FOLIC ACID AND VITAMIN B12 SUPPLEMENTATION ATTENUATES ISOPRENALINE-INDUCED MYOCARDIAL INFARCTION IN EXPERIMENTAL HYPERHOMOCYSTEINEMIC RATS

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Hyperhomocysteinemia (Hhcy) is an independent risk factor for cardiovascular disease. Oxidative stress may contribute to the deleterious effects of homocysteine (Hcy). The aim of the present study is to study the effect of folic acid and Vitamin B12 supplementation on isoprenaline (ISO)-induced myocardial infarction (MI) in hyperhomocysteinemic rats. Hhcy was induced by daily intake of methionine (1 g kg\(^{-1}\) body weight) in the drinking water for 4 weeks. MI was then produced by a single subcutaneous injection of ISO (300 mg kg\(^{-1}\), s.c.). Electrocardiographic parameters, heart rate, ST segment, and blood pressure as well as serum marker enzymes, creatine kinase (CK) and lactate dehydrogenase (LDH) were measured. Lipid peroxidation measured as malondialdehyde (MDA) and reduced glutathione (GSH) concentrations in heart tissue were estimated as indices of oxidative stress. Hhcy resulted in significant blood pressure reduction, ST segment elevation and increase in heart rate and serum CK and LDH levels. Cardiac MDA was significantly increased, while GSH was decreased in Hhcy group compared to the normal control group. All the measured parameters were greatly exaggerated in Hhcy rats treated with ISO in comparison with Hhcy rats alone. Administration of folic acid (10 mg kg\(^{-1}\), orally via gavage) and Vitamin B12 (500 µg kg\(^{-1}\), i.m.) concurrently for 4 weeks during the induction of Hhcy markedly reduced the increase in heart rate, ST segment elevation and blood pressure reduction as well as the increase in serum CK and LDH levels. Cardiac MDA content was decreased while cardiac GSH was elevated in the treated group compared to Hhcy + ISO group. Moreover, the severe cardiac histopathological changes observed in Hhcy + ISO group were attenuated by folic acid and Vitamin B12. These results suggest that Hhcy aggravates MI via oxidative stress mechanisms and that lowering Hcy level with folic acid and Vitamin B12 can ameliorate the detrimental effects of Hhcy and may reduce the risk of MI.

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KEY WORDS: hyperhomocysteinemia, myocardial infarction, isoprenaline, lipid peroxidation, folic acid, Vitamin B12, rats.

INTRODUCTION

Homocysteine (Hcy), a thiol containing amino acid, is an intermediate metabolite of the indispensable amino acid methionine. Hcy is formed by demethylation of methionine and is catabolised to cystathionine and cysteine by pyridoxal phosphate (Vitamin B6)-dependent pathway or remethylated to methionine by folate and cyanocobalamin (Vitamin B12)-dependent reaction [1]. Hyperhomocysteinemia (Hhcy) has been increasingly recognised as an independent risk factor for atherosclerosis [2], peripheral vascular disease [3], coronary heart disease [4], myocardial infarction (MI) [5,6], cerebrovascular disease [7] and venous thromboembolism [8].

Several factors are known to increase plasma concentration of Hcy including deficiencies of folate, Vitamin B12, Vitamin B6 and cystathionine B-synthase [9, 10] as well as thermolability of 5,10-methylene tetrahydrofolate reductase [11]. Genetic deficiency of cystathionine B-synthase leads to elevated levels of plasma Hcy, ranging from 100 to 500 µM and is associated with premature arteriosclerosis, endothelial dysfunction and cardiovascular risk [12]. Furthermore, serum Hcy concentrations are frequently elevated by heavy coffee consumption, in the elderly and in certain disease states as chronic renal disease [13–15].

Hcy is a highly reactive amino acid and is known to produce endothelial cell injury through oxidant-mediated...
mechanisms in cellular, animal and human studies [16–18]. Impaired endothelial function is believed to be an early step in the pathogenesis and pathophysiology of atherosclerosis, thrombosis and other cardiovascular diseases.

