Potential role for adenosine in the pathogenesis of the vascular complications of hyperhomocysteinemia

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Abstract

Hyperhomocysteinemia is an independent risk factor for cardiovascular disease. Most previous investigations focused on the role of homocysteine as direct pathogenetic factor for these adverse vascular events. However, the exact pathophysiological mechanism is still unknown. In this review we discuss the hypothesis that a decreased extracellular concentration of adenosine could contribute to the adverse cardiovascular effects of hyperhomocysteinemia. Fundamental to this hypothesis is that, in vivo, any increase in the plasma concentration of homocysteine reflects an increased intracellular homocysteine concentration, which inevitably will result in a decrease in the adenosine concentration. In this situation, the hydrolyase reaction catalysed by S-adenosylhomocysteine hydrolase will reverse and S-adenosylhomocysteine will accumulate at the expense of adenosine. Stimulation of adenosine receptors by adenosine results in various cardio- and vasoprotective actions, like modulation of vascular resistance, presynaptic inhibition of norepinephrine release, ischaemic preconditioning, inhibition of platelet aggregation, modulation of inflammation and regulation of vascular cell proliferation and death. In this respect, a decrease in the adenosine concentration could contribute significantly to the cardiovascular effects of hyperhomocysteinemia.

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1. Introduction

Patients with homocysteinuria have severe hyperhomocysteinemia and are particularly prone to occlusive vascular disease at young age [1]. Over the last 2 decades it became evident that even a mildly elevated plasma concentration of homocysteine is an independent risk factor for cardiovascular disease [2].

Up to now, the underlying mechanism causing the cardiovascular complications in hyperhomocysteinemia has been poorly understood. Most investigators in the field focused on the role of homocysteine itself and, more recently, of S-adenosylhomocysteine [3]. In this review we discuss the hypothesis that a decreased extracellular concentration of the endogenous nucleoside adenosine could contribute to the pathogenesis of the adverse vascular effects of hyperhomocysteinemia. Fundamental to this hypothesis is that, in vivo, any increase in the plasma concentration of homocysteine will result in a decrease in...
the adenosine concentration. Since adenosine is a potent cardio- and vasoprotective substance, a decrease in the plasma concentration may contribute to the adverse cardiovascular sequelae of hyperhomocysteinemia.

2. Role of homocysteine and S-adenosylhomocysteine

Since McCully, in 1969, linked elevated plasma homocysteine concentrations with vascular disease [4], many investigations have been conducted to define the causes of atherothrombotic complications in hyperhomocysteinemia. However, up to now, no generally accepted mechanism has been described. Most investigations focused on the role of homocysteine itself.

According to most investigators, endothelial cell injury caused by hyperhomocysteinemia plays a key role in the pathogenesis of cardiovascular complications (reviews [3,5]). In vivo, hyperhomocysteinemia rapidly impairs endothelial function [6,7]. Many in vitro studies have presented evidence of effects of homocysteine on platelets, endothelial cells, vascular smooth muscle cells and on coagulation (review [3]). However, these studies were not able to provide conclusive mechanisms for the vascular damage associated with hyperhomocysteinemia. Some reports showed that even micromolar concentrations of homocysteine do not induce platelet aggregation in vitro [8]. Further, many publications which did point towards direct vasotoxic or thrombogenic effects of homocysteine in vitro are hardly relevant to the clinical situation. Often, these studies used very high concentrations reduced homocysteine (1–10 mM). This is highly unphysiologic because total homocysteine concentrations are most often between 10 and 30 μmol/l in plasma of patients with mild hyperhomocysteinemia. Moreover, in blood almost all homocysteine is present in its oxidized disulfide form and only a very small amount of 1%, or even less, of the total pool of blood homocysteine, is present in its reduced form [9]. This means that 10 mM of homocysteine in vitro is about 50 000 times the plasma level normally occurring in patients with mild hyperhomocysteinemia [10].

