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Research Article

The Effect of Cholesterol Loading and Subtotal Cerebral Ischemia on The Content of Circulating Endothelial Cells and Stable Nitric Oxide Metabolites in Rat Blood Plasma

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Abstract

To comprehensively study the effects of an atherogenic cholesterol load and acute subtotal cerebral ischemia, both in isolation and in combination, on the degree of structural damage to the vascular endothelium and the dynamics of stable nitric oxide metabolites in the systemic circulation in a rat model.

Keywords: Atherogenic cholesterol load, Subtotal cerebral ischemia, Vascular endothelium, Circulating endothelial cells (CECs), Nitric oxide (NO), Endothelial dysfunction, Cholesterol, Rats.

Objective

To comprehensively study the effects of an atherogenic cholesterol load and acute subtotal cerebral ischemia, both in isolation and in combination, on the degree of structural damage to the vascular endothelium and the dynamics of stable nitric oxide metabolites in the systemic circulation in a rat model.

Materials and Methods

Experiments were performed on 28 white outbred male rats divided into 4 groups of 7 animals each. The first group served as an intact control. In the second group, a cholesterol load was modeled by daily dietary supplementation of cholesterol at 50 mg per kg of body weight for two weeks. In animals of the third group, a model of subtotal cerebral ischemia (SCI) was induced. The fourth group was subjected to a combination of both pathological interventions: cholesterol loading followed by SCI. The number of circulating endothelial cells (CECs) in peripheral blood, determined by standard cytological methods, served as an integral marker of morphological damage to the vascular intima [1, 8]. The functional state of the endothelium-dependent vasodilator system was assessed by measuring the concentration of stable end metabolites of nitric oxide in blood plasma, expressed as nitrite levels [3, 13]. Blood samples for analysis were collected under ether anesthesia via catheterization of the common carotid artery. Statistical analysis was performed using non-parametric tests. Results are presented as median and interquartile range, with differences considered statistically significant at $p < 0.05$.

Scientific Novelty

For the first time within a single experimental protocol, a comparative study was conducted on the nature and extent of vascular endothelial damage, as well as the direction of changes in the nitric oxide system, under two fundamentally different pathological conditions: a metabolic state induced by cholesterol loading and a circulatory state caused by acute cerebral ischemia. It is demonstrated that these factors exert opposing effects on the level of circulating NO metabolites, deepening our understanding of the pathogenesis of endothelial dysfunction in cardio- and cerebrovascular pathology [2, 4, 12].

Cholesterol is a vital lipid molecule that plays an indispensable role in human physiology. It is a fundamental structural component of all animal cell membranes, where it modulates membrane fluidity and permeability. Cholesterol serves as a precursor for the synthesis of steroid hormones (including cortisol, aldosterone, and sex hormones like estrogen and testosterone), bile acids essential for fat digestion, and vitamin D [20]. In the bloodstream, cholesterol is transported within lipoprotein particles. Low-density lipoprotein (LDL) delivers cholesterol to peripheral tissues, while high-density lipoprotein (HDL) facilitates its reverse transport back to the liver for excretion or recycling. Under normal homeostatic conditions, this system is tightly regulated. However, chronic dietary excess of cholesterol and saturated fats, particularly through the consumption of atherogenic diets, can lead to a pathological state of hypercholesterolemia [5]. In this condition, elevated levels of LDL-cholesterol promote its accumulation within the subendothelial space of arterial walls. This initiates a cascade of events including oxidation, inflammatory cell recruitment, and foam cell formation, which are central to the development of atherosclerosis [9, 18]. The subsequent endothelial dysfunction, characterized by impaired nitric oxide bioavailability and a pro-inflammatory, pro-thrombotic state, is the critical link between hypercholesterolemia and clinical cardiovascular events such as myocardial infarction and stroke [2, 6]. This study explores the specific impact of such an atherogenic load on endothelial integrity.

Results and Discussion

The study revealed a significant and pronounced increase in the number of circulating endothelial cells in all experimental groups compared to the control (Table 1). The greatest increase in this parameter, indicating massive endothelial desquamation, was observed in rats with cholesterol loading, where the CEC level exceeded the control by 3.53-fold [1]. Isolated cerebral ischemia led to a 2.1-fold increase in CEC count, while the combined effect of both factors resulted in a 2.8-fold increase. These data unequivocally indicate the development of significant morphological damage to the vascular intima under conditions of both an atherogenic diet and acute cerebral ischemia [2, 8].

