Review

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Mechanisms of the beneficial effects of vitamin B6 and pyridoxal 5-phosphate on cardiac performance in ischemic heart disease

Abstract

Although vitamin B6 and its metabolite, pyridoxal 5′-phosphate (PLP), have been shown to exert beneficial effects in ischemic heart disease, the mechanisms of their action are not fully understood. Some studies have shown that ventricular arrhythmias and mortality upon the occlusion of coronary artery were attenuated by pretreatment of animals with PLP. Furthermore, ischemia-reperfusion-induced abnormalities in cardiac performance and defects in sarcoplasmic reticular Ca^{2+}-transport activities were decreased by PLP. The increase in cardiac contractile activity of isolated heart by ATP was reduced by PLP, unlike propranolol, whereas that by isoproterenol was not depressed by PLP. ATP-induced increase in $[Ca^{2+}]_i$, unlike KCl-induced increase in $[Ca^{2+}]_i$ in cardiomyocytes was depressed by PLP. Both high- and low-affinity sites for ATP binding in sarcolemmal membranes were also decreased by PLP. These observations support the view that PLP may produce cardioprotective effects in ischemic heart disease by attenuating the occurrence of intracellular Ca^{2+} overload due to the blockade of purinergic receptors.

Keywords: cardiac performance; ischemia-reperfusion injury; myocardial infarction; pyridoxal 5-phosphate; vitamin B6.

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Introduction

Vitamin B6 (pyridoxine) is a water-soluble vitamin and is present in different types of foods including whole grains, legumes, potatoes, nuts, fish, and poultry [1]. It is an essential vitamin because it is known to participate in numerous enzymatic reactions including protein metabolism, neurotransmitter formation as well as conversion of tryptophan to niacin and homocysteine to methionine [2]. Pyridoxine is converted by all organs of the body to pyridoxal 5-phosphate (PLP) and pyridoxamine, which serve as coenzymes for transaminase reaction [3]. Although supplementation of vitamin B6 and its active metabolite, PLP, has been reported to reduce the complications associated with coronary artery disease, diabetes, hypertension, aging, and neurodegenerative disorders [4–10], the underlying mechanisms are not fully understood. The purpose of this article is to review the role of vitamin B6 and PLP in the prevention of ischemic heart disease and to discuss the cellular and molecular mechanisms of their therapeutic actions. In particular, the status of different risk factors for ischemic heart disease in vitamin B6 deficiency with or without vitamin B6 and PLP treatments will be emphasized. Furthermore, the existing information on the mode and site of action of PLP during the development of cardiac dysfunction in ischemic heart disease will be discussed.

Cardiovascular complications in vitamin B6 deficiency

Vitamin B6 deficiency has been shown to be associated with the development of atherosclerosis and coronary artery disease [11]. It has also been reported to promote atherosclerosis directly as a result of its effects on vascular tissue [12] or indirectly as a result of the elevation of plasma homocysteine [11, 13]. In this regard, it should

be noted that hyperhomocysteinemia is considered to be a well-known risk factor for coronary heart disease, myocardial infarction, and heart failure [14–16]. Several reports have also indicated a relationship among vitamin B6 deficiency, elevated levels of homocysteine, and coronary artery disease [11, 17–23]. Low levels of vitamin B6 or PLP and elevated levels of homocysteine have also been observed in patients with rheumatoid arthritis [24] and Alzheimer disease [25]. Vitamin B6 deficiency has been shown to affect the antioxidant defense mechanisms in the liver and the heart [26] and thus can be seen to promote the occurrence of oxidative stress. Low circulating levels of vitamin B6 or PLP have also been reported to be associated with elevated levels of biomarkers for systemic inflammation involving C-reactive proteins and kynurenine [27–29]. Although there is a close relationship among vitamin B6 deficiency, hyperhomocysteinemia, and markers of inflammation with cardiovascular complications, it is not clear if the effects of vitamin B6 deficiency on coronary artery disease are mediated through the elevated levels of homocysteine, C-reactive proteins, or kynurenine. A close analysis of the results in these studies indicates that vitamin B6 deficiency, hyperhomocysteinemia, oxidative stress, and C-reactive proteins may be independent markers of coronary artery disease.