In summary, no coherent, generally accepted view has emerged to explain the pathophysiology of atherosclerotic and thrombotic complications in hyperhomocysteinemia. One potential explanation for these inconsistent results is that not hyperhomocysteinemia itself causes vascular damage, but that high levels of homocysteine are merely a marker for another risk factor. Recently, several studies have provided evidence that not homocysteine, but S-adenosylhomocysteine (SAH), may play an important role in the vascular complications of hyperhomocysteinemia [11–14]. When concentrations of homocysteine are high, the hydrolase reaction catalysed by the SAH hydrolase reverses and SAH is formed from homocysteine and adenosine. SAH in turn is a potent inhibitor of transmethylation reactions in the cell [15,16]. Interestingly, a recent case–control study showed that plasma SAH was a more sensitive marker of cardiovascular disease than plasma homocysteine [17]. Wang et al. showed that incubation of cultured endothelial cells with exogenous homocysteine, only in the presence of adenosine, increases intracellular levels of SAH and inhibits carboxymethylation of p21, resulting in a decreased cellular proliferation [11].

So we can conclude that hyperhomocysteinemia is an independent risk factor for cardiovascular disease and that endothelial dysfunction is the most important mediating mechanism. A direct role for homocysteine itself in the pathophysiology of cardiovascular complications, however, has not been convincingly shown. In conformity with the hypothesis that there might be other risk factors, associated with high levels of homocysteine, for the development of cardiovascular complications, we now focus on the possible role of adenosine in this pathogenesis.

3. Formation and metabolism of homocysteine in man

Fig. 1 shows the biochemical reactions of homocysteine formation, which are relevant to the current discussion. During the conversion of methionine to homocysteine, first S-adenosylmethionine is formed with the use of adenosine-5-triphosphate (ATP). In the human body, S-adenosylmethionine is the ultimate methyl donor of many vital reactions, like DNA, RNA, protein and phospholipid methylation. After demethylation, SAH is generated and further hydrolyzed by the enzyme SAH hydrolase to homocysteine and adenosine. So, the net balance shows that methionine and ATP are converted to homocysteine, a methyl group and adenosine. This pathway may significantly contribute to the production of adenosine in man (Fig. 1, left panel). Metabolic balance studies in normal adults have shown that the generation of adenosine from SAH is about 20 mmol per day [18]. Several in vitro studies have quantified the rate of adenosine production from this transmethylation pathway in rat and guinea pig heart [19–23]. It was shown that during normoxia, SAH hydrolysis significantly contributes to the intracellular adenosine production (up to one third of total). During hypoxia, however, adenosine is predominantly derived from enhanced hydrolysis of adenosine-monophosphate (AMP). It should be realized that the contribution of the transmethylation pathway to adenosine production can differ significantly between various organ systems [15].

4. Adenosine in hyperhomocysteinemia

Of all enzymes in the aforementioned metabolic pathway, only SAH hydrolase is able to function in both directions [3,15]. In fact, the equilibrium of the reaction catalyzed by SAH hydrolase favours SAH formation.
Fig. 1. Schematic representation of the biochemical reactions, which are relevant to the current discussion. Under normal conditions, adenosine is formed from the hydrolysis of SAH in man (left part of the diagram). The extracellular concentration contributes to the homeostasis of the vascular system by stimulation of specific adenosine receptors leading to a variety of effects. Under conditions of increased concentrations of homocysteine, the reaction catalysed by SAH hydrolase will function in the opposite direction (right part of the diagram).

Nonetheless, the reaction is driven in the opposite direction because the products homocysteine and adenosine are both rapidly removed in the normal in vivo situation, causing the enzyme to function in its cleavage direction. However, in case of increased levels of homocysteine, SAH will accumulate at the expense of adenosine (Fig. 1, right panel). Under these circumstances, intracellular adenosine formation is hampered, increasing the transmembrane adenosine concentration gradient that exists under normoxic conditions [24], and, consequently, the extracellular adenosine concentration is decreased.

