PHARMACOLOGY AND TOXICOLOGY

Antithrombogenic and Antiplatelet Activities of Extract from *Maackia amyrensis* Wood

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Antithrombogenic and antiplatelet effects of a new drug, containing isoflavonoids (extract from the wood of *Maackia amyrensis*, a Far Eastern plant), were studied. A course (200 mg/kg intragastrically during 14 days) of *Maackia amyrensis* extract prevented intravascular clotting, initiated by application of 10% iron chloride solution on the vessel. The drug increased antiaggregant activity of the vascular wall and potentiated endothelium-dependent vasodilatation in ovariectomied rats. The reference drug ethinylestradiol (25 μg/kg intragastrically during 14 days) potentiated the antiaggregant effect of the endothelium, but was inferior to *Maackia amyrensis* extract in the capacity to induce endothelium-dependent vasodilatation in ovariectomied rats.

Key Words: *thrombosis; ovariectomy; isoflavonoids; Maackia amyrensis extract; ethinylestradiol*

Thrombosis and microcirculatory disorders are essential for the pathogenesis of cardiovascular diseases. Their probability increases significantly in postmenopausal women [7]. One of the causes of high incidence of arterial hypertension, myocardial infarction, and brain ischemia in women during this period is endothelial dysfunction, caused by hypoestrogenemia [6,13]. Endothelial dysfunction manifests by a reduction of endothelium-dependent vasodilatation and impairment of the antiadhesive properties of the vascular wall, leading to the formation of prothrombotic states and promoting the development of cardiovascular diseases [11]. Substitution hormone therapy (SHT) promotes a reduction of NO basal level and of arterial pressure in the perimenopausal women, indicating endothelium-protecting activity of estrogens [12]. However high risk of breast cancer and liability to thrombosis in patients treated by hormones for a long time suggest phytoestrogens as an effective alternative to SHT, particularly for prevention of cardiovascular complications of the climacteric syndrome [10]. High content of isoflavonoids (phytoestrogens) in extract from the wood of *Maackia amyrensis* (an endemic maritime leguminous plant) [8] suggested studying its effects under conditions of hypoestrogenemia. *Maackia amyrensis* extract (MAE) was registered as a substance for drug making (R No. 003309/01).

We studied MAE effects on the clot formation process in the vessels and on endothelium-dependent effects (platelet aggregation and vasodila-

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tation) in ovariectomied rats in comparison with ethinylestradiol therapy.

MATERIALS AND METHODS

Experiments were carried out on 58 female Wistar rats (300-340 g). The animals received intragastrically MAE and ethinylestradiol during 14 days in doses of 200 and 25 μg/kg, respectively. According to HPLC the content of polyphenols in MAE was 21.6%: 12.7% isoflavonoids and 8.9% stilbenes [8]. Control rats received 1% starch gel in an equivalent volume. The effect of MAE on clot formation in the rat carotid artery was studied under conditions of clot formation induction by applying 10% iron chloride to the vessel in rats narcotized by urethane (1 g/kg) [5]. Bloodflow in the carotid artery was recorded by an MFV-1100 electromagnetic blood flowmeter (Nihon Kohden). The degree of the blood flow reduction by the end of experiment (75 min) was recorded. After 24 h the clot was isolated from the rat carotid artery and weighed.

Endothelium-dependent antiaggregant and vasodilating effects of the vascular wall were studied in experiments on ovariectomied female rats. The ovaries were removed by the common method in experimental and control rats under ether narcosis. The group of sham-operated rats was subjected to laparotomy and wound suturing. Blood for studies of platelet aggregation was collected from the common carotid artery under ether narcosis. Sodium citrate (3.8% solution) served as the stabilizer (stabilizer:blood proportion 1:9). Platelet aggregation was evaluated as described previously [9] on an AT-02 device with registration of aggregograms on an automated Recorder-2210. Platelet-rich and platelet-depleted plasma (PRP and PDP, respectively) were prepared and the platelets were counted by the standard methods [1]. Platelet-rich plasma was standardized by bringing platelet count per mm^3 to 400±30 thousands by adding the needed volume of PDP. One hour after the last dose of the studied compounds, segments of the abdominal aorta (3.0± 0.5 mg) were collected in ether-narcotized animals. The vessel was washed and incubated during 3 min in standardized donor rat PRP [4]. Irreversible platelet aggregation in PRP was initiated by ADP in the final concentration of 4×10^{-5} M.

