Inhibition by dipyridamole of cerebral vasospasm induced in vitro by whole blood

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This study evaluates the effect of dipyridamole, an inhibitor of platelet aggregation, on cerebral artery contraction induced in vitro by the addition of whole blood. Whole fresh arterial blood added to isolated rabbit basilar artery bathed in a physiological buffer produces a sustained contraction in vessels observed for 60 minutes. Significant dose-dependent inhibition of contraction was observed when dipyridamole was added to the vessel bath. This effect was not influenced by preincubating cerebral vessels with aspirin, an inhibitor of prostaglandin synthesis. It is suggested that inhibition of whole blood-induced cerebral artery contraction by dipyridamole does not result from potentiation of cerebral vessel prostaglandin pathways, but possibly from a direct effect on platelets.

KEY WORDS · dipyridamole · cerebral vasospasm · aspirin · prostaglandin I₂ · thromboxane A₂

The management of subarachnoid hemorrhage is frequently complicated by persistent intracranial artery spasm. Many therapeutic approaches have been suggested; however, no effective pharmacological treatment for cerebral vasospasm has been developed. Several studies have suggested that prostaglandin metabolism may play an important role in the pathophysiology of this disorder. Dipyridamole is a vasoactive pyrimido-pyrimidine compound that was first used as a coronary vasodilator for the treatment of chronic angina pectoris. More recently, it has been noted to inhibit platelet aggregation and has been evaluated as an antithrombotic agent. Although its mechanism of action is not entirely clear, it may act by modification of prostaglandin pathways. This study evaluates the effect of dipyridamole on the in vitro contractile response of rabbit basilar artery to whole blood. Moreover, the importance of cerebral vessel prostaglandin synthesis for dipyridamole's action is investigated.

Materials and Methods

New Zealand white rabbits of both sexes, weighing between 4 and 5 kg, were used in this study. The animals were sacrificed by the injection of 100 cc of air in the marginal vein of an ear. Within five minutes the brain stem was removed and placed in cold (5° to 15°C), oxygenated (95% O₂, 5% CO₂), modified Krebs solution (MKS) containing NaCl 117.5 mM, KCl 5.37 mM, CaCl₂ 2.52 mM, MgSO₄ 0.56 mM, NaH₂PO₄ 1.17 mM, NaHCO₃ 15.41 mM, and glucose 5.5 mM, with the pH corrected to 7.40 ± 0.02. The basilar artery was dissected from the surrounding arachnoid and cut into tubular segments, 3 mm in length.

Two stainless steel pins (diameter 0.010 in.) were inserted within the lumen of each vessel segment parallel to each other. The pins were mounted between a fixed support and a tension transducer. Resting tension was applied to the vessel by varying the transducer position. The isometric tension responses of two vessel segments were recorded simultaneously on a Grass model 7B polygraph. Each vessel-pin arrangement was bathed in 25 cc of MKS maintained at 37°C. A mixture of 95% O₂ and 5% CO₂ was bubbled through the MKS bath.

A resting tension of 200 to 400 mg was applied to each vessel segment during a 30- to 45-minute equilibration period. The addition of 2 mEq KCl to the bath resulted in vessel contraction. This response was potentiated by dipyridamole.
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observed for 5 minutes, and the maximal response was defined as the standard contraction. Only vessels that responded briskly and reached a tension of at least 500 mg above resting tension were studied. After three MKS washes, the vessels relaxed to resting tension. For each vessel segment, all subsequent contractions induced by whole rabbit blood (WRB) were considered as a percentage of that vessel's standard contraction.

Next, 0.5 cc of whole fresh arterial blood drawn from another rabbit was rapidly introduced into the bath of each vessel segment. The contractile response was then recorded for 60 minutes. Dipyridamole was dissolved in ethanol and the pH adjusted to 7.40 ± 0.02. An aliquot of 60 μl of ethanol was added to the vessel bath simultaneous to the addition of whole rabbit blood for all trials. The dose of dipyridamole tested was expressed as a final concentration in the MKS bath.

Aspirin was dissolved in MKS at a concentration of 20 mM and the pH adjusted to 7.40 ± 0.02 with NaOH. This solution was warmed to 37°C. The effect of aspirin on the reactivity of rabbit basilar artery was evaluated by incubating the arterial preparation in an aspirin bath for 30 to 45 minutes while the resting tension was applied. Just before the addition of whole rabbit blood and dipyridamole to the bath, the aspirin solution was replaced with plain MKS.