Treatment with folic acid either alone or with Vitamin B6 or B12 has been shown to reduce plasma Hcy levels [19]. Whether supplementation with these substances decreases plasma Hcy concentration but also positively influences the course of coronary heart disease remains to be established.

Mortality associated with acute MI is still a leading cause of death in the developed countries. It has been demonstrated that isoprenaline (ISO) administration produces free radicals that affects membrane integrity with disintegration of polyunsaturated fatty acids in the membrane (lipid peroxidation) which might be responsible for tissue damage and infarcted heart [20, 21]. A better understanding of the processes involved in MI has stimulated the search for drugs which could limit the myocardial injury. The aim of this study is to examine whether lowering Hcy concentration with folic acid and Vitamin B12 supplementation could exert any protective action against ISO-induced MI.

METHODS

Animals

Forty adult male Wistar rats (220 ± 12 g body weight) were obtained from animal house of Department of Pharmacology, College of Medicine, King Saud University. The animals were housed at room temperature (25 ± 2 °C) under a 12 h light–dark cycle and were allowed free access to food and water ad libitum. Experimental protocol was approved by the Institutional Animal Care and Use Committee of our University.

Induction of HhcY

HhcY was induced by methionine (1 g kg−1 body weight) (Sigma, St. Louis, MO, USA) in the drinking water for 4 weeks [22–24]. This model of HhcY has been reported to result in mild to moderate elevation in plasma Hcy level (25–35 μM1−1).

Treatment

Rats were randomly divided into four groups (10 rats each). Group 1 received saline and served as normal control. Group 2, HhcY rats used as control, group 3, HhcY rats treated with ISO (HhcY+ISO); group 4, HhcY rats received folic acid (10 mg kg−1, orally via gavage) [25] and Vitamin B12 (500 μg kg−1, i.m.) [26] daily and concomitantly during the induction of HhcY before treatment with ISO. At the end of 4 weeks, MI was induced by a single injection of ISO (300 mg kg−1, s.c.) [20]. Blood pressure and ECG changes were recorded for 2 h after ISO injection. Then, blood was collected via carotid artery, centrifuged and serum was used for enzyme assays. The heart was quickly excised, and washed immediately with ice-cold physiological saline. Eight hearts from each group were kept at −20 °C after quick freezing in liquid nitrogen. The remaining two hearts were used for the histopathological examination.

Blood pressure and ECG recording

All animals were anaesthetised with urethane (1.7 g kg−1, i.p.). The right carotid artery was cannulated and connected to a pressure transducer (PT400) and an Oscillograph 400 MD 4C-Palmer (Bioscience, Washington, USA). Needle electrodes were inserted under the skin for the limb lead at position II, and ECG parameters, heart rate (beats min−1) and ST segment (expressed in mm) were measured.

Assays

Serum lactate dehydrogenase (LDH) and creatine kinase (CK) were determined kinetically using kits purchased from BIOSYSTEM (Barcelona, Spain) and EliTech Diagnostics (Sees, France), respectively as previously described [27, 28]. The heart lipid peroxide measured as malondialdehyde (MDA) was estimated with thiobarbituric acid according to the method of Buege and Aust [29]. Reduced glutathione (GSH) was measured as previously described by Ellman [30] and modified by Nagi et al. [31]. The MDA and GSH contents were expressed per gram heart tissue.

Histological studies

Heart tissues taken for histological examination were removed and placed in 10% buffered neutral formalin solution. After fixation, tissues were dehydrated in ascending grades of alcohol and embedded in paraffin. Serial sections were cut at 5 μM and stained with haematoxylin and eosin. The sections were examined under light microscope and photomicrographs were taken.

Statistics

All data are expressed as means ± SEM and were subjected to one way analysis of variance (ANOVA) followed by Bonferroni’s test [32].

