Effect of Combined Treatment with Aspirin and Dipyridamole on Oxidative Homeostasis in Mouse Serum

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 7, pp. 28-30, July, 2005
Original article submitted February 11, 2005

Using the method of peroxidative luminol-dependent chemiluminescence we showed that combined treatment with aspirin in low dose and dipyridamole corrects imbalance in oxidative homeostasis in mouse serum. This state resulted from a sharp increase in prooxidant processes after platelet disaggregation with normal dose of aspirin or dose-dependent inhibition of free radical processes with dipyridamole.

Key Words: *aspirin and dipyridamole; platelets; oxidative homeostasis imbalance; normalization; serum*

Antiaggregant therapy is an essential component of treatment for patients with cardiovascular diseases preventing vascular thrombosis [5]. The most widely used antiaggregant aspirin is often prescribed for a life-long therapy in these patients [9]. However, aspirin can cause bronchospasm in salicylate-sensitive patients. Other patients cannot take aspirin due to gastrointestinal disease [7]. Recent studies showed that aspirin can diminish the positive effect of angiotensin-converting enzyme inhibitors in patients with heart failure [8].

The antiaggregant effect of aspirin in low dose is preserved in combined treatment with platelet antiaggregants characterized by another mechanism of action (*e.g.*, dipyridamole with angiogenesis-stimulating activity) [2].

There are contradictory viewpoints on combination therapy with aspirin and dipyridamole. Some authors reported that combination of aspirin in low doses and dipyridamole is effective and pharmacoeconomically validated in patients with myocardial infarction [3,10]. Other researchers showed that administration of dipyridamole for potentiation of the effect of aspirin in coronary angioplasty increases the risk of developing complications [11].

This work was designed to determine the most effective course of aspirin treatment (monotherapy or combined use of aspirin and dipyridamole). We evaluated the degree of imbalance in oxidative homeostasis (OH) in mouse serum.

MATERIALS AND METHODS

Experiments were performed on 150 male (C57Bl×CBA)F_1 mice weighing 18-20 g.

In series I, the mice (n=30) intraperitoneally received 0.2 ml dipyridamole (Persantin, Boehringer Ingelheim) in a daily dose of 30 mg/kg for 30 days. Control animals (n=30) were treated with 0.2 ml physiological saline.

In series II, the mice (n=30) received aqueous solution of aspirin in a daily dose of 0.5 mg for 30 days. Control animals (n=30) received drinking water.

In series III, the mice (n=30) intraperitoneally received dipyridamole in a daily dose of 30 mg/kg and drank aqueous solution of aspirin in a dose of 0.25 mg. The blood and serum from control animals of series I and II served as the control.

The mice were decapitated 2, 7, and 30 days after administration of antiaggregants and physiological saline (10 specimens in each period). The blood was

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0007-4888/05/1401-0022

sampled to prepare the smears and obtain the serum. Blood smears were prepared routinely. Platelet count was estimated by the method of Fonio (per 1000 erythrocytes in several fields of view). The serum was obtained by centrifugation of blood at 2000 rpm for 15 min.

OH in mouse serum was studied by the oxidation index. It was calculated as the ratio between luminol-dependent chemiluminescence of free radical oxidation (FRO) and total antioxidant activity (TAA).

Chemiluminescence of the reaction mixture containing 700 ml phosphate buffer (pH 7.4), 50 ml 0.1 mM luminol, and 50 ml mouse serum was recorded on an Emilite-1105 chemiluminometer [1]. H_2O_2 (20 mM, 200 ml) was added to the mixture to initiate chemiluminescence. Total chemiluminescence was recorded at 37°C for 2 min.

TAA was estimated by the interaction of riboflavin with H_2O_2 in the presence of Fe^{2+}.

Chemiluminescence study of mouse serum was performed taking into account chemiluminescence of luminol in the reaction mixture with H_2O_2 (control) [1]. Serum protein concentration was measured by the method of Lowry.

