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Research Paper

Dipyridamole enhances inhibitory effect of adenosine on neutrophils in human peripheral blood

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Abstract

Objective: It was known that neutrophils play an important role in ischemic-reperfusion injury. In this study we tested the effect and its mechanism of dipyridamole on neutrophils. Methods:Hydrogen peroxide(H_2O_2) production by neutrophils was determined using luminol amplified chemiluminescence and the percentage of activity was calculated by observing the uninhibited peak height. Results: Dipyridamole per se produced a concentration-dependent inhibition of H_2O_2 by formyl-MetleuPhe(fMLP)-stimulated neutrophils. Dipyridamole at a low concentration(0.3 μ mol • L^{-1}) that per se affected neutrophils only slightly, enhanced markedly the effects of adenosine on neutrophils. On the other hand, dipyridamole did not alter the inhibitory effect of NECA(5'-N-ethylcarboxamidoadenosine) on neutrophils. However, propentofylline, a known inhibitor of adenosine uptake, also gotten the same result. Conclusion:Dipyridamole inhibited the production of H_2O_2 by fMLP-stimulated neutrophils. Dipyridamole at a low concentration enhanced the inhibitory effect of adenosine on neutrophils. The mechanism involved is probably due to the effect of dipyridamole on adenosine uptake.

Key words: dipyridamole; hydrogen peroxide; adenosine; neutrophils

INTRODUCTION

Neutrophils are the most abundant circulating leukocytes and are the first cells to arrive at an infected or inflamed site. Activated neutrophils phagocytize bacteria, debris, bactericidal oxygen metabolites and lysosomal enzymes. Generally the neutrophils play a protective role in host defense in appropriate neutrophil activation. However, the poor selectivity in the target tissue allows neutrophils to attack everything surrounding them when they are activated. The migration and accumulation of neutrophils at the site of tissue injury may exacerbate the potential for tissue destruction. The growing body of evidence suggests that infiltrating polymorphonuclear may contribute to ischemia and reperfusion injury[1,2]. Therefore drugs may exert beneficial effects secondary to an action on neutrophils, either to prevent their migration or to inhibit their function[3,4]. Dipyridamole has been used in several clinical and experimental studies due to its action on platelets, endothelial cells, macrophages and neutrophils. It was shown that this drug inhibited platelet aggregation, induced coronary vasodilation, and inhibited leukotriene B_4 as well as O_2^- generation by neutrophils[3,5]. In this study we tested the effect and mechanism of dipyridamole on neutrophil H_2O_2 production.

MATERIAL AND METHODS

Neutrophil preparation

Human peripheral blood neutrophils were separated by one-step peroll technique[2]. Contaminating erythrocytes were lysed by explosion to 2 ml of distilled water for 30 s. Cells were centrifuged at 200 g and then resuspended to the desired cell concentration in HBSS(Hank's balanced salt solution). The final preparation was over 95% neutrophils by Giemsa stain and 98% was viable by trypan blue exclusion.

Measurement of H_2O_2 production

H_2O_2(hydrogen peroxide) production by neutrophils was determined using luminol amplified chemilumi-

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nescence[2]. 100 μl of neutrophil suspension(1×10^6 cells) was added to 850 ml of the test solution. The mixture was incubated with either 40 μl control or agonists at different concentration for 5 minutes in a plastic cuvette. 10 μl of 1 μmol • L^{-1} fMLP(formylmethionyl-leucyl-phenylalanine) was given to the cuvette and luminol amplified chemiluminescence was measured by a luminometer(Chrono-log Corporation, USA) and the percentage of H_2O_2 production was calculated by observing the uninhibited peak height.

Statistical analysis

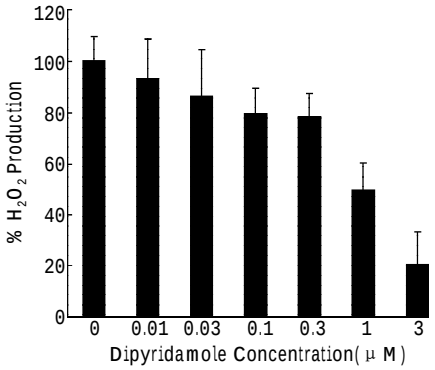
All results were given as mean ± SD. Results were compared by one-way analysis of variance or Student's *t*-test using the Primer program on a Macintosh computer.