RESULTS

Haemodynamic parameters

Figure 1 illustrates the effect of folic acid and Vitamin B12 on mean blood pressure. In HhcY animals, blood pressure was significantly reduced to (90 ± 4, 86 ± 6, 25, 90 ± 4, 28, 85 ± 4, 14, 84 ± 5, 68, and 83 ± 5, 36 mmHg) as compared to (115 ± 4, 18, 111 ± 4, 112 ± 3, 6, 110 ± 5, 35, 117 ± 6 and 113 ± 4 mmHg) the normal control group at 0, 10, 20, 30, 60 and 120 min, respectively (P < 0.05). ISO-induced MI caused drastic decrease in blood pressure of HhcY rats that started 10 min after ISO injection and lasted for 120 min recording. Mean blood pressure values were 89 ± 5.36, 58 ± 4.53, 60 ± 4, 1, 56 ± 3.65, 57 ± 4.9 and 59 ± 3.57 mmHg in HhcY + ISO group at 0, 10, 20, 30, 42, 60 and 120 min.
Fig. 1. Time course of the effect of folic acid (10 mg kg$^{-1}$, orally) and Vitamin B$_{12}$ (500 µg kg$^{-1}$, i.m.) for 120 min on blood pressure in ISO-induced MI in hyperhomocysteinemic rats. Each point represents the mean of eight experiments; vertical lines show standard error mean; (∗) $P<0.05$ compared with the control group, (#) $P<0.05$ compared with Hhcy group, (@) $P<0.01$ compared with the Hhcy + ISO group.

Treatment of Hhcy rats with ISO induced a severe increase in heart rate (430 ± 18, 560 ± 26, 578 ± 17, 565 ± 20, 591 ± 26 and 600 ± 15 beats min$^{-1}$, vs the corresponding values in Hhcy rats alone, $P<0.05$). Tachycardia was attenuated with folic acid + Vitamin B$_{12}$ treatment (396 ± 23, 428 ± 20, 493 ± 18, 499 ± 15, 500 ± 16 and 510 ± 15 beats min$^{-1}$ vs the corresponding values in Hhcy + ISO rats, $P<0.05$).

Figure 2 shows the effect of folic acid and Vitamin B$_{12}$ on heart rate. Heart rate was significantly increased from (340 ± 13, 339 ± 16, 369 ± 18, 358 ± 16, 370 ± 18 and 374 ± 20 beats min$^{-1}$) in control group to (425 ± 17, 430 ± 21, 475 ± 15, 479 ± 18, 497 ± 19 and 502 ± 19 beats min$^{-1}$, $P<0.05$) in Hhcy group at 0, 10, 20, 30, 60 and 120 min, respectively. Treatment of Hhcy rats with ISO induced a severe increase in heart rate (430 ± 18, 560 ± 26, 578 ± 17, 565 ± 20, 591 ± 26 and 600 ± 15 beats min$^{-1}$, vs the corresponding values in Hhcy group alone, $P<0.05$). Treatment of Hhcy rats with ISO induced a severe increase in heart rate (430 ± 18, 560 ± 26, 578 ± 17, 565 ± 20, 591 ± 26 and 600 ± 15 beats min$^{-1}$, vs the corresponding values in Hhcy group alone, $P<0.05$). Tachycardia was attenuated with folic acid + Vitamin B$_{12}$ treatment (396 ± 23, 428 ± 20, 493 ± 18, 499 ± 15, 500 ± 16 and 510 ± 15 beats min$^{-1}$ vs the corresponding values in Hhcy + ISO rats, $P<0.05$).

