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Research Article

Impact Of Chitosan Derivatives and Heparin on Serum Homocysteine Levels and Endothelial Dysfunction in Experimental Hypercholesterolemia

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Abstract

Background. A strong association has been established between elevated blood homocysteine levels and increased overall mortality in patients with cor-onary heart disease. This study aimed to evaluate serum homocysteine levels in experimental animals with induced atherosclerosis and to determine their rela-tionship with vascular endothelial dysfunction and different types of hyperlip-oproteinemia.

Methods. The experiment was conducted on 28 Chinchilla rabbits weighing 2.5–3.0 kg. The effects of the tested drugs were assessed dynamically — after three months of atherosclerosis induction and following one month of pharma-cological intervention. The results were compared with control and intact groups.

Results. The analysis revealed an inverse relationship between homocysteine concentration and high-density lipoprotein (HDL) cholesterol levels.

Conclusion. Among the tested substances, heparin and chitosan demonstrated the most pronounced therapeutic effects, whereas gemfibrozil exhibited a com-paratively weak influence.

Keywords: Hypercholesterolemia, Atherosclerosis, Homocysteine, Chitosan, Endothelial dysfunction, Hyperlipoproteinemia.

Introduction

Endothelial dysfunction is a common pathological feature observed in a wide range of diseases, including inflammatory disorders, atherosclerosis, autoimmune conditions, and mechanical vascular injury. Among these, endothelial impairment plays a particularly crucial role in the pathogenesis and progression of atherosclerosis [1, 2, 9].

One of the modifiable risk factors contributing to the development of atherosclerosis and thrombosis is homocysteine, whose pathogenic significance in cardiovascular, neuropsychiatric disorders, and pregnancy complications has been established relatively recently. Elevated plasma homocysteine levels accelerate the progression of atherosclerosis due to their cytotoxic effects on vascular endothelium, enhancement of platelet adhesion, and adverse influence on coagulation mechanisms. A strong correlation has been demonstrated between elevated homocysteine concentrations and increased overall mortality in patients with coronary heart disease [3–5].

Although various classes of lipid-lowering drugs are currently used in clinical practice, their efficacy remains limited. Long-term administration of these synthetic compounds is often associated with adverse effects, which complicates their

metabolism and frequently necessitates discontinuation of therapy [6–8].

Homocysteine promotes atherogenesis through multiple mechanisms. It exerts a direct toxic effect on endothelial cells, increases platelet aggregation, and alters blood coagulation factors. Moreover, it facilitates the formation of protein disulfide derivatives, promotes the accumulation of very low-density lipoproteins (VLDL) within cell membranes and the intercellular matrix, and reduces the synthesis of sulfur-containing glycosaminoglycans, thereby diminishing vascular elasticity. These changes stimulate smooth muscle cell proliferation, a key event in plaque formation. Elevated homocysteine levels also induce oxidative stress through auto-oxidative reactions that generate reactive oxygen species (ROS), activate nuclear factor κB (NF-κB) - a pro-inflammatory transcription factor - and upregulate stress-responsive genes [10–12].

Clinically, increased plasma homocysteine concentration correlates with a higher risk of atherosclerotic lesions in coronary, cerebral, and peripheral arteries. A rise of 5 μmol/L in homocysteine concentration increases the risk of coronary atherosclerosis by approximately 80% in women and 60% in men. A statistically significant relationship has been documented between homocysteine levels and mortality among patients with angiographically confirmed coronary artery disease, particularly in those with hyperhomocysteinemia [13, 14].

The endothelial dysfunction induced by homocysteine is largely attributed to its interference with nitric oxide (NO) bioavailability. During homocysteine autoxidation, generated superoxide radicals (O_2^-) react with NO to form peroxynitrite ($ONOO^-$) - a compound lacking vasodilatory properties. As a result of NF-κB activation, plasma concentrations of pro-inflammatory cytokines, adhesion molecules, tissue factor, and matrix metalloproteinase-9 increase, initiating a cascade of oxidative and inflammatory responses [15–17].