RESULTS

Dipyridamole per se produced a concentration-dependent inhibition of fMLP-stimulated oxidative burst(Fig. 1). The effect was minimal(less than 20%) up to 0.3 μmol • L^{-1}(no difference $P > 0.05$) but was pronounced thereafter, reaching more than 80% at 3 μmol • L^{-1}, $P > 0.001$. Dipyridamole at a low concentration(0.3 μmol • L^{-1}) that per se only slightly affected neutrophils, enhanced the inhibitory effects of adenosine on H_2O_2 production(Fig. 2, $P < 0.001$). On the other hand, dipyridamole(0.3 μmol • L^{-1}) did not alter the inhibitory effect of NECA on H_2O_2 production (Fig. 3, $P > 0.05$). This is to be expected if dipyridamole acts as an uptake inhibitor because NECA is not eliminated by the transport system that removes adenosine. In order to test further whether uptake inhibition could potentiate the effect of adenosine on neutrophils, we examined the effect of a known inhibitor of adenosine uptake, propentofylline[5]. As with dipyridamole, propentofylline per se reduced the effect of fMLP-activated neutrophil H_2O_2 production. The inhibitory effect of adenosine on fMLP-activated neutrophil H_2O_2 production was also markedly enhanced by the concentration of propentofylline(100 μmol • L^{-1}), (Fig. 4, $P < 0.001$). However, the ability of NECA to inhibit fMLP-induced neutrophil H_2O_2 production was unaffected by propentofylline(Fig. 5, $P > 0.05$).

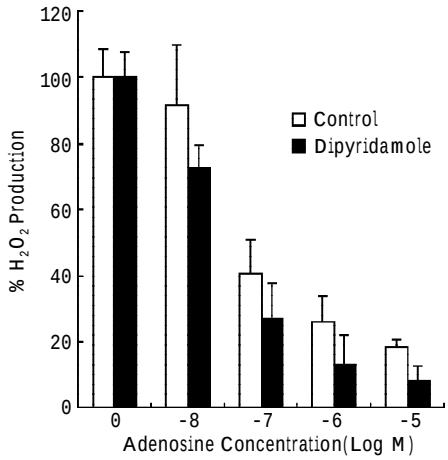
DISCUSSION

Dipyridamole is an antithrombotic drug that has been shown to influence not only platelet function but also some aspects of leukocyte activation[5]. Dipyridamole was found directly inhibiting active oxygen metabolites generated by human PMN(polymorphonuclear leukocyte) at therapeutic concentration[6]. Preoperative treatment with oral dipyridamole significantly reduces both neutrophil superoxide anion generation and extent of neutrophil adhesion to endothelial cells after coronary bypass grafting procedures with cardiopulmonary bypass[6]. In a recent report, dipyridamole has shown the marked hepatoprotective effects against severe ischemia and reperfusion injury in canine livers. Dipyridamole is a promising agent for liver surgery and transplantation[7]. But the effective mechanism of dipyridamole on neutrophil leukocytes was not understood. In agreement with previous reports, we found that dipyridamole per se produced a dose-dependent inhibition of fMLP-induced oxidative burst[5,8,9]. The novel finding is that dipyridamole significantly enhanced the inhibitory effect of adenosine on H_2O_2 by stimulated neutrophils. On the other hand, it did not enhance the effect of the adenosine analogue NECA(5'-N-ethylcarboxamidoadenosine). The mechanism involved is probably the effect of dipyridamole on adenosine



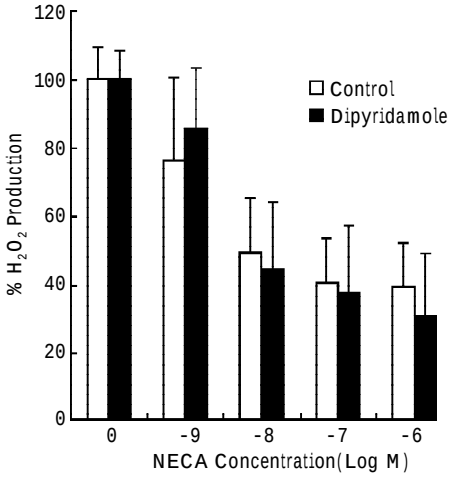
Dipyridamole was added 5 min before fMLP(1 μmol • L^{-1}). The data showed the means and standard errors of four experiments from different donors. Values represented % of control.

Fig. 1 The effects of dipyridamole alone on H_2O_2 production



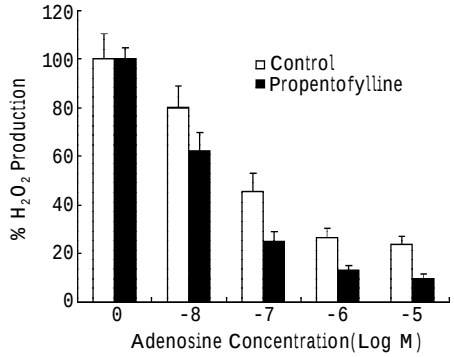
Dipyridamole(0.3 μmol • L^{-1}) was added with adenosine 5 min before fMLP(1 μmol • L^{-1}). The data showed the means and standard errors of four experiments from different donors. Values represented % of 0 concentration. Control and dipyridamole were adenosine alone and adenosine with dipyridamole(0.3 μmol • L^{-1}), respectively. $P < 0.001$ compared between two groups.