Figure 3 demonstrates the effect of folic acid and Vitamin B$_{12}$ on ST segment. Hhcy produced significant ST segment elevation compared to normal control group (0.21 ± 0.009, 0.22 ± 0.012, 0.23 ± 0.008, 0.21 ± 0.009, 0.29 ± 0.016, 0.29 ± 0.016, 0.37 ± 0.015, 0.38 ± 0.017, 0.37 ± 0.018, 0.39 ± 0.013 and 0.38 ± 0.017 mV vs 0.21 ± 0.009, 0.22 ± 0.012, 0.23 ± 0.008, 0.21 ± 0.009, 0.29 ± 0.016, 0.29 ± 0.016 mV, $P<0.05$).
Fig. 1. Time course of the effect of folic acid (10 mg kg\(^{-1}\), orally) and Vitamin B\(_{12}\) (500 µg kg\(^{-1}\), i.m.) for 120 min on ST segment in ISO-induced MI in hyperhomocysteinemic rats. Each point represents the mean of eight experiments; vertical lines show standard error mean. (a) * P < 0.01 compared with the control group. (b) ** P < 0.001 compared with the Hhcy group. (c) + P < 0.05 compared with the Hhcy + ISO group.

Biochemical results

In Table I, Hhcy significantly (P < 0.001) increased serum CK and LDH levels. CK and LDH levels increased 2.45- and 1.84-fold, respectively compared to the normal control groups. Hhcy exacerbated ISO-induced MI as indicated by significant (P < 0.001) remarkable increase in serum CK and LDH levels up to 2.1 and 2 times, respectively the corresponding values in Hhcy rats alone. Folic acid and Vitamin B\(_{12}\) treatment significantly (P < 0.001) reduced the increase in CK and LDH levels compared to Hhcy and ISO group.

As given in Table II, heart lipid peroxide concentration measured as MDA was elevated while GSH content was reduced in Hhcy rats (by approximately 59 and 32%, respectively of the corresponding control values). Hhcy rats treated with ISO showed a dramatic increase in cardiac MDA concentration and a marked depletion of GSH content. MDA concentration was increased by about 73% while GSH was reduced by about 38.5% of the corresponding control values.

Histopathological studies

Figure 4(A) depicted the normal architecture of heart on histological examination. A massive necrosis of heart muscle fibres with haemorrhage was observed in Hhcy + ISO [Fig. 4(C)] while, it was only moderate in Hhcy rats [Fig. 4(B)]. Folic acid and Vitamin B\(_{12}\) supplementation resulted in mild degree of necrosis [Fig. 4(D)].

Table I

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDH (U l(^{-1}) min(^{-1}))</th>
<th>CK (U l(^{-1}) min(^{-1}))</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>174 ± 13.29</td>
<td>196 ± 8.79</td>
</tr>
<tr>
<td>Hhcy</td>
<td>321 ± 11.51</td>
<td>260 ± 9.81</td>
</tr>
<tr>
<td>Hhcy + ISO</td>
<td>647 ± 28.29</td>
<td>547 ± 30.36</td>
</tr>
<tr>
<td>Folic acid + B(_{12})</td>
<td>282 ± 17.17</td>
<td>219 ± 16.04</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± s.e.m. of eight animals. * P < 0.01 compared with the control group. ** P < 0.001 compared with the Hhcy group. + P < 0.05 compared with the Hhcy + ISO group.

Table II

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol g(^{-1}) tissue)</th>
<th>GSH (µmol g(^{-1}) tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>103 ± 4.17</td>
<td>1.67 ± 0.050</td>
</tr>
<tr>
<td>Hhcy</td>
<td>164 ± 5.71*</td>
<td>1.14 ± 0.044*</td>
</tr>
<tr>
<td>Hhcy + ISO</td>
<td>284 ± 8.41*</td>
<td>0.70 ± 0.042*</td>
</tr>
<tr>
<td>Folic acid + B(_{12})</td>
<td>118 ± 3.32</td>
<td>0.91 ± 0.038*</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± s.e.m. of eight animals. * P < 0.01 compared with the control group. ** P < 0.001 compared with the Hhcy group. + P < 0.05 compared with the Hhcy + ISO group.
Fig. 4. (A) A photograph of heart from control rats (H&E, ×200). (B) A photograph of Hcy rats showing moderate necrotic areas of the cardiac tissue with haemorrhage (H&E, ×200). (C) A photograph of Hcy + ISO rats showing severe necrotic cardiac areas (H&E, ×200). (D) A photograph of rats supplemented with folic acid + Vitamin B₁₂ showing mild necrosis (H&E, ×200).
DISCUSSION

There is clinical and epidemiological evidence that elevated plasma Hcy levels are associated with increased myocardial mortality [5, 6, 33]. Treatment with folic acid alone or combined with Vitamin B12 has been shown to reduce plasma Hcy levels but it is not clear to what extent such treatment may reduce the incidence of MI.