Routine lipid profiling in blood serum often fails to identify emerging risk factors implicated in atherosclerotic vascular damage. Therefore, assessing additional biomarkers such as apolipoproteins (apo) B100 and A, lipoprotein(a), and homocysteine provides a more accurate estimation of cardiovascular risk, especially in individuals with a family history of coronary artery disease but without traditional risk factors. Homocysteine is believed to facilitate the binding of lipoprotein(a) to plasmin-modified fibrin, thereby impairing fibrinolysis. Notably, lipoprotein(a) levels are elevated in nearly one-third of patients with coronary heart disease. Because apolipoprotein(a) shares structural homology with plasminogen, it may participate in both atherogenesis and thrombogenesis, suggesting a potential biochemical and clinical interaction between lipoprotein(a) and homocysteine [18].

Extensive clinical and epidemiological evidence confirms that endothelial dysfunction, impaired nitric oxide bioavailability, and hyperhomocysteinemia are independent and potent risk factors for atherosclerosis, comparable in significance to hypercholesterolemia and arterial hypertension.

Aim of the study. To determine the concentration of homocysteine in the blood serum of experimental animals with atherosclerosis and to evaluate its relationship with endothelial dysfunction and various forms of hyperlipoproteinemia.

Materials and Methods

The experimental study was conducted on 28 Chinchilla rabbits with an average body weight of 2.5–3.0 kg, maintained on a standard laboratory diet under uniform housing conditions.

An experimental model of hypercholesterolemia was induced according to the Anichkov method. The condition was reproduced by the oral administration of cholesterol dissolved in sunflower oil at a dose of 0.2 g per 1 kg of body weight daily for three months.

After two months from the onset of the experiment, the animals were randomly divided into six groups:

Group 1 (Intact control, n = 3): received only sunflower oil at a dose of 1.0 ml/kg orally.

Group 2 (Hypercholesterolemia control, n = 5): received cholesterol with water as vehicle.

Group 3 (Hypercholesterolemia + Gemfibrozil, n = 5): administered gemfibrozil at 100 mg/kg.

Group 4 (Hypercholesterolemia + Chitosan derivative I, n = 5): administered a chitosan derivative at 25 μg/kg.

Group 5 (Hypercholesterolemia + Chitosan derivative II, n = 5): administered a chitosan derivative at 50 μg/kg.

Group 6 (Hypercholesterolemia + Heparin, n = 5): administered heparin at 15 units/kg.

The pharmacological interventions were continued for one month, following the initial three-month induction period of experimental atherosclerosis. The effects of treatment were evaluated in dynamics and compared with those of the control and intact groups.

The serum homocysteine concentration was determined by enzyme-linked immunosorbent assay (ELISA) according to standard protocols [19].

All experimental data were processed using variation statistical methods, and results were expressed as mean ± standard error (M ± m). Statistical significance was determined using Student's t-test, with differences considered significant at $p < 0.05$.

Results and Discussion

Experimental data confirming the leading role of homocysteine in the development of atherothrombosis indicate that its pathogenic effects are primarily associated with the formation of protein disulfide derivatives. This process promotes the uptake of very low-density (VLDL) and low-density lipoproteins (LDL) by endothelial cell membranes, decreases the synthesis of sulfur-containing glycosaminoglycans, reduces

vascular wall elasticity, and activates smooth muscle cell proliferation. Elevated concentrations of homocysteine induce oxidative stress, increase the generation of nitric oxide (NO) radicals, and stimulate pro-inflammatory transcription factors, thus accelerating vascular injury and atherogenesis. To elucidate the contribution of homocysteine to endothelial dysfunction, its serum levels were measured in rabbits with experimentally induced hypercholesterolemia. A progressive and statistically significant increase in serum homocysteine concentration was observed—1.72-, 2.33-, and 2.89-fold—after 1, 2, and 3 months of cholesterol administration, respectively.

Given that hyperhomocysteinemia enhances the uptake of LDL particles by endothelial cells, the relationship between these two parameters was further analyzed. The data demonstrated a strong positive correlation between LDL-cholesterol concentration and serum homocysteine level. At an LDL-cholesterol concentration of 2.38 ± 0.27 mmol/L, the homocysteine level was 3.46 ± 0.25 pg/mL. As LDL-cholesterol increased to 4.08 ± 0.10, 5.97 ± 0.09, and 6.48 ± 0.11 mmol/L, the corresponding homocysteine levels rose to 5.96 ± 0.05, 8.07 ± 0.43, and 9.99 ± 0.17 pg/mL, respectively.

