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Dipyridamole in Antithrombotic Treatment

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Abstract

The antithrombotic activity of dipyridamole was initially discovered in an in vivo experiment about half a century ago. At that time science had not appreciated the complexity of the regulation of local thrombus formation. Inhibition of platelets has been the main focus for the prevention of arterial thrombus formation. Unfortunately, established in vitro test systems have to take away several important components of the hemostatic system. Rather than directly inhibiting platelet aggregation, dipyridamole amplifies endogenous antithrombotic systems and modulates or downregulates prothrombotic processes. While for many years the main focus had been on preventing acute thrombus formation in the case of a rupture of an atherosclerotic plaque in large coronary arteries, it now has been appreciated that perfusion of tissue and patency of small vessels and capillaries is equally important for preventing further damage to the tissue. Here dipyridamole was experimentally shown to improve perfusion and function in chronic hypoperfused tissue unrelated to its vasodilatory properties. Recently, several clinical trials have shown the benefit of dipyridamole when given in a formulation that assures a sufficient plasma concentration. Its potential to scavenge particularly peroxy radicals, its direct reduction of innate inflammation, and a chronic elevation of interstitial adenosine seems to be of more importance for the prevention of vascular and tissue damage than its adenosine- and prostacyclin-mediated antithrombotic effect. In its extended-release preparation with the tartaric acid nucleus, not only does it not seem to add significantly to the risk of bleeding, but seems to hold potential for protecting tissue from oxidative and metabolic stress.

Preventing thrombosis and thromboembolic complications as well as maintaining tissue viability without the risk of major bleeding has still not been satisfactory addressed despite many decades of research. Most approaches and innovations have been driven by the concept that prevention of thrombosis is best served by increasing the strength of inhibition of platelets. In fact, modern reversible and particularly irreversible inhibitors of receptors triggering platelet activation have proven to block platelet activation and aggregation much more powerfully than earlier approaches such as acetylsalicylic acid (ASA).

The more powerful inhibition of platelet aggregation as tested in vitro by turbidimetry has also translated into stronger platelet inhibition in patients, particularly whenever different modes of inhibition were applied simultaneously. Stronger inhibition of platelets allowed controlling hyperactivation of the thrombotic system caused, for example, by the exposure of foreign material within the blood stream. This allowed the development of today's modern intravascular interventional treatment and in particular the placement of a metal stent to keep stenosed vessel lumen open.

While powerful platelet inhibition seems to be acceptable for a short period of time in patients following cardiovascular intervention, its long-term or chronic use, however, exposes the downside of high-strength platelet inhibition: a significant increase in the risk of bleeding complications – the most feared of which are intracranial bleeds and hemorrhagic strokes. In stroke prevention trials, no or only marginal improvement over conventional treatment with ASA has been shown by treatment with more modern or potent inhibitors of platelets [1–3]. This confirms that platelet inhibition is of benefit, but there seems to be significant differences in the risk of bleeding in different clinical settings, particularly when comparing chronic platelet inhibition for the prevention of thromboembolic complications in the coronary circulation with that of the cerebrovascular circulation.

In chronic preventive settings, even high doses of ASA as used in the past (as high as 1 g or more per day) have had no additional benefit over doses as low as 150 mg/day or even 81 mg/day, which is currently recommended despite not being independently tested in a clinical trial.

Focusing on potent inhibition of platelets, however, obscured the view of other major players controlling the formation of a thrombus in vivo for quite some time. The endothelial lining of blood vessels and other cellular and noncellular components of the vessel wall were only discovered later as being of critical importance for formation and even prevention of thrombus formation. Endothelial cells, smooth muscle cells, and different compositions of the subendothelial matrix are important modulators not only of platelet activation but also of the interaction with the clotting system. Endothelial cells convert endoperoxides into prostacyclin rather that prothrombotic thromboxane A_2. At higher ASA doses, the blockade of prostacyclin synthesis becomes clinically important, particularly when blockade is prolonged over time. This supports the importance of the vascular wall on formation or prevention of thrombosis. It also opens new opportunities to pharmacologically modulate the pro- or antithrombotic properties of the vessel wall.