The present study shows that Hhcy exacerbates ISO-induced MI via oxidative mechanisms as assessed by ECG changes and indicators of myocardial oxidative injury (increased heart lipid peroxides and GSH content). Folate and Vitamin B12 treatment counteracted the harmful hyperhomocysteinemic actions and improved MI.

The precise mechanism by which Hcy causes vascular disease is not fully understood but several mechanisms have been suggested. Hcy is toxic to the vascular endothelium and impairs endothelial function [34] by inhibiting the synthesis of endothelium-derived relaxing factor, nitric oxide or by increasing its degradation via the generation of oxygen-derived radicals such as superoxide radical, peroxynitrite and hydrogen peroxide [35, 36]. These free radicals in turn can promote the growth of vascular smooth muscle [36], modification of proteins and oxidation of lipids [37] resulting in formation of oxidised low density lipoprotein which can impair expression of nitric oxide synthase and directly degrade nitric oxide [38]. In addition to alteration of nitric oxide pathway by Hcy, other mechanisms may be involved including inhibition of prostacyclin [39], enhancement of thromboxane synthesis [29] and induction of monococyte tissue factor expression [40] thus, promoting platelet aggregation leading to atherothrombotic vascular disease [8].

In the current investigation, ISO-induced MI produced oxidative stress as indicated by increased heart lipid peroxides and decreased heart GSH content, an action that was exaggerated by Hhcy and abrogated by folic acid and Vitamin B12 supplementation. The implication of oxidative insult in ISO-induced MI have been demonstrated [28]. The ability of Hhcy to induce lipid peroxidation and oxidative stress was demonstrated in endothelial dysfunction [41], atherosclerosis [42] and cerebrovascular stroke [9]. Moreover, Hhcy decreased intracellular GSH concentration in endothelial cells [43]. On the contrary, no change in GSH level was reported in another study [44].

In the present study, folic acid and Vitamin B12 supplementation was able to abrogate the effect of Hhcy on ISO-induced MI. Our results are in accordance with other studies that have shown that either folic acid alone or in combination with Vitamin B12 is protective against Hhcy-induced vascular or renal injury [45–47]. This beneficial effect of the combined therapy may be attributed to their ability to increase the metabolism of Hcy back to methionine via methionine synthase enzyme that requires methylenetetrahydrofolate as methyl donor and Vitamin B12 as cofactor.

The unique feature of the present study is that folic acid and Vitamin B12 not only abolished the action of Hhcy but also was able to improve ISO-induced MI. This improvement in MI may be attributed to other beneficial actions of the combined therapy, most notably folic acid. Folic acid was able to improve flow-mediated vasodilation in healthy volunteers with mildly elevated or even normal plasma Hcy levels [47, 48]. It has been suggested that folic acid may have other protective actions against triglycerides and cholesterol [49, 50]. Moreover, folate deficiency has been reported to result in increased lipid peroxidation and oxidative stress [44]. Therefore, another possibility for the protective action of folic acid is through antioxidant effects that have been recently shown [51].

Although the present study tested the effect of combined folic acid and Vitamin B12, other studies show that this combination seems not to be markedly more effective than folic acid monotherapy for Hhcy [52]. Vitamin B12 supplementation is only effective against Hhcy in Vitamin B12-deficient subjects [52, 53]. However, it is well known that erroneous treatment of cobalamin deficiency with folic acid may elicit and deteriorate Vitamin B12 neuropathy. Therefore, such a combination could be an innocuous means of reducing risk of Hhcy.

In conclusion, the present study suggested that dietary supplementation of folic acid and Vitamin B12 might have greater significance in the protection against cardiovascular disease.

REFERENCES