The results were analyzed by Student's *t* test for small samples. The differences were significant at $p<0.05$.

RESULTS

No differences were revealed between control mice in series I and II. These animals were combined into a common control group for series I, II, and III.

Individual treatment with aspirin or dipyridamole significantly decreased blood platelet count (Table 1).

TABLE 1. Platelet Count in Mouse Peripheral Blood after Individual or Combined Treatment with Aspirin and Dipyridamole ($\times 10^9$, $M \pm m$)

Group	Treatment, days		
	2	7	30
Control	253.1±12.4	257.5±9.3	215.2±9.6
Dipyridamole	355.4±6.2	268.5±7.5	276.7±9.7
Aspirin	380.7±5.4	467.5±9.9	307.0±8.4
Dipyridamole and aspirin	483.5±7.0	4019.2±281.7	89.4±6.6

Combined administration of these preparations was accompanied by significant variations in platelet count. Seven days after combined treatment with the test preparations the number of platelets was 11.6-fold higher compared to that observed in experiments with administration of aspirin alone. However, combined treatment with aspirin and dipyridamole caused thrombocytopenia by the end of the experiment.

Significant variations in blood platelet count probably serve as a compensatory response, which determines enzyme activity of the antioxidant protection system and further blockade of this pathway. Platelets contain antioxidant enzyme superoxide dismutase, which contributes to inhibition of FRO in the serum and maintenance of OH [7]. Previous studies showed that long-term administration of dipyridamole (Curantyl) leads to destruction of abnormal bone marrow megakaryocytes (source of platelets). These data substantiate enzymatic pathway of antioxidant protection under these conditions [4].

Administration of dipyridamole modulated parameters of OH. By the end of the experiment this pre-

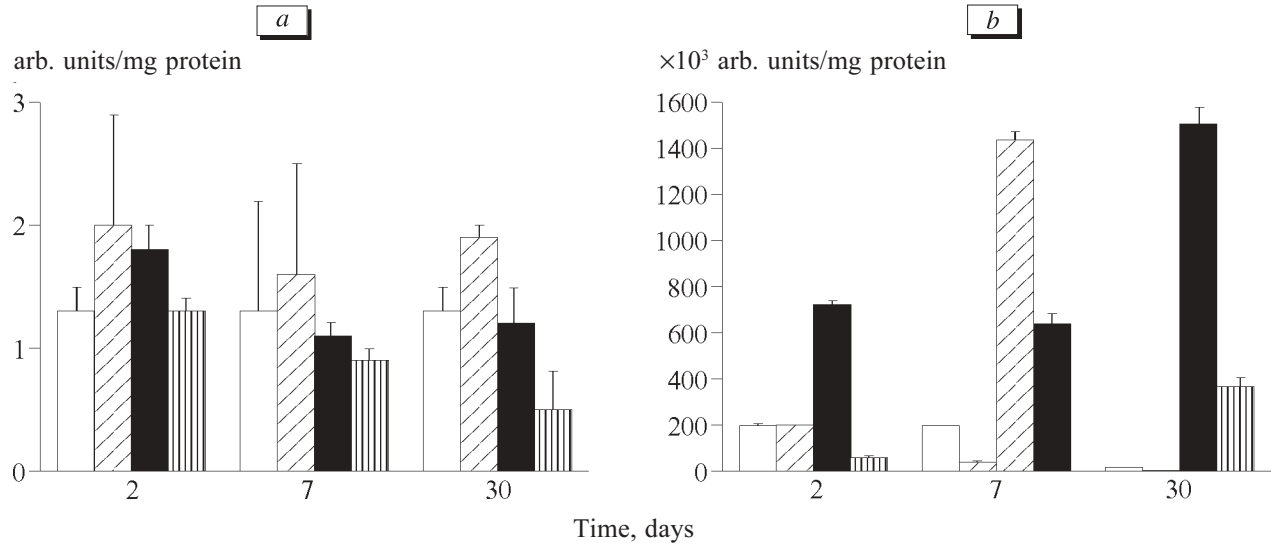

Fig. 1. Total antioxidant activity (*a*) and free radical oxidation (*b*) in the serum from mice receiving physiological saline (light bars), dipyridamole (slant shading), aspirin (dark bars), and aspirin in low dose and dipyridamole (vertical shading). $p<0.05$ compared to the control.