Here is where the discovery of antithrombotic properties of dipyridamole was made. Mitchell [4] observed in vivo (particularly intracerebral arteries investigated for other reasons) that dipyridamole treatment resulted in the prevention of thrombus formation. This finding was further investigated in vitro, but direct inhibition of platelet aggregation was only seen in supratherapeutic concentration. It was also established, that dipyridamole blocked the reuptake of adenosine into cells, which led to increased adenosine concentration in blood [5, 6]. However, by preparing platelet-

rich plasma for turbidimetric analysis of platelet function, elevated adenosine concentration is lost and only later when tested in the presence of red blood cells does the inhibition of platelet activation by adenosine become apparent [7]. Earlier, this led many to believe that dipyridamole is only – if at all – a very weak inhibitor of thrombosis [8]. Later it was shown that dipyridamole's amplification of endogenous antithrombotic mechanisms adds well to the direct inhibition of platelet function by low-dose ASA [9].

Antithrombotic Rather than Antiplatelet Activity by Dipyridamole

While platelet function can be tested in a purified in vitro system using platelet-rich plasma, the antithrombotic function of cells lining the vessel wall can either be studied in vivo or in flow chambers recreating the interaction of whole blood with the elements of the vessel wall, i.e. endothelial lining on top of subendothelial matrix. Such a system then allows for differential treatment of the different elements contributing (endothelial cells, smooth muscle cells, subendothelial matrix, red blood cells, white blood cells, and platelets) and a quantification of its effect on thrombus formation. A test system employing endothelial cells as well as subendothelial matrix was used in an ex vivo setting to investigate dipyridamole and ASA. The treatment regimen was similar to the ESPS-2 trial. This test system was able to show very similar results as found later in the ESPS-2 trial [10, 11]. The complexity of such in vitro test systems limits its widespread use; consequently, the notion that platelet inhibition represents only a small segment of the spectrum of antithrombotic treatment is not broadly accepted. Instead, a number of different platelet tests have been developed; these are broadly used despite failing to prove predictive value for individual patient management.

The Importance of Sufficient Plasma Concentration

A more subtle modulation of prothrombotic pathways by amplifying endogenous antithrombotic mechanisms offers the opportunity to provide antithrombotic protection on a more local basis rather than shifting the balance systematically and increasing the risk of not being able to control bleeding where it is needed.

In addition to the more refined array of antithrombotic mechanisms dipyridamole addresses, its reversible activity at the many targets requires continuous and sufficient blood levels for its activities. This is in contrast to most of the commonly used pharmacological platelet inhibitors which use irreversible blockade of receptors, such as the adenosine diphosphate receptor by the thiopyridines, or irreversible blockade of cyclooxygenase by ASA.

In today's therapeutic environment, not only has individual response to drug treatment and inherent metabolic differences leading to less potent inhibition of platelet

function become an issue, but drug-drug or drug-food interaction have again come into focus as a limitation for drug treatment.

Absorption, in particular, is often dependent on sufficient acidity of the stomach. Both in elderly patients and with increased use of stomach acid suppressants such as proton pump inhibitors, the pH in the stomach may no longer allow sufficient absorption of the drug. This is also a problem for dipyridamole and, based on current knowledge, has most likely influenced older studies using an instant-release formulation. This problem had been identified and a particular formulation carrying a tartaric acid nucleus in order to provide the necessary low pH for absorption even in the presence of proton pump inhibitors has been developed [12].

Protecting Tissue – Pleiotropic Effects

Dipyridamole predominantly blocks phosphodiesterase (PDE) 5 rather than PDE3. The latter results in direct inhibition of platelet function, whereas the inhibition of PDE5 results in amplifying the nitric oxide (NO) pathway, i.e. even under reduced NO availability, inhibition of PDE5 reduces the decay of cGMP, thus showing additive effects to therapies resulting in NO increase (fig. 1). Other medications known to increase NO, such as statin treatment, have been shown to be additive to dipyridamole when it comes to reduction of ischemic damage such as in experimental myocardial infarction or stroke [13, 14].