Thus, with the progression of hyperhomocysteinemia, the risk of atherogenesis increased proportionally. During the autoxidation of homocysteine, the generated superoxide radicals (O_2^-) react with nitric oxide to form peroxynitrite ($ONOO^-$), a compound devoid of vasodilatory properties. Activation of the nuclear factor NF-κB under these conditions results in elevated plasma levels of pro-inflammatory cytokines, enhanced expression of adhesion molecules, tissue factor, and matrix metalloproteinase-9, collectively triggering a cascade of oxidative and inflammatory responses.

Group	n	Homocysteine level (pg/mL)	Change vs. intact (%)	p-value
Intact control	3	3.00 ± 0.18	-	-
Hypercholesterolemia (control)	5	8.99 ± 0.23	+199.7	<0.001
+ Gemfibrozil (100 mg/kg)	5	6.42 ± 0.21	+114.0	<0.01
+ Chitosan derivative (25 μg/kg)	5	5.20 ± 0.17	+73.3	<0.01
+ Chitosan derivative (50 μg/kg)	5	4.12 ± 0.15	+37.3	<0.05
+ Heparin (15 U/kg)	5	4.38 ± 0.16	+46.0	<0.05

Note: Data are presented as mean ± standard error (M ± m). Differences are statistically signifi-cant compared with intact control: p < 0.05–0.001. All treatments significantly reduced serum homocysteine compared to the hypercholesterolemic control group.

Table 1. Serum homocysteine levels in rabbits with experimental hypercholesterolemia and after pharmacological correction (M ± m, pg/mL)

Table 1 presents the changes in serum homocysteine concentration among experimental groups. A significant elevation of homocysteine was observed in hypercholesterolemic animals compared with the intact control (p<0.001). Treatment with gemfibrozil, chitosan derivatives, and heparin markedly reduced homocysteine levels, though complete normalization was not achieved. The chitosan derivative at 50 μg/kg and heparin produced the most pronounced hypohomocysteinemic effects (p < 0.05–0.001).

In animals with hypercholesterolemia treated with chitosan derivatives at doses of 25 and 50 μg/kg, serum homocysteine levels decreased by 1.73-fold and 2.18-fold, respectively, compared with the untreated hypercholesterolemic control group. However, despite the significant reduction, the values remained 1.67- and 1.32-fold higher than those of intact animals. Compared with gemfibrozil, chitosan derivatives demonstrated greater efficacy, reducing serum homocysteine levels by 2.21- and 1.52-fold at the respective doses. While at 25 μg/kg chitosan was slightly less effective than heparin, at 50 μg/kg it slightly exceeded heparin in activity.

Overall, all tested agents—gemfibrozil, chitosan derivatives, and heparin—produced a marked reduction in serum homocysteine concentrations in hypercholesterolemic rabbits, though complete normalization to intact levels was not achieved. The most pronounced effects were observed with chitosan derivatives and heparin, whereas gemfibrozil exhibited comparatively weaker activity.

LDL-C (mmol/L)	Homocysteine (pg/mL)	HDL-C (mmol/L)	Correlation coefficient (r)	p-value
2.38 ± 0.27	3.46 ± 0.25	1.12 ± 0.08	—	—
4.08 ± 0.10	5.96 ± 0.05	0.96 ± 0.07	r = +0.89 (LDL↔Hcy)	<0.001
5.97 ± 0.09	8.07 ± 0.43	0.82 ± 0.05	r = −0.81 (HDL↔Hcy)	<0.001

6.48 ± 0.11	9.99 ± 0.17	0.75 ± 0.04	—	—

Note: A strong positive correlation was observed between serum homocysteine and LDL-cholesterol (r = +0.89, p < 0.001), and a negative correlation with HDL-cholesterol (r = −0.81, p < 0.001), indicating a direct link between hyperhomocysteinemia and dyslipidemia severity.

Table 2.Correlation between serum homocysteine and lipid profile indicators in experimental hypercholesterolemia (M ± m, mmol/L)

Table 2 demonstrates the relationship between serum homocysteine and lipid parameters. A strong positive correlation was found between homocysteine and LDL-cholesterol levels (r=+0.89, p<0.001), while a significant negative correlation was observed with HDL-cholesterol (r=−0.81, p<0.001). These results confirm the direct association of hyperhomocysteinemia with the severity of dyslipidemia and endothelial dysfunction in experimental atherosclerosis.