Patients with myocardial infarction were found to have low levels of vitamin B6 [20, 22]. Furthermore, the risk of developing myocardial infarction in patients with degenerative diseases was observed to be markedly reduced upon treatment with vitamin B6 [21]. Vitamin B6 deficiency in pregnant women or in females on anovulatory steroid therapy has also been shown to be associated with hypertension [30, 31]. Rats on vitamin B6-deficient diet were also observed to exhibit hypertension [32, 33]. Because the norepinephrine turnover in hearts of vitamin B6-deficient animals was markedly increased [34], it has been suggested that hypertension in vitamin B6 deficiency may be mediated through the augmented activity of the sympathetic nervous system. The induction of hypertension due to vitamin B6 deficiency was also reported to be due to an increase in Ca^{2+} influx in the vascular smooth muscle [35]. The Ca^{2+}-handling abilities of cardiomyocytes obtained from vitamin B6-deficient rats were also examined in the absence and presence of KCl or ATP [36]. In this study, KCl-induced increase in $[Ca^{2+}]_i$ was observed to be augmented without any changes in the basal level of $[Ca^{2+}]_i$. Such an increase in $[Ca^{2+}]_i$ due to depolarization of cardiomycytes by KCl can be seen to induce intracellular Ca^{2+} overload in the myocardium and lead to the development of cardiac dysfunction in vitamin B6-deficient rats [36]. Meanwhile, ATP-induced increase in $[Ca^{2+}]_i$ in cardiomyocytes from vitamin B6-deficient animals was reduced due to a depression in the number of ATP-binding sites in the sarcolemmal membrane [36]. These alterations in KCl- and ATP-induced increases in $[Ca^{2+}]_i$ as well as loss of ATP-binding in vitamin B6 deficiency were reversible upon treatment with vitamin B6 and can be explained on the basis of membrane abnormalities in cardiac membranes of the vitamin B6-deficient animals.

Cardioprotective effects of vitamin B6 and PLP in ischemic heart disease

Several studies have shown that vitamin B6 and its major metabolite, PLP, exert anti-ischemic effects in the heart [1, 9, 21, 22, 37]. PLP has also been reported to reduce ischemic injury to the brain [38]. In a rat model of myocardial infarction, PLP has been demonstrated to reduce infarct size and improve cardiac function [9, 37]. A reduction of the ischemia-reperfusion (I/R) injury and infarct size was also seen in isolated rat hearts [9, 37]. A phase II clinical trial, in which PLP was given to patients undergoing percutaneous coronary intervention, showed a decrease in infarct size 24 h after angioplasty [39]. In 901 high-risk patients undergoing coronary artery bypass graft (CABG) surgery, PLP resulted in a significant decrease in preoperative myocardial infarction but did not affect the prespecified primary end point [40]. A reduction in cardiovascular death and myocardial infarction by PLP in the high-risk patients undergoing CABG was found to be independent of cross clamp time [41]. However, in another study, in which 3023 intermediate- to high-risk patients undergoing CABG were used as subjects, PLP did not show any significant effect on cardiovascular death or non-fatal myocardial infarction [42, 43]. Although the exact reason for the negative results of this study using intermediate- to high-risk patients for CABG [42, 43] is not clear, the clinical studies using high-risk patients for CABG [40, 41] as well as in patients following angioplasty [39] show a high potential for PLP therapy in ischemic heart disease.

In view of the close association of arrhythmias and mortality in subjects with ischemic heart disease, the beneficial effects of vitamin B6 and PLP on ischemia-induced arrhythmias were tested upon occluding the coronary artery in normal healthy rats [44, 45], and the results are shown in Table 1. Pretreatment of animals for 2 days with PLP, unlike vitamin B6, was observed to delay the onset of arrhythmias upon coronary occlusion.