Dipyridamole is a potent blocker of the nucleotide transporter which results in the inhibition of adenosine reuptake into cells. This increases the level of circulating adenosine in plasma, but more so in the intracellular space. Adenosine is not only known for its strong inhibition of platelets, but also for its tissue protective effects (preconditioning) in the chronic setting.

Ischemic preconditioning of tissue is thought to be caused by the accumulation of adenosine in the intercellular space. Pharmacologically, this cannot be achieved by infusion of adenosine, but has been demonstrated by microdialysis after treatment with extended-release dipyridamole.

Recently, the PRoFESS trial [15] has shown that under dipyridamole treatment, fewer new or worsening of heart failure patients have been observed. This confirmed earlier experimental and clinical data showing improved perfusion of ischemic tissue under dipyridamole unrelated to vasodilatation [16]. Initially, only vasodilatation was assumed to be of significant value [17]. Improved tissue perfusion without dilatation of large vessels or increasing perfusion pressure indicates that the microcirculation is protected and significant improvements in cardiac output and oxygen extraction and consumption has been reported in clinical studies with chronic dipyridamole therapy [18, 19].

Besides these well-known properties, several more hidden beneficial effects of dipyridamole have recently become apparent (fig. 2). It is well known that following depravation of oxygen and nutrients, derailed energy pathways lead to the intensive

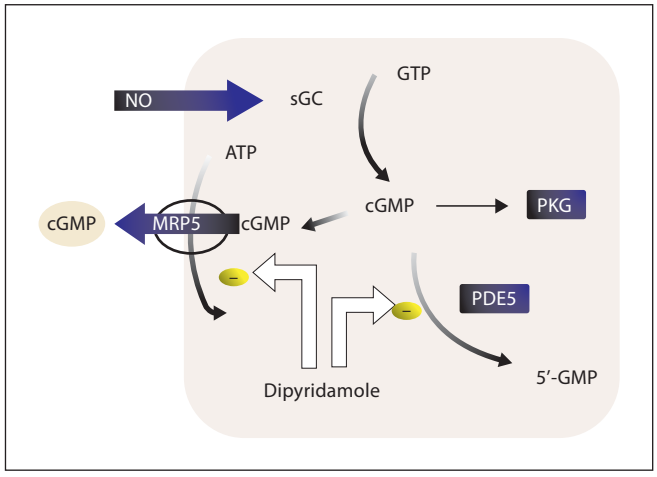

Fig. 1. Dipyridamole inhibits intracellular PDE5, which leads to an increase of intracellular concentration of cGMP. This amplifies the effect of NO which has been described to be elevated by statin treatment. Dipyridamole also inhibits the transmembranal nucleoside transporter, which has been associated with multidrug resistance.

intracellular formation of radicals. Most of the attention has been directed in the past toward scavenging of oxyradicals, which are water soluble. Potentially more dangerous, however, are lipid soluble peroxy radicals. Dipyridamole has been shown to be the most potent orally available scavenger of peroxy radicals, stronger than vitamin E.

In contrast to water-soluble oxyradicals, which propagate spherically within the cell and whose damaging density will therefore decline with distance from the source, lipid soluble peroxy radical species are confined to the lipids within the cell, i.e. intracellular membrane structures [20]. This also indicates that spatial concentration of peroxy radical species may be very high within lipid structures/membranes and therefore has a greater opportunity to interact and potentially damage transmembranal proteins. The significance of three-dimensional organizations within the cell given by intracellular membranes has become apparent in the past. With significant perturbation of membrane structures, the organization of interacting proteins within the cell become jeopardized and in many cases dysfunctional [20]. Maintaining the structure of membranes stabilizes those intracellular protein-protein interactions which require hydrophobic binding to membranes to maintain function. Scavenging peroxy radicals seems to protect tissue function after metabolic/oxidative stress [21].

