The effects of adenosine on plasma homocysteine levels and some other biochemical parameters

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Özet

Adenozinin Plazma Homosistein ve Bazı Biyokimyasal Parametreler Üzerine Etkileri

Adenozin kardiyovasküler hastalıkların fizyolojisi ve gelişiminde önemli bir role sahiptir. Bazı klinik ve epidemiyolojik çalışmalar ateroskleroz gelişiminde yüksek plazma homosistein düzeylerinin önemini ortaya koymuştur. Yüksek plazma homosistein düzeylerinin koroner, kardiyovasküler ve periferik damar hastalıklarında bağımsız bir risk faktörü olduğu da gösterilmiştir. Bu çalışmada, ekzojen olarak adenozin verilmesinin plazma homosistein düzeylerine ve bazı biyokimyasal parametreler üzerine olan etkisi araştırıldı. 20 yetişkin Balb-c cinsi sıçan çalışmaya dahil edildi. Deney grubuna (n=10) 0,2 ml intraperitoneal yolla 3 gün (2 kez/gün) 30 mg/kg adenozin verildi. Kontrol grubuna (n=10) ise 0.2 ml 0.09% NaCl aynı yolla uygulandı. Eter anestezisi altında intrakardiyak yoldan alınan kan örnekleri heparinli tüplere konuldu. Plazma homosistein, total kolesterol, HDL-kolesterol, trigliserid, C-reactif protein, alkalen fosfataz, aspartat amino transferaz, alanin amino transferaz, gama-glutamil transferaz düzeyleri ölçüldü. LDL-kolesterol and VLDL-kolesterol düzeyleri uygun formüllerle hesaplandı. Gruplar arasındaki farklılıklar Mann-Whitney U testi ile analiz edildi. Gruplar arasında plazma homosistein ve diğer biyokimyasal parametrelerde anlamlı farklılık bulunamadı. Bu sonuçlar, eksojen olarak adenozin uygulanmasının plazma homosistein, total kolesterol, HDL-kolesterol, trigliserid, C-reactif protein, alkalen fosfataz, aspartat amino transferaz, alanin amino transferaz, gama-glutamil transferaz, LDL-kolesterol ve VLDL-kolesterol düzeylerini etkilemediğini göstermektedir.

Anahtar kelimeler: Adenozin, homosistein, fare, biyokimyasal parametreler

Abstract

Adenosine has an important role in (physiology and pathological cardiovascular aspects) the pathogenesis of cardiovascular diseases. Some clinical and epidemiological studies revealed the importance of high plasma homocysteine levels in the progression of atherosclerosis. It was also shown that high level of plasma homocysteine is an independent risk factor for coronary, cerebrovascular and peripheral occlusive vascular diseases. In this study we investigated the effects of exogenous adenosine administration on plasma homocysteine levels and some other biochemical parameters. Twenty adult male Balb-c mice were included to the study. To the experimental group (n=10) intraperitoneal 0.2 ml, 30 mg/kg adenosine was applied twice in a day for three consecutive days. To the control group (n=10) intraperitoneal 0.2 ml 0.09% NaCl was applied twice in a day for 3 consecutive days. After blood was collected into heparinized tubes under the ether anaesthetized mice by intracardiac puncture. Plasma homocysteine, total cholesterol, HDL-cholesterol, triglyceride, C-reactive protein, alkaline phosphatase, aspartat amino transferase, alanin amino transferase, gama-glutamyl transferase were measured. LDL-cholesterol and VLDL-cholesterol levels were calculated by using appropriate formulas. Differences between the groups were analysed by Mann-Whitney U test. There was no difference in plasma homocysteine and other biochemical parameters between both groups. These results show that exogenous adenosine application did not effect the plasma homocysteine, total cholesterol, HDL-cholesterol, triglyceride, C-reactive protein, alkaline phosphatase, aspartat amino transferase, alanin amino transferase, gama-glutamyl transferase, LDL-cholesterol and VLDL-cholesterol levels.