Parameters	Untreated MI (n=7)	Vitamin B6-treated MI (n=8)	PLP-treated MI (n=8)
Time of onset of arrhythmias, s	45±9.6	47±8.4	125±11.2[a]
ST segment, mV	0.18±0.02	0.19±0.01	0.10±0.02[a]
QTc interval, ms	554±26	566±31	427±22[a]
Number of PVC, %	5.0	5.2	1.4[a]
Incidence of ventricular tachycardia, %	67	61	12[a]
Mortality within first day, %	35	30	16[a]

Table 1 Effects of vitamin B6 and its metabolite PLP on ventricular arrhythmias and mortality due to myocardial infarction (MI) in rats. Treatment of rats with or without vitamin B6 (50 mg/kg, daily) or PLP (25 mg/kg, daily) was started 2 days before inducing MI by occluding the coronary artery. The procedures for inducing MI and monitoring electrocardiographic changes (lead II) and the analysis of data for ST segment, QTc interval, PVCs, incidence of ventricular tachycardia, and mortality at the end of first day are similar to those described elsewhere [44, 45]. [a]p<0.05 in comparison with untreated MI.

In addition, marked alterations in different electrocardiographic parameters, including ST segment, QTc interval, number of premature ventricular contraction(PVC), and incidence of tachycardia as well as mortality, observed in animals at 1 day after occluding the coronary artery were attenuated by PLP (Table 1). The ineffectiveness of vitamin B6 in modifying the coronary occlusion-induced arrhythmias under this acute experimental setting may be related to insufficient dose or duration of vitamin B6 treatment. To gain information if PLP exerts any direct beneficial effects on the I/R-induced changes in cardiac performance, isolated perfused hearts were subjected to 30 min of global ischemia followed by reperfusion for 30 min [46]. The results in Table 2 show that depressions in left ventricle developed pressure (LVDP), rate of pressure development (+dP/dt), and rate of pressure decay (-dP/dt) as well as increase in left ventricular end diastolic pressure due to I/R injury were reduced by PLP in a dose-dependent manner. Because the sarcoplasmic reticulum (SR), by virtue of its ability to regulate intracellular Ca^{2+}, plays a central role in determining the status of cardiac contraction and relaxation [47], SR Ca^{2+}-uptake and SR Ca^{2+}-release activities of hearts subjected to I/R injury were examined [46] with or without PLP treatment. The results in Table 2 indicate that the I/R-induced defects in SR Ca^{2+} transport were attenuated significantly by PLP treatment. These observations provide evidence regarding the potential use of PLP as a therapeutic intervention for the prevention of ischemic heart disease.

Mechanisms of PLP action in the ischemic heart disease

Despite several studies showing the anti-ischemic effects of vitamin B6 and PLP [19, 20–22, 37], the mechanisms of the PLP effect on the ischemic myocardium are far from clear. Because PLP has been reported to inhibit acetyl-CoA carboxylase [48], which is known to play an important role in synthesis, elongation, and oxidation of long-chain fatty acids, it can be argued that the beneficial effects of PLP are mediated through the reduction of excessive utilization

Parameters	Control (n=6)		I/R injury	
		Untreated (n=4)	10 μM PLP (n=4)	25 μM PLP (n=4)
A. Cardiac performance				
LVDP, mm Hg	118±5.4	39±2.1[a]	72±4.4[b]	96±5.2[b]
LVEDP, mm Hg	7.8±0.6	62±3.7[a]	44±2.8[b]	25±3.1[b]
+dP/dt, mm Hg/s	2680±134	488±16.7[a]	1420±21.9[b]	2278±87[b]
-dP/dt, mm Hg/s	2346±125	396±15.4[a]	1360±18.6[b]	2042±74[b]
B. SR Ca^{2+} transport				
Ca^{2+} uptake, nmol Ca^{2+}/mg/min	28.6±0.77	7.9±0.46[a]	13.6±1.2[b]	20.4±0.65[b]
Ca^{2+} release, nmol Ca^{2+}/mg/15 s	7.2±0.24	2.3±0.18[a]	4.5±0.31[b]	5.9±0.23[b]