Inhibition of Thrombin Formation

Perturbation of membrane structures – particularly the exposure of negatively charged phospholipids towards the outside of the cell surface – is not only the early sign of cell apoptosis, but also will allow the formation of protein complexes such as the prothrombinase complex, accelerating the formation of thrombin. Dipyridamole reduces the number of binding sites for the prothrombinase complex in platelets presumably

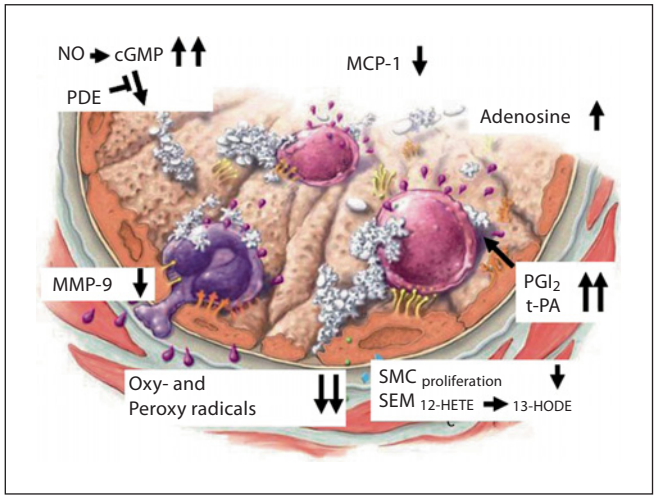

Fig. 2. Dipyridamole shows a number of effects which are more directed towards supporting an endogenous vessel wall-based antithrombotic mechanism: (1) increase of circulating adenosine, (2) increased release of t-PA and PGI_2 from endothelial cells, (3) direct blockade of gene expression of inflammatory protein MCP-1, (4) inhibits release of matrix metalloproteinase 9 (MMP-9), (5) inhibits reversibly proliferation of smooth muscle cells, (6) changes subendothelial matrix component 12-HETE to less thrombogenic 13-HODE, (7) scavenges peroxy- and oxyradicals under oxidative and metabolic stress, and (8) reduces cell membrane disturbance, which leads to increased binding of prothrombinase complex.

by its peroxy radical scavenging properties [20, 21]. The number of prothrombinase-binding sites was found to be almost doubled in platelets showing limited or no inhibition by conventional platelet inhibitors. In vitro treatment of these platelets with dipyridamole reduced the number of annexin 5-binding sites (prothrombinase complex-binding sites) [22, 23], suggesting that in platelets with highly disturbed membrane structures, excessive thrombin formation might override the inhibition by conventional platelet inhibitors, which might be corrected by simultaneous treatment with dipyridamole. This scavenging property is also assumed to be the reason for reducing the accumulation of fibrinogen/fibrin in in vivo studies following severe vessel wall damage by overstretching the artery by balloon angioplasty. The level of reduction of fibrinogen/fibrin accumulation at this site was found to be even higher when compared to therapeutic doses of heparin [24]. This was surprising as the treatment with dipyridamole did not change the clotting or platelet function tests ex vivo.

Inhibition of Smooth Muscle Cell Proliferation – Prevention of Restenosis

Mechanical damage to the wall of larger vessels such as during balloon angioplasty, surgical interventions, or stenting leads to reactive proliferation of smooth muscle

cells in about 30% of the cases. Besides minimizing the trauma during intervention, only local application of cytostatic medication, such as coating of stents, has provided protection; however, there is the disadvantage of the endothelium of the vessel not being able to cover the stent and restore endogenous protection from thrombosis. It has been shown in vitro and experimentally in vivo that dipyridamole reversibly blocks smooth muscle cell proliferation after balloon angioplasty in animals. Later in a large multicenter clinical trial, it was shown that dipyridamole, when given in its present form of extended-release dipyridamole plus low doses of ASA (Aggrenox), does inhibit smooth muscle cell proliferation at the anastomosis of the dialysis shunt, and it was shown for the first time that pharmacological intervention can extend the unassisted life span of a newly implanted dialysis shunt [25].

A smaller clinical study showed that the addition of extended-release dipyridamole plus low-dose ASA (Aggrenox) on top of conventional platelet inhibition by a high dose of clopidogrel and ASA leads to the inhibition of restenosis after placement of uncoated stents in the carotid artery (personal communication from J. English).

