied *in vitro* and *in vivo* modulation of its effects in mouse BMC with doxorubicin (DR).

MATERIALS AND METHODS

Experiments were performed on male outbred albino mice weighing 20-25 g. Each series was conducted on 5 animals.

Apoptosis was detected immunocytochemically by the expression of phosphatidylserine on the outer membrane using FITC-labeled annexin and luminescence microscopy. In each preparation 200 cells were analyzed under an immersion objective (×900). The preparations were obtained after incubation of BMC (10^6 cells/ml) in medium 199 at 37°C for 1 h. The cells

Department for Animal Anatomy and Physiology, Krasnoyarsk State Agrarian University; *Department of Biochemistry, Course of Medical Chemistry, Krasnoyarsk State Medical Academy. ***Address for correspondence:*** onmpi@krsk.info.

0007-4888/04/1382-0116

were washed 2 times with Hanks solution, resuspended in binding buffer, treated with annexin V-FITC and propidium iodide (Annexin V Apoptosis Detection Kit, Caltag Lab.), and incubated in dark at room temperature for 15 min.

The *in vitro* effect of ATP on BMC was studied during incubation of cells with ATP (1 mM, Reanal) at 37°C for 1 h. DR in a dose of 1×10^{-6} M was added to the culture medium. The mice *in vivo* received single intramuscular injection of DR in a maximum permissible dose (6 mg/kg, acute treatment) or were repeatedly treated with 0.95 mg/kg DR (1/10 LD_{50}, at 24-h intervals for 10 days, subacute treatment). The cells were preincubated with purinergic receptor antagonists PPADS (100 μM, Sigma) and suramin (100 μM, Sigma) for 15 min and then coincubated with ATP for 1 h.

The results were analyzed by Student's *t* test.

RESULTS

During 1-h incubation of BMC with 1 mM ATP (this concentration of ATP corresponds to the amount of this compound detected in the intercellular space during cytolysis and inflammation [12]) the count of cells in the early stage of apoptosis and necrosis increased, while the number of viable cells decreased (Fig. 1).

Suramin in a concentration of 100 μM did not modulate the apoptotic and necrotic effects of ATP. Preincubation of cells with 100 μM PPADS abolished the apoptotic effect of ATP and decreased the percentage of cells in the early stage of apoptosis and secondary necrosis, but potentiated induction of necrosis by this compound (Fig. 1). Therefore, the apoptotic and necrotic effects of ATP on BMC are realized via P_{2X} receptors. Blockade of these receptors induces necrosis in cells incapable of undergoing apoptosis. These findings and results of our previous experiments with induction of blebbing in the plasma membrane of BMC incubated with ATP [1] are consistent with published data [5,11]. It was reported that binding of ATP to $P2X_7$ receptors is followed by changes in the cytoskeleton, opening of cationic channels, blebbing of the plasma membrane, and cell death.

DR modulates the ability of ATP to cause transitory blebbing of the plasma membrane in BMC [1]. Single and repeated (10 days) administration of DR *in vivo* affects the sensitivity of BMC to ATP. Treatment with DR for 1 day decreased apoptotic activity of ATP. It should be emphasized that this xenobiotic had no apoptotic and necrotic properties. Ten-day treatment with DR prevented ATP-induced necrosis in cells. The relative number of viable cells returned no normal in both series. These data indicate that the alternative mechanism for cell death is not induced in cells incapable of undergoing apoptosis (Table 1, Fig. 2).

Incubation of BMC with DR in a concentration of 1×10^{-6} M producing a membranotoxic effect [1] was followed by an increase in the count of apoptotic cells, induction of necrosis, and decrease in the ratio of viable cells.

TABLE 1. *In Vitro* and *in Vivo* Apoptosis and Necrosis of BMC Produced by DR (% of total cell count, *M±m*)

Series	Cell population			
	living	apoptosis	secondary necrosis	necrosis
Control	73.70±2.89	13.78±1.79	10.33±1.30	5.40±0.67
DR, 10^{-6} M	59.50±3.56**	21.71±3.19*	10.86±0.60	9.00±1.08**
In vivo DR, 1 day	69.25±0.99	16.50±2.13	7.25±0.99	7.0±1.7
In vivo DR, 10 days	61.40±2.05**	20.80±1.78**	10.80±1.78	7.00±1.58

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control.

TABLE 2. Effect of Purinergic Receptor Antagonists PPADS and Suramin on Cytotoxicity of DR *in Vitro* (% of total cell count, *M±m*)

Series	Cell population			
	living	apoptosis	secondary necrosis	necrosis
Control	73.70±2.89	13.78±1.79	10.33±1.30	5.40±0.67
DR, 10^{-6} M	59.50±3.56**	21.71±3.19*	10.86±0.60	9.0±1.08**
PPADS and DR, 10^{-6} M	65.20±2.77	11.40±2.41	3.60±0.84***	21.25±1.52***
Suramin and DR, 10^{-6} M	63.20±4.04	8.67±1.54*	2.17±0.66***	25.0±4.37***