Results

Control Group

The addition of 0.5 cc of whole rabbit blood to the MKS bath resulted in a progressive contraction which continued over the 60 minutes of observation (Fig. 1, open circles). Sixty minutes following the addition of blood, a mean value of 50.5 ± 8.9% (SEM) standard contraction was noted for eight trials.

Effect of Dipyridamole on Preparation

The addition of 0.5 cc of whole rabbit blood to the vessel bath with dipyridamole at concentrations of 0.025, 0.1, and 0.4 mM resulted in dose-dependent inhibition of contraction compared to control vessels. This inhibition was statistically significant (Student's t-test) at concentrations of 0.1 and 0.4 mM (Fig. 1). Sixty minutes following the simultaneous addition of whole rabbit blood and dipyridamole, mean values of 30.8 ± 8.1% (SEM), 17.3 ± 8.8%, and 1.6 ± 0.8% standard contraction were observed for dipyridamole concentrations of 0.025, 0.1, and 0.4 mM, respectively.

Effect of Preincubation of Vessels in Aspirin

When cerebral arteries were preincubated for 30 to 45 minutes in 20 mM aspirin, the addition of 0.5 cc of whole rabbit blood resulted in a contraction greater than was noted for untreated vessels (Fig. 2, closed circles). This augmented response was significantly greater than the response for control vessels at both 50 and 60 minutes (p < 0.025). Sixty minutes following the addition of blood, a mean value of 143.7 ± 45% (SEM) standard contraction was noted for seven trials.

Effect of Dipyridamole on Vessels Preincubated in Aspirin

The inhibition of contraction produced by 0.1 mM dipyridamole was preserved even when cerebral vessels were preincubated in 20 mM aspirin (Fig. 3, closed circles).

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†Dipyridamole supplied by Sigma Chemical Co., St. Louis, Missouri.
§Acetylsalicylic acid manufactured by Aldrich Chemical Co., Milwaukee, Wisconsin.

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Dipyridamole is a vasoactive substance which was first used as a coronary vasodilator. It is one of a number of pyrimido-pyrimidine compounds that inhibit platelet aggregation. This property has led to its increasing use in the treatment of thrombotic disorders. Several theories have been proposed to explain its mode of action.

The tendency of platelets to aggregate appears to be regulated by the level of platelet cyclic adenosine monophosphate (cAMP). Cyclic AMP is formed from adenosine triphosphate (ATP) by the enzyme adenylyl cyclase. It is degraded to 5'AMP by the enzyme phosphodiesterase. Thromboxane A2 (TXA2), an unstable prostaglandin synthesized in platelets, causes platelet aggregation by inhibiting adenylyl cyclase which results in low platelet cAMP levels. Conversely, prostaglandin I2 (PGI2), an unstable prostaglandin synthesized by vascular endothelium, inhibits platelet aggregation by stimulating this enzyme, resulting in elevated platelet cAMP.

Previous work has suggested that dipyridamole acts primarily on blood platelets. It has been proposed that this drug antagonizes the effects of TXA2 on platelet cAMP by potentiating the effects of adenylyl cyclase. Alternatively, dipyridamole may directly inhibit platelet TXA2 synthesis. Dipyridamole also interferes with platelet adenosine metabolism, which may inhibit platelet aggregation.

Other reports suggest that dipyridamole acts primarily on blood vessels by potentiating PGI2 synthesis, which results in vasodilation and inhibition of platelet aggregation. Finally, it has been proposed that, by inhibiting the enzyme phosphodiesterase, dipyridamole may act synergistically with PGI2 to elevate platelet cAMP.

These data demonstrate that dipyridamole inhibits in vitro vasoconstriction induced by whole blood. Augmentation of the contractile response of rabbit basilar artery to whole rabbit blood has been noted previously when vessels are preincubated in 20 mM aspirin. Studies in this department suggest that this effect of aspirin, a prostaglandin synthesis inhibitor, may be due to inhibition of PGI2 production (unpublished data). These results demonstrate that the inhibitory effects of dipyridamole are preserved even when cerebral vessels are preincubated in aspirin. Vascular prostaglandin synthesis may not be obligatory for dipyridamole to alleviate cerebral smooth-muscle contraction in response to whole rabbit blood. Dipyridamole may have a direct effect on blood platelets.

Extrapolation of the results of the present in vitro study to in vivo vasospasm may not be valid. These data should encourage the development of in vivo laboratory protocols to evaluate whether dipyridamole might also have a favorable effect on chronic cerebral vasospasm.

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