Table 2 Effects of PLP on cardiac performance and SR Ca^{2+} transport in isolated rat hearts subjected to ischemia-reperfusion injury. I/R injury was induced in isolated rat hearts by subjecting to 30 min global of ischemia followed by 30 min of reperfusion. The data for LVDP, LVEDP, +dP/dt, and -dP/dt are mean±SE of the number of hearts shown in each column. Hearts were treated with 10 or 25 μM PLP 10 min before inducing global ischemia as well as throughout the reperfusion period. At the end of the experiments, hearts were removed from the perfusion apparatus, SR vesicles were isolated, and Ca^{2+}-uptake as well as Ca^{2+}-release activities were determined. All the procedures for the perfusion of isolated hearts, induction of I/R injury, recording of parameters for cardiac performance, isolation of SR vesicles, and determination of Ca^{2+}-uptake as well as Ca^{2+}-release activities were the same as described earlier [46]. [a]p<0.05 vs. control; [b]p<0.05 vs. untreated.
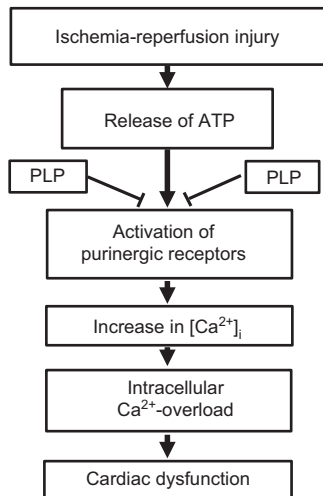
Figure 1 Proposed site of action of PLP in events involving the release of ATP and activation of purinergic receptors and thus leading to the development of intracellular Ca^{2+} overload and cardiac function due to I/R injury.

of fatty acids in the ischemic heart. In fact, different PLP-related compounds have been reported to exert cardioprotective effects on the ischemic myocardium by inducing a shift from fatty acid oxidation toward glucose oxidation [49]. Vitamin B6 compounds, including PLP, have also been shown to prevent the oxygen radical generation and lipid peroxidation caused by oxidative stress [50] and are thus likely to protect the ischemic myocardium through their antioxidant properties. In view of their inhibitory effect on the sympathetic nervous system [51, 52], vitamin B6 and PLP can also be seen to produce anti-ischemic actions on the heart by reducing the sympathetic system activity and associated adrenoceptor-linked signal transduction mechanisms. In addition, the improvement of cardiac function by vitamin B6 and PLP may be occurring through the protection of endothelium in the ischemic heart because vitamin B6 has been reported to improve the endothelial function in cardiac transplant recipients [53]. Nonetheless, the involvement of purinergic receptor blockade in the beneficial actions of PLP on ischemic myocardium is most likely because suramin and various PLP derivatives such as pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) with purinergic P2 receptor antagonist properties have been reported to prevent I/R injury [54–58]. Thus, vitamin B6 and PLP appear to be acting on multiple sites including acetyl-CoA carboxylase, oxidative stress, sympathetic nerves, endothelium, and purinergic receptors for attenuating different abnormalities in the ischemic heart disease.

Although cardiac dysfunction in the heart due to I/R injury has been suggested to be due to the occurrence of oxidative stress and development of intracellular Ca^{2+}