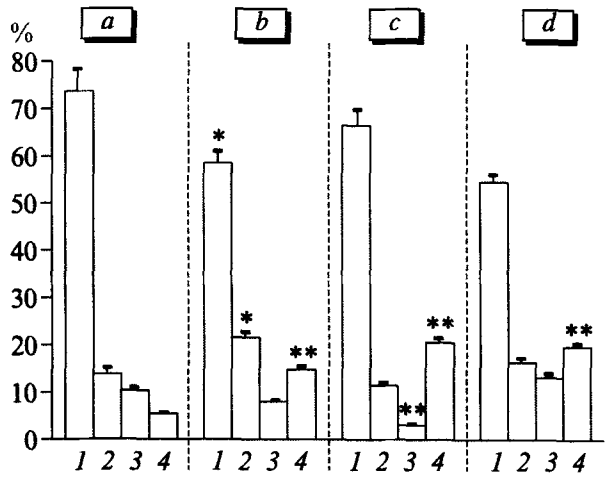

Fig. 1. *In vitro* modulation of the effect of ATP (1 mM) on bone marrow cells with PPADS (100 μM) and suramin (100 μM): control (*a*), ATP (*b*), PPADS and ATP (*c*), and suramin and ATP (*d*). Here and in Fig. 2: living cells (*1*), apoptosis (*2*), secondary necrosis (*3*), and necrosis (*4*). *$p<0.01$ and **$p<0.001$ compared to the control.

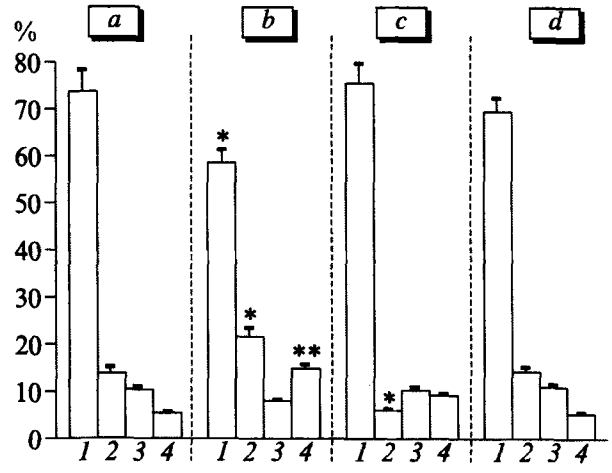

Fig. 2. Effect of doxorubicin on apoptotic and necrotic activity of ATP (1 mM) in bone marrow cells: control (*a*); ATP (*b*); and *in vivo* doxorubicin and ATP, 1 day (*c*) and 10 days (*d*).

Preincubation of BMC with PPADS or suramin *in vitro* modified cytotoxicity of DR. The number of cells in the state of apoptosis or secondary necrosis significantly decreased, while the count of necrotic cells increased (Table 2). The ability of purinergic receptor antagonists to decrease apoptotic activity of DR is related to the xenobiotic-induced release of ATP into the extracellular space and paracrine effect of ATP. Despite high selectivity of antagonists in relation to exogenous ATP, PPADS and suramin caused apoptosis in cells *in vitro* treated with DR. The purinergic receptor antagonist suramin violates coupling of G proteins with receptors and inhibits phospholipase D. Moreover, suramin acts as an antagonist of P_{2X} receptors [7]. It can be concluded that observed suppression of apoptosis-inducing activity of DR with suramin is probably mediated by these mechanisms.

Our results show that extracellular ATP induces apoptosis of BMC via P_{2X} purinergic receptors. Acute and subacute administration of DR decreases BMC sensitivity to the apoptotic and necrotic effects of ATP. The observed properties of DR are of considerable pathophysiological importance. They probably contribute to myelotoxic activity of this xenobiotic associated with the impairment of purinergic regulation in hemopoietic cells.

REFERENCES

1. Yu. A. Uspenskaya, A. B. Egorova, and V. P. Nefedov, *Byull. Eksp. Biol. Med.*, **133**, No. 5, 487-491 (2002).
2. O. R. Baricordi, D. Ferrari, L. Melchiorri, *et al.*, *Blood*, **87**, 682-690 (1996).
3. R. Beigi, E. Kobatake, M. Aizawa, *et al.*, *Am. J. Physiol.*, **276**, No. 1, C267-C278 (1999).
4. P. Costantini, E. Jacotot, D. Decauding, and G. Kroemer, *J. Natl. Cancer Inst.*, **92**, 1042-1053 (2000).
5. P. C. Dagher, *Am. J. Physiol.*, **279**, No. 4, C1270-C1277 (2000).
6. G. R. Dubyak and C. El-Moatassim, *Ibid.*, **265**, C577-C606 (1993).
7. E. Nakamura, Y. Uezono, K. Narusawa, *et al.*, *Ibid.*, **279**, No. 2, C510-C519 (2000).
8. X.-M. Sun, M. MacFarlane, J. Zhuang, *et al.*, *J. Biol. Chem.*, **274**, No. 8, 5053-5060 (1999).
9. A. L. Taylor, B. A. Kudlow, K. L. Marrs, *et al.*, *Am. J. Physiol.*, **275**, No. 5, C1391-C1406 (1998).
10. F. Virgilio, P. Chiozzi, D. Ferrari, *et al.*, *Blood*, **97**, No. 3, 587-600 (2001).
11. H. L. Wilson, S. A. Wilson, A. Surprenant, *et al.*, *J. Biol. Chem.*, **277**, No. 37, 34,017-34,023 (2002).
12. L. M. Zheng, A. Zychlinsky, C. C. Liu, *et al.*, *J. Cell Biol.*, **112**, 279-288 (1991).