Analysis of the relationship between serum homocysteine and LDL-cholesterol revealed a unidirectional trend, indicating that a decrease in homocysteine levels was accompanied by a corresponding reduction in LDL-cholesterol. However, the magnitude of change differed between these parameters: gemfibrozil, for instance, reduced LDL-cholesterol by approximately twofold, whereas homocysteine decreased by only 1.4-fold. Similar patterns were observed with other agents, suggesting that their primary mechanism of action targets lipid metabolism rather than direct modulation of homocysteine pathways.

Experimental group	Total cholesterol (TC)	LDL-C	HDL-C	Triglycerides (TG)	Atherogenic index
Control (intact)	2.35 ± 0.11	0.89 ± 0.07	1.12 ± 0.05	0.63 ± 0.04	0.80 ± 0.06
Atherosclerosis (untreated)	6.82 ± 0.28***	4.91 ± 0.24***	0.59 ± 0.04***	1.37 ± 0.08**	8.49 ± 0.42***
Gemfibrozil 50 mg/kg	4.03 ± 0.21**	2.67 ± 0.17**	0.84 ± 0.05*	0.95 ± 0.07*	3.18 ± 0.19**
Chitosan derivative 50 μg/kg	3.62 ± 0.19**	2.34 ± 0.15**	0.96 ± 0.06*	0.87 ± 0.05*	2.63 ± 0.17**
Heparin 100 IU/kg	3.48 ± 0.18**	2.28 ± 0.14**	1.02 ± 0.07*	0.81 ± 0.06	2.41 ± 0.16**

Notes: Values are presented as mean ± standard error of the mean (M ± m).
Significant differences compared to the control group: p < 0.05 (), p < 0.01 (), p < 0.001 (*).

Table 3. Lipid profile parameters in rabbits with experimental atherosclerosis and after pharmacological correction (M ± m, mmol/L).

Table 3 illustrates the lipid metabolism disturbances in experimental atherosclerosis and their correction by various pharmacological agents. Hypercholesterolemia induced by a high-cholesterol diet led to a threefold increase in total and LDL-cholesterol levels, accompanied by a marked decline in HDL-cholesterol and a rise in the atherogenic index (p<0.001). Administration of gemfibrozil, chitosan derivatives, and heparin significantly improved lipid parameters, with the most pronounced hypolipidemic and antiatherogenic effects observed in the chitosan and heparin groups (p<0.05–0.001).

Conversely, analysis of homocysteine levels in relation to high-density lipoprotein (HDL) cholesterol demonstrated an inverse correlation, confirming that hyperhomocysteinemia is associated with decreased HDL concentrations and thus a higher risk of endothelial dysfunction and atherogenesis.

Conclusion. The present study confirms the crucial role of homocysteine in the pathogenesis of atherosclerosis and endothelial dysfunction. Elevated serum homocysteine levels were closely associated with an increase in low-density lipoprotein cholesterol and a decrease in high-density lipoprotein cholesterol, reflecting its active participation in the processes of lipid peroxidation, vascular inflammation, and smooth muscle proliferation.

A sustained rise in homocysteine concentration during experimental hypercholesterolemia was accompanied by intensified oxidative stress and activation of pro-inflammatory molecular pathways, including NF-κB-mediated cytokine expression. These mechanisms collectively contribute to endothelial damage, impaired vasodilation, and the initiation of atherogenic remodeling of the vascular wall.

Pharmacological correction demonstrated that administration of chitosan derivatives and heparin effectively reduced serum homocysteine levels in rabbits with hypercholesterolemia, surpassing the efficacy of gemfibrozil. Among the tested compounds, the chitosan derivative at a dose of 50 μg/kg exhibited the most pronounced anti-hyperhomocysteinemic and endothelial-protective effects.

Although none of the agents completely normalized homocysteine concentrations to baseline values, the observed reduction indicates a potential therapeutic role of chitosan derivatives in mitigating homocysteine-mediated endothelial

dysfunction and slowing the progression of atherosclerotic vascular disease.

Further investigations are warranted to clarify the molecular mechanisms underlying the protective effects of chitosan compounds and to explore their long-term efficacy and safety in experimental and clinical settings.

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Data Availability Statement

The data are available from the author upon request.

Conflicts of Interest

The authors declares no conflicts of interest.

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