overload [59, 60], there is evidence to suggest that ATP is released in the extracellular space in the ischemic myocardium [52, 61–63]. The extracellular ATP has been shown to induce Ca^{2+} influx in cardiomyocytes through the activation of purinergic receptors [64–67] and thus may contribute to the development of intracellular Ca^{2+} overload and subsequent cardiac dysfunction. These events due to I/R injury are shown in Figure 1, where the blockade of purinergic receptors by PLP has been indicated to attenuate the I/R-induced cardiac dysfunction. In view of the therapeutic potential of PLP for the prevention of ischemic heart disease [37], it seems appropriate to review the evidence for the action of PLP at the purinergic receptors in the myocardium. By using an isolated perfused heart, an extensive study was undertaken to examine if the positive inotropic effect of ATP was attenuated by PLP [58]. It was observed that the time-dependent increases in LVDP, +dP/dt, and -dP/dt by ATP were prevented by pretreatment with PLP. The depressant effect of PLP on ATP-induced increase in LVDP was evident at different concentrations of ATP and was observed to be a concentration-dependent action [58]. Furthermore, the antagonistic effect of PLP on ATP-induced increase in LVDP was of a specific nature because PLP did not affect the isoproterenol-induced increase in LVDP, whereas the ATP-induced increase in LVDP was not affected by propranolol, a well-known β-adrenoceptor blocker [58]. In another set of experiments, PLP was observed to depress the ATP-induced increase in $[Ca^{2+}]_i$ without affecting the basal level of $[Ca^{2+}]_i$ in adult cardiomyocytes [58]. Because the KCl-induced increase in $[Ca^{2+}]_i$ in cardiomyocytes was not affected by PLP, it appears that the effect of PLP on ATP-induced changes in $[Ca^{2+}]_i$ was of a specific nature. Furthermore, the depressant effect of PLP on ATP-induced increase in $[Ca^{2+}]_i$ was evident at different concentrations of ATP, and PLP produced a dose-dependent inhibitory action on an ATP-induced increase in $[Ca^{2+}]_i$ [58] in cardiomyocytes. It was also observed that PLP reduced the maximal binding of ATP at both the high- and low-affinity sites in the sarcolemmal membrane isolated from the myocardium; this action of PLP was simulated by suramin, a well-known purinergic receptor antagonist [58]. In addition, PLP was observed to depress ATP binding at both high- and low-affinity binding sites in the sarcolemmal membrane in a concentration-dependent manner [58]. These observations provide a compelling evidence that PLP is a specific purinergic receptor antagonist. It is thus evident that the improvement of cardiac function in ischemic heart disease upon treatment with PLP is partly mediated through the blockade of purinergic P2 receptors.

It should be mentioned that PLP was found to exert cardioprotective effects with respect to I/R-induced changes

in the rate-pressure product, creatine kinase release, and necrotic area of the isolated rate heart [55]. This cardioprotection by PLP was suppressed by treatment with protein kinase inhibitor (H89) and phospholipase C blocker (U73122), indicating the involvement of P2Y receptor-mediated signal transduction in PLP-induced cardiac preconditioning [55]. Purinergic P2Y antagonists, suramin and Reactive Blue, have also been reported to exert ischemic preconditioning in the rat heart [68]. In control hearts, diadenosine pentaphosphate was observed to produce transient coronary vasoconstriction followed by marked vasodilatation, which are alterations modified by I/R [69]. Furthermore, the vasoconstriction response was inhibited by the blockade of P2X receptors by PPADS, whereas the vasodilatation was attenuated by the P2Y blocker, Reactive Blue in the I/R hearts [69]. The blockade of P2X receptors by PPADS was also found to attenuate the exercise-induced pressor reflex in heart failure [70] as well as after circulatory occlusion [71]. The activation of cardiac sympathetic afferents, which leads to chest pain and reflex cardiovascular response, by the endogenously released ATP during ischemia was blocked by PPADS [52]. ATP-induced alterations in both the excitatory and the inhibitory neurotransmission to cardiac vagal neurons in the brainstem were also prevented by PPADS [72, 73]. Both suramin and PPADS were observed to attenuate ATP-induced arrhythmias in isolated hearts as well as ATP-induced activation of depolarizing membrane currents and increase in $[Ca^{2+}]_i$ in isolated cardiomyocytes [74]. PPADS was also reported to reduce the purinergic receptor-mediated permeation of vascular endothelial cells [75]. On the basis of these observations, it is suggested that the cardioprotective effects of the purinergic receptor blockade by PLP, PPADS, suramin, and Reactive Blue in ischemic heart disease may be occurring at the level of neurons, endothelium, coronary vasculature, and cardiomyocytes.