It is essential to realise that under baseline normoxic conditions, adenosine is produced continuously in several cell types, originating from two different pathways. The first is the hydrolysis of SAH by SAH hydrolase. A second important pathway in adenosine formation is the intra- and extracellular hydrolysis of AMP by 5'-nucleotidase. Under hypoxic conditions, the hydrolysis of AMP greatly increases and predominates the other pathway. Under normoxic conditions, adenosine is quickly taken up by neighbouring cells via nucleoside transporters and is degraded to inosine by adenosine deaminase or rephosphorylated by adenosine kinase [25]. As such, the plasma concentration of adenosine is determined by the sum of these processes, and a fall in the formation of adenosine originating from the hydrolysis of SAH should inevitably result in a lower plasma adenosine level, provided that other routes of adenosine metabolism remain constant.

Indeed, several studies showed that the administration of homocysteine or homocysteine thiolactone decreases adenosine release [26,27]. In the study by Sciotti and van Wylen, microdialysis probes were used to measure the adenosine concentration in cerebral interstitial fluid (ISF) in a rat model under different conditions [26]. This study showed that infusion of homocysteine thiolactone in the probe, in concentrations of $10^{-2}$–$10^{-3}$ M, decreases basal ISF adenosine concentration and cerebral blood flow. Moreover, it showed that homocysteine thiolactone attenuates the increase in dialysate adenosine during ischaemia. A comparable experiment was conducted in perfused isolated guinea-pig hearts, in which under hypoxic conditions, homocysteine thiolactone was shown to decrease the adenosine concentration in the perfusate by more than 50%, while the tissue content of SAH increased significantly [27]. However, not all studies on this subject showed similar unequivocal results. Deussen et al. found attenuation of adenosine release with homocysteine during hypoxia, but were not able to show an effect of homocysteine on coronary flow during hypoxia [28]. Also, in the dog heart, homocysteine thiolactone was shown not to influence reactive hyperaemia, which is believed to be mediated by adenosine [29]. This discrepancy could be
explained by the multitude of mechanisms, other than adenosine, which contribute to reactive hyperaemia [30]. Although these studies convincingly illustrated the mechanism we described of the homocysteine-induced decrease in adenosine concentration, it has to be emphasized that in these experiments, supraphysiological homocysteine concentrations were used and that they do not mimic the situation of hyperhomocysteinaemia in man. Moreover, these studies showed that, although the contribution of SAH hydrolysis to basal adenosine formation is limited, under hypoxic conditions (when adenosine production is mostly from AMP hydrolysis) and with high homocysteine levels, this pathway can contribute significantly to intracellular adenosine trapping. Very recently, Chen et al. found that acute as well as chronic hyperhomocysteinaemia in rat decreases plasma and renal interstitial adenosine concentration through the inhibition of SAH hydrolase [31]. This study more closely resembled the human situation of hyperhomocysteinaemia, in that a plasma homocysteine concentration of approximately 15 μM was induced.

5. Effects of endogenous adenosine

Extracellular adenosine may exert several physiological effects by stimulation of specific adenosine receptors. These adenosine receptors can be subdivided into \( \Lambda_1 \), \( \Lambda_{2A} \), \( \Lambda_{2B} \) and \( \Lambda_3 \) receptors [32]. By stimulation of these receptors, adenosine exerts a multitude of cardio- and vasoprotective effects by interfering with numerous mechanisms that contribute to the pathogenesis of atherosclerosis and thrombosis.

Firstly, adenosine exhibits direct and indirect effects on vascular tone. Directly, predominantly via activation of \( \Lambda_2 \) receptors, adenosine induces vasodilatation [25]. Indirectly, adenosine blocks the synthesis of potent vasoconstrictor factors such as angiotensin II and norepinephrine by inhibiting renin release [33]. At the level of the vascular smooth muscle cell, adenosine has been reported to inhibit the release of the neurotransmitter norepinephrine and to reduce the postsynaptic vasoconstrictor response to \( \alpha \)-adrenoceptor-stimulation in man [34,35].

Secondly, several direct cardioprotective effects of adenosine are known. It exerts negative inotropic, chronotropic and dromotropic cardiac effects [36]. Adenosine has also been reported to play a key role in ischaemic preconditioning of the heart. This phenomenon concerns the observation that short periods of ischaemia render the myocardium resistant to a subsequent more serious ischaemic event [37]. A number of studies have shown that this endogenous cardioprotective mechanism can be reduced by adenosine receptor antagonism [38], and potentiated by dipyridamole, an adenosine uptake inhibitor [39].