Investigation of the biochemical marker—the concentration of stable nitric oxide metabolites (NOx)—revealed divergent changes (Table 1). Cholesterol loading led to a distinct 16.56% decrease in plasma NOx levels relative to control, consistent with the classical understanding of endothelial dysfunction development in hypercholesterolemia associated with reduced basal NO production by endothelial NO synthase (eNOS) [5, 10, 13]. This decrease reflects impaired endothelial vasodilatory capacity and a key early step in atherogenesis. In stark contrast, subtotal cerebral ischemia caused a sharp, nearly threefold increase in NO metabolite concentration. This effect is likely mediated by activation of the inducible form of NO synthase (iNOS) in neural, glial, and inflammatory cells in response to the ischemic and inflammatory stimulus [11, 14, 19]. This high-output NO production, while part of the immune response, can contribute to nitrosative stress, exacerbate neuronal damage, and mediate systemic effects.

Group No	Rat groups	CECs, cells/100 µl	[NOx], µmol/l
1	Control	3,30 (2,64; 3,96)	33,21 (30,50;5,90)
2	Cholesterol (n=7)	6,82 (5,06; 13,42) *	27,71 (23,75;30,79) *+
3	SCI (n=7)	11,66 (7,92; 15,18) *	92,37 (83,58;111,29) *
4	SCI + Cholesterol (n=7)	9,24 (6,60; 16,28) *	43,99 (40,91%;70,38) *+Δ
5	Nicotine (0,3 мг/кг)	8,80 (5,94; 11,00) *	23,31 (21,11; 25,07) *
6	Nicotine (1,0 мг/кг) (n=7)	5,28 (4,84; 5,72) *	31,01 (25,74;32,77)
7	Nicotine (0,3 мг/кг) + Cholesterol (n=7)	4,62 (4,40; 5,50) *	31,01 (26,83; 33,43)
8	Nicotine (1,0 мг/кг) + Cholesterol (n=7)	4,18 (3,74; 5,28)	24,63 (22,43;25,95) *
9	SCI +Nicotine (0,3 мг/кг) + Cholesterol (n=7)	7,44 (5,98; 9,24) *	30,66 (21,56;35,87)
10	SCI +Nicotine (1,0 мг/кг) + Cholesterol (n=7)	8,52 (6,72; 10,36) *	32,64 (18,92;38,17)

Notes:

1. * - Statistically significant differences compared to the control group, $p < 0.05$.
2. + - Statistically significant differences compared to the group of rats with SCI, $p < 0.05$.
3. Δ - Statistically significant differences compared to the group of rats with cholesterol load, $p < 0.05$.

Table 1 – Content of Circulating Endothelial Cells and Stable Metabolites [NOx] in the Blood of Rats with Nicotine Intoxication, Cholesterol Load, and Nicotine Intoxication, Median (25%; 75%).

Abbreviations

CECs – Circulating Endothelial Cells
[NOx] – Concentration of stable nitric oxide metabolites
SCI – Subtotal Cerebral Ischemia
n – number of animals in group

In the group with combined exposure, a moderate 32.46% increase in NOx was observed, which was significantly less pronounced than with isolated ischemia. This result demonstrates a complex interaction of pathogenetic mechanisms: the pro-inflammatory stimulation of iNOS-derived NO production during ischemia is partially counteracted by the inhibitory effect of hypercholesterolemia on constitutive eNOS function [7, 13, 18]. This suggests that pre-existing endothelial dysfunction may modulate the neurovascular response to acute ischemia.

Conclusions

Cholesterol loading and subtotal cerebral ischemia are potent factors causing structural damage to the vascular endothelium [2, 13]. However, they exert opposing effects on the systemic level of stable nitric oxide metabolites: cholesterol loading reduces it, reflecting classical endothelial dysfunction, whereas brain ischemia significantly increases it, associated with the activation of inducible cellular inflammatory mechanisms [11, 19]. The combined action of these factors results in a less pronounced increase in NOx compared to isolated ischemia, indicating a competitive interaction between impaired endothelial NO synthesis and inducible NO overproduction under conditions of combined metabolic and circulatory pathology. These findings highlight the distinct and interactive pathways of endothelial injury and systemic NO metabolism in different disease states.