A catheter was inserted into the carotid artery to animals narcotized by sodium thiopental (50 mg/kg) for registration of arterial pressure after intravenous injection of agents causing endothelium-dependent (acetylcholine, 5 μg/kg) and non-endothelium-dependent (sodium nitroprusside, 30 μg/kg) vasodilatation. The area above the arterial pressure recovery curve after acetylcholine and sodium nitroprusside tests was calculated. The endothelial dysfunction coefficient (EDC) was estimated as the proportion of area above the curve after injection of sodium nitroprusside and the same characteristic after injection of acetylcholine [3].

The results were statistically processed using Statistica 6.0 software.

RESULTS

The effect of MAE on clot formation in the rat carotid artery, initiated by application of iron chloride solution, was evaluated at stage 1 of the study. Clotting and complete obstruction of the bloodflow were observed in the vessels of control animals 20±1 min after the application. Twenty-four hours after occlusion the clot remained in the vessels, its mean weight being 1.2 mg (Table 1). In experimental animals simulation of intravascular thrombosis after a course of MAE did not lead to complete obstruction of the bloodflow, which reduced by 26% of its initial value by min 75 of experiment. No clots were detected in the carotid arteries of these rats on the next day (Table 1). These results indicate that MAE inhibited or prevented intravascular clotting caused by application of iron chloride solution.

Clotting under the effect of iron chloride application results from initiation of LPO processes and hence, activation of the vascular platelet component of hemostasis [2]. These data suggested further studies of the probable mechanisms of anti-

TABLE 1. Effects of a Course of MAE Treatment on Intravascular Clotting, Induced by Application of 10% Iron Chloride Solution to the Carotid Artery (*M*±*m*)

Group	Bloodflow in carotid artery, ml/min		Clot weight after 24 h, mg
	initial value	by min 75 of experiment	
Control (*n*=6)	4.9±0.9	0.4±0.2*	1.2±0.2
MAE (*n*=6)	5.0±0.6	3.7±0.6*	0

Note. *$p<0.05$ *vs.* initial value.

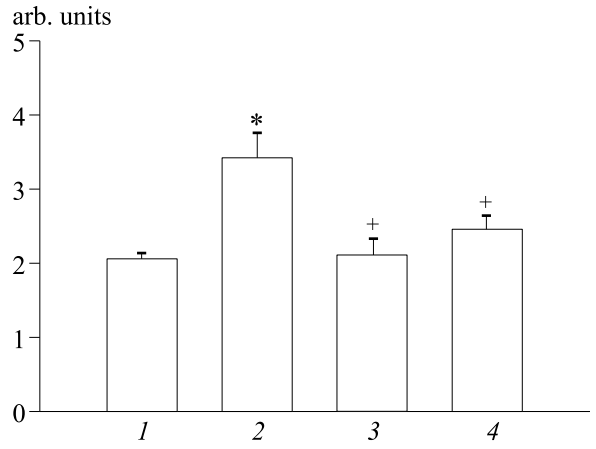

Fig. 1. Endothelial dysfunction coefficient in sham-operated rats (*1*), in rats on day 21 after ovariectomy (*2*; control), and in ovariectomied rats treated by MAE (*3*) and ethinylestradiol (*4*). $p<0.05$ *vs.* sham-operated rats, +controls.

thrombogenic effect of MAE by evaluating the effect of the studied extract on antiaggregant and vasodilating activities of the vascular wall endothelium in ovariectomied rats in comparison with ethinylestradiol. Ovariectomy as a model for studies of these pharmacological effects of MAE was selected because endothelial dysfunction under conditions of hypoestrogenemia is a proven fact [13], while isoflavonoids (the main fraction of MAE polyphenol complex [8]) are selective modulators of estrogen receptors.