paration dose-dependently decreased serum prooxidant activity, but increased TAA (Fig. 1, *a*). The oxidation index in dipyridamole-treated and control mice was 45.3 and 105.3, respectively.

Activation of FRO in mouse serum was observed 2 days after the start of treatment with aspirin (Fig. 1, *b*). The intensity of oxidative processes reached maximum on days 7 and 30. These data show that administration of aspirin in the follow-up period has no effect on FRO. TAA in aspirin-treated animals progressively decreased and was below the normal by the end of the experiment (Fig. 1, *a*).

Individual administration of aspirin and dipyridamole produced opposite changes in the serum oxidant status. Our results support published data that the effects of these antiaggregants are mediated by different mechanisms [3,10]. Chemiluminescence of the prooxidant processes only slightly decreased by the 30th day of aspirin treatment. However, serum TAA in control mice remained unchanged under these conditions. These data illustrate high effectiveness of the antioxidant protection system in mouse serum. The oxidation index in animals receiving aspirin was 986.3.

Chemiluminescence of FRO in mouse serum decreased on day 2 of combined treatment with aspirin and dipyridamole. In the follow-up period these antiaggregants normalized the concentration of free oxygen radicals. The content of free oxygen radicals sharply increased after administration of aspirin, but decreased after treatment with dipyridamole (Fig. 1, *b*). The test preparations dose-dependently decreased TAA in mouse serum (Fig. 1, *a*). Under these conditions the oxidation index in mouse serum was below the level observed after individual administration of aspirin (496.6). It can be hypothesized that combined treatment with aspirin and dipyridamole stimulates the reserve mechanism of the maintenance of OH. Our study confirms that the effect of combined treatment with aspirin and dipyridamole can be evaluated by measuring chemiluminescence untensity.

REFERENCES

1. A. V. Arutyunyan, E. E. Dubinina, and N. N. Zybina, *Methods for Study of Free Radical Oxidation and Antioxidant System in the Organism. Methodical Recommendations* [in Russian], St. Petersburg (2000).
2. Yu. I. Buziashvili, E. Picano, S. G. Ambatiello, *et al*., *Kardiologiya*, No. 12, 82-86 (2000).
3. K. G. Gurevich and E. G. Lobanova, *Ibid*., No. 12, 87-90 (2000).
4. I. N. Ivasenko, Yu. A. Blyudzin, D. N. Chernyakova, and V. A. Almazov, *Byull. Eksp. Biol. Med*., **114**, No. 12, 611-613 (1992).
5. V. A. Kryzhanovskii, *Kardiologiya*, No. 7, 72-84 (2001).
6. I. L. Chertkov, *Gematol. Transfuziol*., **38**, No. 4, 3-4 (1993).
7. M. I. Burgess, C. G. Densem, N. H. Brooks, *et al*., *Postgrat. Med. J*., **76**, No. 90, 734-735 (2000).
8. D. Hall, *Cardiol. Clin*., **19**, No. 4, 597-603 (2001).
9. C. H. Hennekens, M. L. Dyken, and V. Fuster, *Circulation*, **96**, No. 8, 2751-2753 (1997).
10. P. S. Hervey and K. L. Goa, *Drugs*, **56**, No. 3, 466-477 (1999).
11. N. Lembo, A. J. Black, G. S. Roubin, *et al*., *Am. J. Cardiol*., **65**, No. 7, 422-426 (1990).