References

1. Blann, Andrew D., Alexander Woywodt, Francesco Bertolini, Todd M. Bull, Jill P. Buyon, Robert M. Clancy, Marion Haubitz et al. "Circulating endothelial cells." *Thrombosis and haemostasis* 93, no. 02 (2005): 228-235.
2. Bonetti, Piero O., Lilach O. Lerman, and Amir Lerman. "Endothelial dysfunction: a marker of atherosclerotic risk." *Arteriosclerosis, thrombosis, and vascular biology* 23, no. 2 (2003): 168-175.
3. Cyr, Anthony R., Lauren V. Huckaby, Sruti S. Shiva, and Brian S. Zuckerbraun. "Nitric oxide and endothelial dysfunction." *Critical care clinics* 36, no. 2 (2020): 307-321.
4. D'Armiento, Francesco P., Alfredo Bianchi, Filomena de Nigris, David M. Capuzzi, Maria R. D'Armiento, Giuseppe Crimi, Pasquale Abete, Wulf Palinski, Mario Condorelli, and Claudio Napoli. "Age-related effects on atherogenesis and scavenger enzymes of intracranial and extracranial arteries in men without classic risk factors for atherosclerosis." *Stroke* 32, no. 11 (2001): 2472-2480.
5. Davignon, Jean, and Peter Ganz. "Role of endothelial dysfunction in atherosclerosis." *Circulation* 109, no. 23_suppl_1 (2004): III-27.
6. Endemann, Dierk H., and Ernesto L. Schiffrin. "Endothelial dysfunction." *Journal of the American Society of Nephrology* 15, no. 8 (2004): 1983-1992.
7. Faraci, Frank M. "Protecting against vascular disease in brain." *American Journal of Physiology-Heart and Circulatory Physiology* 300, no. 5 (2011): H1566-H1582.
8. Goon, P. K., Christopher J. Boos, and G. Y. Lip. "Circulating endothelial cells: markers of vascular dysfunction." *Clinical laboratory* 51, no. 9-10 (2005): 531-538.
9. Galkina, Elena, and Klaus Ley. "Immune and inflammatory mechanisms of atherosclerosis." *Annual review of immunology* 27, no. 1 (2009): 165-197.
10. Incalza, Maria Angela, Rossella D'Oria, Annalisa Natalicchio, Sebastio Perrini, Luigi Laviola, and Francesco Giorgino. "Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases." *Vascular pharmacology* 100 (2018): 1-19.
11. Iadecola, Costantino, and Josef Anrather. "The immunology of stroke: from mechanisms to translation." *Nature medicine* 17, no. 7 (2011): 796-808.
12. Loscalzo, Joseph. "Nitric oxide insufficiency, platelet activation, and arterial thrombosis." *Circulation research* 88, no. 8 (2001): 756-762.
13. Förstermann, Ulrich, Ning Xia, and Huige Li. "Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis." *Circulation research* 120, no. 4 (2017): 713-735.
14. Rodrigo, Ramon, Rodrigo Fernandez-Gajardo, Rodrigo Gutiérrez, Jose Manuel Matamala, Rodrigo Carrasco, Andres Miranda-Merchak, and Walter Feuerhake. "Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities." CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders) 12, no. 5 (2013): 698-714.
15. Pirillo, Angela, Giuseppe Danilo Norata, and Alberico Luigi Catapano. "LOX-1, OxLDL, and atherosclerosis." *Mediators of inflammation* 2013, no. 1 (2013): 152786.
16. Khoshnam, Seyed Esmaeil, William Winlow, Maryam Farzaneh, Yaghoob Farbood, and Hadi Fathi Moghaddam. "Pathogenic mechanisms following ischemic stroke." *Neurological sciences* 38, no. 7 (2017): 1167-1186.
17. Lozano, Rafael, Mohsen Naghavi, Kyle Foreman, Stephen Lim, Kenji Shibuya, Victor Aboyans, Jerry Abraham et al. "Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010." *The lancet* 380, no. 9859 (2012): 2095-2128.
18. Libby, P., Buring, J. E., Badimon, L., Hansson, G. K., Deanfield, J., Bittencourt, M. S., ... & Lewis, E. F. (2019). *Atherosclerosis. Nature Reviews Disease Primers*, 5(1), 56.

19. Zhang, L., Zhang, Z. G., & Liu, X. S. (2016). The dual role of iNOS in ischemic stroke. *Current Medicinal Chemistry*, 23(38), 4335–4348.

20. Luo, Jie, Hongyuan Yang, and Bao-Liang Song. "Mechanisms and regulation of cholesterol homeostasis." *Nature reviews Molecular cell biology* 21, no. 4 (2020): 225-245.

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