Inhibiting Innate Inflammation

The most intriguing finding of the recent past has been dipyridamole's ability to reduce innate inflammation (fig. 2). It was found that dipyridamole directly inhibits the gene expression of MCP-1 and reduces the production of matrix metalloproteinase-9 [26]. Innate inflammation has been described as a common pathway of endothelial and vascular damage in patients with smoking habits, hypertension, or metabolic disease, and has been seen as responsible for the destruction of vessel wall structures as seen in stroke victims. Therefore, lowering innate inflammation has been viewed as important for preventing vessel wall disease and in particular dysfunction of small vessels and microcapillaries. This might also explain the reduction in new and worsening heart failure in a recent stroke trial (PRoFESS). This study confirmed an earlier pilot study of improvement of cardiac output and oxygen consumption measured during a 6-month treatment period by functional MRI [19].

Conclusion

While dipyridamole in its early life was simplistically wrongly named a platelet inhibitor, we now know that dipyridamole is effective if sufficient plasma concentration can be maintained, such as with the modern formulation (Aggrenox), in many ways as an antithrombotic rather than an antiplatelet treatment.

Through its radical scavenging properties (which may be the explanation for the previous observation of improved tissue protection in organ transplantation), reduction of innate inflammation (hereby counteracting a well-accepted risk factor for

vascular events), additivity to statin treatment by sustaining the NO production, inhibition of smooth muscle cell proliferation and prevention of restenosis, and indirect antithrombotic properties, dipyridamole seems to be able to help in many ways in patients with vascular or thromboembolic complications; however, by itself it will not be a sufficiently potent inhibitor of platelets to replace existing antiplatelet therapy in the acute setting. Much like in the prevention of stroke, dipyridamole has shown properties which might help patients in the chronic setting in addition to antiplatelet therapy. However, even though older literature has shown positive data, it needs to be studied in patients in today's clinical setting.

References

1 Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, Buring J, Hennekens C, Kearney P, Meade T, Patrono C, Roncaglioni MC, Zanchetti A: Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet 2009;373:1849–1860.

2 The Dutch TIA Trial Study Group: A comparison of two doses of aspirin (30 mg vs 283 mg a day) in patients after a transient ischemic attack or minor ischemic stroke. N Engl J Med 1991;325:1261–1266.

3 Diener HC, Bogousslavsky J, Brass LM, Cimminiello C, Csiba L, Kaste M, Leys D, Matias-Guiu J, Rupprecht HJ, MATCH Investigators: Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): randomised, double-blind, placebo-controlled trial. Lancet 2004; 364:331–337.

4 Mitchell JRA: Theory and practice in prevention of arterial thrombosis. Br Heart J 1982;48:417.

5 Klabunde RE, Althouse DG: Adenosine metabolism in dog whole blood: effects of dipyridamole. Life Sci 1981;28:2631.

6 Klabunde RE: Dipyridamole inhibition of adenosine metabolism in human blood. Eur J Pharmacol 1983;93:21–26.

7 Eisert WG: How to get from antiplatelet to antithrombotic treatment. Am J Ther 2001;8:443–449.

8 Emmons PR, Harrison MJG, Honour AJ, Mitchell JRA: Effect of dipyridamole on human platelet behaviour. Lancet 1965;286:603.

9 Eisert WG, Müller TH: Dipyridamole – evaluation of an established antithrombotic drug in view of modern concepts of blood cell-vessel wall interactions. Thrombosis Research 1990;57(suppl 3):65–72.

10 Mueller TH, Su CAPF, Weisenberger H, Brickl R, Nehmiz G, Eisert WG: Dipyridamole alone or combined with low-dose acetylsalicylic acid inhibits platelet aggregation in human whole blood ex vivo. Br J Clin Pharmac 1990;30:179–186.

11 Diener HC, Cunha L, Forbes C, Sivenius J, Smets, Lowenthal A: European Stroke Prevention Study 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. J Neurol Sci 1996; 143:1–13.

12 Derendorf H, VanderMaelen CP, Brickl RS, MacGregor TR, Eisert W: Dipyridamole bioavailability in subjects with reduced gastric acidity. J Clin Pharmacol 2005;45:845–850.