Although ATP and PLP are considered to be broad-based purinergic P2 receptor agonists and antagonists, respectively, some specific P2X and P2Y receptor agonists and antagonists have been identified. In this regard, α,β-methylene ATP and the *N*-methanocarba derivative of 2-chloro-AMP (MRS-2339) were found to serve as P2X receptor agonists [52, 76, 77], whereas 2-methylthio-ATP and ATPγS were observed to activate P2Y receptor agonists [78–80]. Meanwhile, compound NF-279 was shown to serve as a selective P2X receptor antagonist [81], whereas agents such as PPADS, suramin, and Reactive Blue are considered to be P2Y receptor antagonists [69, 74, 82]. Although extensive studies have to be carried out to establish the specificity of these purinergic receptor agonists and antagonists, it appears that PLP and P2Y receptor antagonists may produce beneficial effects in reducing the I/R injury

[9, 37, 39, 52, 74]. It is pointed out that novel P2X receptor agonists, MRS-2339 as well as 2-methylthio-ATP, have been found to improve the survival of calsequestrin knockout mice with cardiomyopathy [76]. Accordingly, it has been claimed that P2X receptors may represent an important therapeutic target for the treatment of heart failure [76]. In view of the beneficial effects of PLP and related agents in hypertension, atherosclerosis, stroke, I/R injury, myocardial infarction, and congestive heart failure [10, 11, 21, 37, 39], it is suggested that the development of drugs based on both purinergic P2 receptor agonist and antagonist properties should be encouraged for the prevention and treatment of ischemic heart disease.

Because vitamin B6, unlike PLP, was ineffective in attenuating cardiac abnormalities due to the occlusion of coronary artery under acute experimental conditions, it is likely that the reported beneficial effects of vitamin B6 [20–22] in patients with myocardial infarction may be occurring when a sufficient level of its active metabolite (PLP) is achieved in cardiomyocytes. Such an action can be seen to occur through the alterations in cardiac gene expression because moderate variations in intracellular concentration of PLP have been observed to exert profound modulatory effects on steroid-induced gene expression [83, 84]. In fact, the elevation of intracellular PLP levels have been shown to decrease transcriptional responses to glucocorticoid, progesterone, androgen, or estrogen hormones, whereas vitamin B6 deficiency exhibited enhanced responsiveness to steroid hormones [83]. These gene expression-modulatory effects of PLP were reported to involve the interruption of functional interactions between steroid hormone receptors and the nuclear transcription factor NF1 [83–87]. PLP has been shown to depress the binding of steroid hormone receptor to DNA, inhibit the ATP-stimulated translocation promoter, and serve as a transcriptional coregulator [88–90]. PLP as an active form of vitamin B6 is not only involved in a multitude of reactions including decarboxylation and transamination, it can also inhibit DNA polymerases, and several steroid receptors [91]. From the foregoing discussion on the interaction of purinergic P2 receptor agonists and antagonists, it is evident that PLP acts on purinergic receptors in the cell membrane and intracellularly on the nucleus.

Concluding remarks

In this article, we have reviewed the existing literature concerning the beneficial effects of vitamin B6 and its metabolite, PLP, in heart disease. We have presented the evidence to show that vitamin B6 may not exert its

therapeutic effects in the ischemic heart disease directly but PLP may attenuate the myocardial infarction-induced arrhythmias. The attenuation of cardiac dysfunction due to I/R injury by PLP was associated with the reduction of I/R-induced defects in SR Ca^{2+}-transport activities. Because of the observations that PLP markedly suppressed the action of ATP on cardiac contractile activity, it is evident that this agent may serve as a purinergic receptor antagonist. This view is further attested by findings that the ATP-induced increase in $[Ca^{2+}]_i$ in cardiomyocytes, as well as ATP binding at both high- and low-affinity sites in the sarcolemma, was reduced by PLP. Such information supports our contention that the preventive action of PLP in attenuating cardiac complications, associated with ischemic heart disease, may be a consequence of the blockade of purinergic receptors.

Conflict of interest statement

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