Thirdly, adenosine potently inhibits the aggregation of platelets [40,41]. Moreover, adenosine exerts anticoagulant activity by downregulation of tissue factor expression on endothelial cells and by suppressing the expression of P-selectin on platelets [42,43]. These effects may well contribute to the well-known antithrombogenicity of an intact endothelial lining.

Fourthly, adenosine, at concentrations similar to those observed in vivo, is an important anti-inflammatory agent [44,45]. Adenosine receptor stimulation inhibits the activation of neutrophils and protects vascular endothelium from damage by neutrophils [46]. Moreover, adenosine inhibits TNF-α production in macrophages and monocytes [47], suppresses arachidonic acid release and leukotriene biosynthesis in human neutrophils [48], and is shown to act as an endogenous activator of cellular antioxidant enzyme systems [49].

Fifthly, adenosine has been shown to play an important role in the regulation of vascular cell proliferation and death, which plays a key role in the vascular remodelling process that leads to vaso-occlusive diseases [50]. Smooth muscle cell derived adenosine inhibits the proliferation and collagen synthesis of these muscle cells in an autocrine manner via adenosine \( \Lambda_{2B} \) receptor stimulation [51–53]. Moreover, a selective \( \Lambda_2 \) receptor agonist was shown to reduce neointimal thickening in an animal model [54]. In contrast to the growth-inhibitory effect on vascular smooth muscle cells, adenosine \( \Lambda_{2B} \) receptor stimulation induces mitogenic effects on endothelial cell [55]. Besides inhibiting the growth of vascular smooth muscle cells, adenosine has been shown to induce apoptosis of human vascular smooth muscle cells via \( \Lambda_{2B} \) receptor stimulation [56]. In this way, adenosine could restrict intimal hyperplasia in the early phase of atherosclerosis, but on the other hand, could also play a role in the formation of the necrotic core in advanced atherosclerosis. Regarding the effects of adenosine on vascular cell proliferation and death, the net effect would be to facilitate the recovery of blood vessels from injury by the inhibition of inappropriate migration and proliferation of vascular smooth muscle cells into the intima layer and promoting re-endothelialization via its mitogenic effects on endothelial cells [55].

All these effects make adenosine a powerful endogenous protector against arteriosclerotic and vaso-occlusive disorders and are thought to contribute to the well-documented cardioprotective properties of adenosine receptor stimulation [57,58]. In this respect, a decreased plasma and interstitial adenosine concentration in hyperhomocysteinaemia could significantly contribute to the cardiovascular complications of this disorder. The recent study by Chen et al. strongly supports this view by indeed showing a decreased plasma and renal interstitial adenosine concentration in hyperhomocysteinaemia in vivo [31].

A significant role for adenosine in vascular complications is not unique. Recently this was also established for hypertension in the spontaneously hypertensive rat (SHR). Extracellular adenosine levels in cultured vascular smooth muscle cells were significantly lower in SHR than in Wistar Kyoto rats (WKY) and this difference mediated the
enhanced proliferation of vascular smooth muscle cells in SHRs [59]. These results imply that a decrease in adenosine concentration may play a role in the cardiovascular complications of hypertension, in line with the present discussion concerning the pathophysiology of hyperhomocysteinemia. To explore the situation in hyperhomocysteinemia further, the latter study could easily be replicated in CBS deficient mice, an animal model for hyperhomocysteinemia [60]. Moreover, in future experiments, using microdialysis techniques, we will measure interstitial adenosine concentrations in patients with hyperhomocysteinemia and controls to evaluate the role of adenosine in the human situation.

6. Conclusion

Increased levels of homocysteine will reverse the biochemical reaction catalysed by SAH hydrolase, leading to a decreased adenosine formation, or even to net extraction of adenosine from the extracellular compartment. Because of the beneficial effects of adenosine in the cardiovascular system, reduced adenosine formation during hyperhomocysteinemia may be a significant factor in the pathogenesis of the cardiovascular sequelae of this condition.

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