Key Words: Adenosine, homocysteine, mice, biochemical parameters

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Introduction

Adenosine forms as a result of nucleotide breakdown and occurs in tissue during hypoxia and ischemia (1). Increased levels of adenosine have been reported to exert a homeostatic and cytoprotective role in the body (2, 3). Homocysteine (Hcy) is formed by the demethylation of the essential amino acid methionine. Hyperhomocysteinemia in adults may be acquired by an excess dietary intake of methionine or a decreased intake of folate (4). Individuals with elevated plasma levels of either Hcy or cholesterol are at increased risk of cardiovascular disease (5). Elevated levels of plasma Hcy have been associated with an increased incidence of arterial thrombosis, and are recognized to be an independent risk factor for coronary heart disease, stroke and peripheral vascular disease (6). Under experimental conditions, increased Hcy concentrations have been found to result in endothelial dysfunction, leukocyte adhesion, and smooth muscle and collagen proliferation (7-10). Animal experiments demonstrated that hyperhomocysteinemia could be a pathogenic factor responsible for arterial damages such as endothelial injury, cell proliferation, increased matrix formation, and arteriosclerosis (11). The underlying molecular and metabolic links have not been conclusively elucidated (12). S-adenosylhomocysteine hydrolase (SAHase) plays a critical role in the regulation of tissue adenosine and Hcy concentrations (13). When adenosine, Hcy, or both increased, S-adenosylhomocysteine (SAH) synthesis was markedly enhanced, resulting in reduction of adenosine levels in different tissues (14). In addition, several studies showed that the administration of Hcy or Hcy thiolactone decreases adenosine release (14, 15). Given a wide variety of protective effects of adenosine in the cardiovascular homeostasis, regulation of various organ function, and cell growth or proliferation, it seems that adenosine has the opposite effects in different organ systems compared to Hcy (16, 17). Hyperhomocysteinemia decreases plasma and tissue adenosine concentrations associated with inhibition of SAHase (13). Decrease in plasma and tissue adenosine may be an important mechanism mediating the pathogenic effects of Hcy (13). In this study under the scope of this information we investigated the effect of exogenous adenosine on Hcy levels. Also we investigated the possible relation between adenosine, Hcy and other parameters as total cholesterol (TC), High-density lipoprotein-cholesterol (HDL-C), Low-density lipoprotein-cholesterol (LDL-C), Very low-density lipoprotein-cholesterol (VLDL-C), triglyceride (TG), C-reactive protein (CRP), alkaline phosphatase (ALP), aspartat amino transferase (AST), alanin amino transferase (ALT), -glutamyl transferase (GGT) which are related with cardiovascular events.

Materials and Methods

In this study 20 adult Balb-c mice were used. The mice were fed ad libitum. To the study group consisting of 10 mice intraperitoneal 0.2 ml, 30 mg/kg adenosine (Merck) was applied twice in a day for three consecutive days. To the control group (n=10) intraperitoneal 0.2 ml 0.09% NaCl was applied twice in a day for 3 consecutive days. At the end of this period blood was collected into heparinized tubes under ether anaesthetized mice by intracardiac puncture. The mice were sacrified while under anaesthetic. Plasma was separated by centrifugation at 4C and stored at -70C until assayed. The biochemical tests were performed using automatic analysers. TC, TG, ALP, AST, ALT, GGT measurements were performed by using enzymatic assays (Instrumentation Lab, MA, USA). HDL-C was measured by a direct enzymatic assay without precipitation (Instrumentation Lab, MA, USA). LDL-C and VLDL-C levels were estimated by using Friedewald and triglyceride/5 formulae respectively. Hcy levels were measured by competitive solid phase chemiluminescance immunoassay (IMMULITE; DPC Biosystems, CA, USA). CRP was measured by using enhanced latex immuno-turbidimetric assay (Scil Diagnostics GmbH, Martinsried). Differences between the groups were analysed by Mann Whitney U test. Animal care and all experimental procedures used were in accordance with those detailed in the Guide for Care and Use of Laboratory Animals, which was published by the U.S. Department of Health and Human Services.

Results

Plasma Hcy, TC, TG, HDL-C, LDL-C, VLDL-C, AST, ALT, ALP, GGT, CRP measurements of the mice are illustrated in Table 1. No significant changes in the plasma levels of these parameters were found between the two groups.

Discussion

Hcy is regarded as an independent risk factor for occlusive vascular diseases (18, 19). In animal studies, Hcy administration leads to accelerated atherosclerosis and thrombosis (20, 21). Elevated plasma triglyceride

Table 1: Plasma Homocysteine, TC, TG, HDL-C, LDL-C, VLDL-C, AST, ALT, ALP, GGT, CRP levels of the mice (Mean±S.D.).

	Control Group (n=10)	Study Group (n=10)	P
Homocysteine (mol/L)	7.02±2.71	7.02±3.02	1.000
TC (mg/dl)	96.30±15.98	98.30±17.06	0.970
TG (mg/dl)	71.40±33.71	79.20±34.60	0.241
HDL-C (mg/dl)	50.50±9.74	52.80±13.15	0.850
LDL-C (mg/dl)	31.80±11.31	29.70±8.77	0.544
VLDL-C (mg/dl)	14.30±6.60	15.70±6.93	0.287
AST (U/L)	183.80±62.82	193.20±70.60	0.596
ALT (U/L)	29.40±15.74	41.10±29.01	0.405
ALP (U/L)	12.75±9.37	14.35±10.39	0.791
GGT (U/L)	1.54±1.11	1.65±1.28	0.739
CRP (mg/dl, 10^{-3})	0.21±0.34	0.14±0.24	1.000