At stage 2 of the study we found that the amplitude of platelet aggregation in donor rat PRP without pre-incubation with vascular segments from the studied groups of animals was 50%. Incubation of donor PRP with segments of the abdominal aorta of sham-operated rats reduced the platelet aggregation activity by 38%, with segments of ovariectomied rats by 26% (Table 2). Hence, ovariectomy reduced the antiaggregant activity of the vascular wall in comparison with sham-operated animals. Endothelial dysfunction coefficient (proportion of area above the curve of arterial pressure recovery after injection of sodium nitroprusside, inducing non-endothelium-dependent vasodilatation, and the parameter after injection of acetylcholine, causing endothelium-dependent vasodilatation) was 66% higher than in sham-operated animals (Fig. 1). Hence, estrogen insufficiency, induced by ovariectomy, promotes the development of endothelial dysfunction, manifesting by reduction of its antiaggregant and endothelium-dependent vasodilatation activities. These results are in line with clinical data, indicating progressive deterioration of endothelial function in postmenopausal women [13].

TABLE 2. Effect of Incubation of Abdominal Aorta Segment from Sham-Operated Rats and from Rats Treated Intragastrically by MAE and Ethinylestradiol during 14 Days on the Amplitude of ADP-Induced (4×10^{-5} M) Platelet Aggregation in Donor Rat PRP ($M\pm m$)

Group	Before incubation with vascular segment, %	After incubation with vascular segment, %
Sham-operated (n=5)	50±2	31±1
Ovariectomied		
control (n=5)		37±2*
MAE (n=6)		30±2+
ethinylestradiol (n=6)		31±2+

Note. $p<0.05$ *vs.* *sham-operated rats, +control rats.

The amplitude of platelet aggregation in PRP, incubated with vascular segments from ovariectomied animals, treated by MAE and ethinylestradiol, was lower than in untreated rats by 19 and 16%, respectively (Table 2). In addition, ethinylestradiol therapy led to a 28% reduction of EDC in comparison with untreated ovariectomied rats (control). The coefficient reduced even more markedly (by 38%) in animals treated by MAE in comparison with the control group (Fig. 1). This value virtually reached the level of sham-operated rats, indicating that the drug restored the balance between endothelium-dependent and non-endothelium-dependent vasodilatation under conditions of reduced production of NO by the endothelium in ovariectomied animals.

The endothelium-protective effect of substitute hormone therapy is well known [7,12]. Characterizing MAE effect on endothelial dysfunction by its capacity to restore two major endothelium-dependent processes (antiaggregant and vasodilating), we should like to emphasize the advantages of isoflavonoids of the polyphenol complex of the studied extract in comparison with ethinylestradiol. Presumably, this is explained by the fact that the mechanism of the isoflavonoid endothelium-protective effect (modulation of estrogen receptors) is wider than that of hormones. In addition to isoflavonoid (specifically, genistein, a component of MAE [8]) capacity to simulate the receptor mechanism of estradiol effect, the complex effect of the new drug includes direct antiradical and antioxidant activities. The antioxidant effect of MAE is explained by neutralization of free radicals in the course of reversible oxidation into quinones and transformation of polyenic fatty acid hydroperoxide into nontoxic hydroxyacids with reduction of the production of conjugated dienes, Shiff's bases, and MDA [9].

Hence, MAE is characterized by antithrombogenic effect. One of the mechanisms of this effect realization is pronounced endothelium-protective activity of the drug, detected in ovariectomied rats. Ethinylestradiol reduced, but did not arrest the endothelial dysfunction in ovariectomied rats.

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