Fig. 2 The effect of adenosine on H_2O_2 production in the presence or absence of dipyridamole



Dipyridamole (0.3 μmol • L^{-1}) was added with NECA 5 min before fMLP(1 μ mol • L^{-1}). The data showed the means and standard errors of four experiments from different donors. Values represented % of 0 concentration. Control and dipyridamole were NECA alone and NECA with dipyridamole(0.3 μmol • L^{-1}), respectively. No difference between two groups.

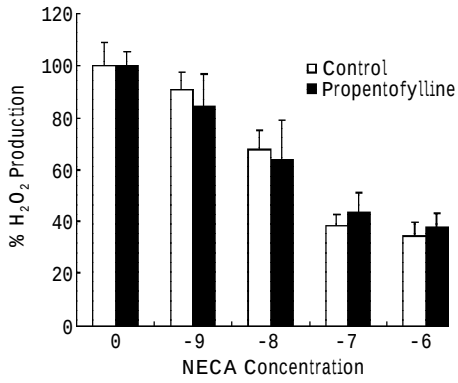
Fig. 3 The effect of NECA on H_2O_2 production in the presence or absence of dipyridamole



Propentofylline(100 μmol • L^{-1}) was added with adenosine 5 min before fMLP(1 μmol • L^{-1}). The data showed the means and standard errors of four experiments from different donors. Values represented % of 0 concentration. Control and propentofylline were adenosine alone and adenosine with propentofylline(100 μmol • L^{-1}), respectively. $P < 0.001$ compared between two groups.

Fig. 4 The effect of adenosine on H_2O_2 production in the presence or absence of propentofylline

uptake[10,11]. As propentofylline, a known inhibitor of adenosine uptake, has achieved the same results as dipyridamole in our study. In addition, adenosine deaminase (which metabolize adenosine to inactive product) prevents the effects of dipyridamole on superoxide anion generation and on the expression of procoagulant activity by leukocytes[6].



Propentofylline(100 μmol • L^{-1}) was added with NECA 5 min before fMLP(1 μmol • L^{-1}). The data showed the means and standard errors of four experiments from different donors. Values represented % of 0 concentration. Control and propentofylline were NECA alone and NECA with propentofylline(100 μmol • L^{-1}), respectively. No difference between two groups.

Fig. 5 The effect of NECA on H_2O_2 production in the presence or absence of propentofylline

References

[1] Nolte D, Lehr HA, Sack FU, Messmer K. Reduction of post-ischemic reperfusion injury by the vasoactive drug buflometidil. *Blood Vessels* 1991; 28(suppl): 8-14.

[2] Haga Y, Dnmitrescu A, Zhang Y, Stain_Malmgren K, SjÖuist PO. Effects of calcium blocker on the calcium, H_2O_2 production and elastase in human neutrophils. *Pharmacol Toxicol* 1996; 79(6): 312-7.

[3] Colli S, Remoli E. Multiple affects of dipyridamole on neutrophils and leukocytes: adenosine-dependent and adenosine-independent mechanisms. *J Lab Clin Med* 1991; 118(2): 136-45.

[4] Suzuki S, Sugai K, Sato H, Sakatume M, Arakawa M. Inhibition of active oxygen generation by dipyridamole in human polymorphonuclear leukocytes. *Eur J Pharmacol* 1992; 227(4): 395-401.

[5] Zhang Y and Fredholm BB. Propentofylline enhancement of the actions of adenosine on neutrophil leukocytes. *Biochem Pharmacol* 1994;48(11): 2025-32.

[6] Chello M, Mastroroberto P, Malta E, Cirillo F, Celiv. Inhibition by dipyridamole of neutrophil adhesion to vascular endothelium during coronary bypass surgery. *Ann Thorac Surg* 1999; 67(5): 1277-82.

[7] Taniguchi M, Magata S, Suzuki T, ShimamuraT, Jin MB Iida J, *et al*. Dipyridamole protects the liver against warm ischemia and reperfusion injury. *J Am Coll Surg* 2004;198(5):758-69.

[8] Vargas F, Rivas C, Diaz Y, Contreras N, Silva A, Ojeda LE, *et al*. Antioxidant properties of dipyridamole as assessed by chemiluminescence. *Pharmazi* 2003; 58(11):817-23.

[9] Wykretowicz A, Filipiak A, Szczepanik A, Wysocki H. Dipyridamole inhibits hydroxylamine augmented nitric oxide(NO) production by activated polymorphonuclear neutrophils through an adenosine-independent mechanism. *Physiol Res* 2004; 53 (6): 645-52.

[10] Wykretowicz A, Filipiak A, Szczepanik A, Wysocki H. Dipyridamole inhibits hydroxylamine augmented nitric oxide(NO) production by activated polymorphonuclear neutrophils through an adenosine-independent mechanism. *Physiol Res* 2004; 53(6): 645-52.

[11] Yuan L, Yang JG, Zhu GP. The effects of dipyridamole on mouse-liver ischemia refusion injoury. *J Abdominal Surgery* 2006; 19(4): 244-6.