13 Ye Y, Perez-Polo RR, Birnbaum Y: Protecting against ischemia-reperfusion injury: antiplatelet drugs, statins, and their potential interactions. Ann NY Acad Sci 2010;1207:76–82.

14 Kim H-H, Sawada N, Soydan G, Lee H-S, Zhou Z, Hwang S-K, Waeber C, Moskowitz MA, Liao JK: Additive effects of statin and dipyridamole on cerebral blood flow and stroke protection. J Cereb Blood Flow Metab 2008;28:1285–1293.

15 Sacco RL, et al; PRoFESS Study Group: Aspirin and extended-release dipyridamole versus clopidogrel for recurrent stroke. N Engl J Med 2008;359:1238–1251.

16 Roberts AJ, Jacobstein JG, Cipriano PR, Alonso DR, Combes JR, Gay WA Jr: Effectiveness of dipyridamole in reducing the size of experimental myocardial infarction. Circulation 1980;61:228–236.

17 Packer M, Gorlin R, Meller J, Medina N: Central hemodynamic effects of dipyridamole in severe heart failure: comparison with hydralazine. Clin Pharmacol Ther 1982;32:54–61.

18 Sanada S, Asanuma H, Koretsune Y, Watanabe K, Nanto S, Awata N, Hoki N, Fukunami M, Kitakaze M, Hori M: Long-term oral administration of dipyridamole improves both cardiac and physical status in patients with mild to moderate chronic heart failure: a prospective open-randomized study. Hypertens Res 2007;30:913–919.
19 Akhtar M, Ordovas K, Martin A, Higgins CB, Michaels AD: Effect of chronic sustained-release dipyridamole on myocardial blood flow and left ventricular function in patients with ischemic cardiomyopathy. Congest Heart Fail 2007;13:130–135.
20 Kusmic C, Picano E, Busceti C L, Petersen C, Barsacchi R: The antioxidant drug dipyridamole spares the vitamin E and thiols in red blood cells after oxidative stress. Cardiovasc Res 2000;47:510–514.
21 Chakrabarti S, Vitseva O, Iyu D, Varghese S, Freedman JE: The effect of dipyridamole on vascular cell-derived reactive oxygen species. J Pharmacol Exp Ther 2005;315:494–500.
22 Serebruany V, Malinin A, Ziai W, Atar D, Pokov A, Jilma B, Hanley D: Dipyridamole decreases protease-activated receptor and annexin-V binding on platelets of post stroke patients with aspirin nonresponsiveness. Cerebrovasc Dis 2006;21:98–105.
23 Serebruany VL, Malinin AI, Pokov AN, Hanley DF: Antiplatelet profiles of the fixed-dose combination of extended-release dipyridamole and low-dose aspirin compared with clopidogrel with or without aspirin in patients with type 2 diabetes and a history of transient ischemic attack: a randomized, single-blind, 30-day trial. Clin Ther 2008;30:249–259.
24 van Ryn J, Lorenz M, Merk H, Buchanan MR, Eisert WG: Accumulation of radiolabelled platelets and fibrin on the carotid artery of rabbits after angioplasty: effects of heparin and dipyridamole. Thromb Haemost 2003;90:1179–1186.
25 Dixon BS, Beck GJ, Vazquez M A, Greenberg A, Delmez J A, Allon M, Dembe LM, Himmelfarb J, Gassman JJ, Greene T, Radeva M K, Davidson IJ, Ikizler TA, Braden GL, Fenves AZ, Kaufman JS, Cotton JR, Martin KJ, McNeil JW, Rahman A, Lawson JH, Whiting JF, Hu B, Meyers CM, Kusek JW, Feldman HI, for the DAC Study Group: Effect of dipyridamole plus aspirin on hemodialysis graft patency. N Engl J Med 2009;360:2191–2201.
26 Weyrich A, Denis MM, Kuhlmann-Eyre JR, Spencer ED, Dixon DA, Marathe GK, McIntyre TM, Zimmerman GA, Prescott SM: Dipyridamole selectively inhibits inflammatory gene expression in platelet-monocyte aggregates. Circulation 2005;111:633–642.

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