TC; Total cholesterol, HDL-C; High-density lipoprotein-cholesterol, LDL-C; Low-density lipoprotein cholesterol, VLDL-C; Very low-density lipoprotein -cholesterol, TG; Triglyceride, CRP; C-reactive protein, ALP; Alkaline phosphatase, AST; aspartat amino transferase, ALT; alanin amino transferase, GGT; -glutamyl transferase.

and cholesterol levels are also accepted as independent risk factors for coronary artery disease. Adenosine that is an active biological compound modulates various physiological mechanisms such as cardiovascular tone and immune responses (2, 3, 22). During ischemic or chronic atherosclerotic processes, an increase up to micromolar levels in local adenosine is observed. Adenosine has anti-inflammatory and cytoprotective effects (2, 3, 22). The well-known protective effects of adenosine in the cardiovascular system are mediated by purine surface receptors (23, 24). The increased removal of adenosine, due to increased Hcy concentrations, would significantly lower the adenosine concentration at these receptors and by diminishing the protective actions potentially permit harmful effects on the cardiovascular system (25). Extracellular adenosine may have several physiological effects by stimulation of specific adenosine receptors as A_1, A_{2A}, A_{2B} and A_3 receptors (26). The stimulation of these receptors results with the cardio and vasoprotective effects of adenosine by interfering with numerous mechanisms that contributes to the pathogenesis of atherosclerosis and thrombosis (27). All these effects make adenosine a powerful endogenous protector against arteriosclerotic and vaso-occlusive disorders and are thought to contribute to the cardioprotective properties of adenosine receptor stimulation (28, 29). In this way, adenosine could restrict intimal hyperplasia in the early phase of atherosclerosis, but could also play a role in the formation of the necrotic core in advanced atherosclerosis (30). 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 (30).

S-adenosylhomocysteine (SAH), is hydrolyzed to simultaneously produce Hcys and adenosine by SAH hydrolase in a variety of mammalian cells (13). SAH hydrolase is bi-directional and the equilibrium of the reaction favours the condensation of Hcys and adenosine forming SAH (27, 31). But, as homocysteine and adenosine are removed rapidly in vivo, the reaction proceeds in the direction of producing homocysteine and adenosine (27).

Recently some studies revealed the evidence that SAH may play an important role in the vascular complications in atherosclerosis. In high homocysteine concentrations hydrolyse of SAH to homocysteine and adenosine by SAHase reverses and homocysteine and adenosine condenses to form SAH (8, 14, 32) resulting in a decrease of adenosine (13). The inhibition of SAHase is attributed to a possible product feedback inhibition on SAHase (13). It was also shown that in tissue homogenates adenosine level decreased by a specific SAHase inhibitor (13). In in vitro experimental studies elevated SAH level was observed when cultured endothelial cells were incubated with exogenous homocysteine in the presence of adenosine (27). It was also postulated that in the development of atherosclerosis there might be some other risk factors associated with high homocysteine levels including adenosine (27). In several studies a decrease in adenosine release by homocysteine administration was shown in normoxic (14, 15) and hypoxic conditions (33). It was postulated that in the studies supraphysiological amounts homocysteine were used which might not resemble to the conditions of hyperhomocysteinemia in man (27). Because adenosine has a protective effect in the cardiovascular system, during hyperhomocysteinemia the protective effect of adenosine on cardiovascular system will be diminished so this will be an important factor in the pathogenesis of cardiovascular disease (13, 27). SAHase inhibiton by adenosine or with adenosine analogies were observed in some experimental studies (34, 35).

We hypothesized that, as SAHase is inhibited by elevated homocysteine-one of the two products in SAHase reaction- results with decreased adenosine, adenosine which is known to inhibit SAHase may

have an effect on homocysteine concentration. However in this study, administration of exogenous adenosine didn't result with any change in plasma homocysteine concentration. This result can be observed because of many conditions. 1) The half life of adenosine is very short as in seconds, and for a change in homocysteine concentration a longer period should be needed; 2) In this study we applied 30 mg/kg adenosine twice in a day. This dose was applied according to the information obtained from the literature. If adenosine concentration was higher or lower the result may be different; 3) Adenosine application could be longer than three days that may result with a chronic effect; 4) In many of the homocysteine and adenosine studies done in animals rat was used. In our study we used mice and this difference may be one of the factors affecting the result. To our opinion, for assessing the effect of adenosine on Hcy levels it may be worthy on studying this possible effect by using a different adenosine application protocol. Also kinetic studies also may be helpful to explain the effect of adenosine on homocysteine levels.

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