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MISOPROSTOL AS AGONIST OF IP₂ RECEPTOR.

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Fourteen patients with peripheral vascular disease received 200 ug of misoprostol 3 times a day during one month. The therapy with misoprostol caused clinical and biochemical improvement in all 14 patients. An elongation of pain free and maximum walking distance, shortening of pain duration and increase in arterial blood flow in both calves were observed. At the same time an activation of the fibrinolytic system, rise in the platelet aggregate ratio and increase in cAMP levels were noticed. It is suggested that misoprostol in human being caused rather activation of IP₂ receptor.

Key words: *misoprostol, IP₂ receptor,*

INTRODUCTION

In clinical trials alprostadil (PGE₁) has shown beneficial effects in patients with peripheral arterial disease (PAD) (1). It inhibited platelet and leukocyte activation and enhanced the fibrinolysis action. Alprostadil, like prostacyclin, is probably an agonist of IP₂ receptor with lack of oral activity.

A few years ago we performed a pilot study with misoprostol, a derivate of alprostadil, in patients with intermittent claudication (2). Misoprostol treated PAD patients showed some clinical improvement.

Moreover, the treatment with misoprostol activated fibrinolysis and inhibited platelet aggregation. . In experimental tests after misoprostol intake we observed thrombolytic activity preceded by short lived thrombogenic phase (3). That suggested activation of EP₂ (thrombogenic phase) and IP₂ (thrombolytic phase) receptor. Research has been undertaken to explain the receptor activation of misoprostol in humans.

PATIENTS AND METHODS.

Fourteen patients with PAD (Fontaine stage IIa and II b) received 200 ug of misoprostol 3 times a day, during one month.

Patients with diseases of the liver, kidney, bile tract, thyroid disease and diabetes mellitus were excluded from the study. All patients underwent standard medical examination (ECG, chest X-rays, eye fundus, and laboratory tests including routine hematology, blood chemistry and urine analysis). The study was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from each patient.

Clinical estimations

Clinical estimations were done before treatment, after two and four weeks of treatment.

Pain-free and maximum walking distance on treadmill at the speed of 4 km/h expressed in meters, was evaluated twice, with an interval of 30 min.

Time of pain relief in the ischaemic limb after maximum walking distance (seconds) was estimated.

Arterial blood flow in both calves with the use of mercury gauge pletysmograph (ml of blood/dl tissue/min) was registered.

Ratio of tibial posterior arterial pressure to brachial arterial pressure with use of ultrasound Doppler technique (APAPR) was determined.

Functional laboratory tests

Functional laboratory tests were made before treatment and 2 hours after intake of the first dose (200 ug of misoprostol). These estimations were repeated after two and four weeks of the treatment, except cAMP level which was checked only before the treatment and after the intake of the first dose.

Euglobulin clot lysis time (ECLT) was estimated according to von Kaulla and expressed in minutes (4).

The platelet aggregate ratio (PAR) was calculated using the method of Wu and Hoak (5).

Threshold proaggregatory concentrations of ADP was determined in platelet rich plasma using a Born aggregometer (6).

cAMP plasma levels were measured by radioimmunoassay using commercially available kits.

RESULTS

Short term administration of misoprostol induced therapeutic benefits to patients with PAD. We observed an elongation of the pain-free and maximum walking distance (*Tabl 1*). At the same time a shortening of pain duration was observed (*Tabl 2*). An increase in AAPR and an improvement in arterial blood flow were observed in both limbs (*Tabl 3*).

Moreover two hours after intake of misoprostol we observed activation of the fibrinolytic system (*Fig. 1*). The ECLT shortened on the 14th and 28th day of therapy. The platelets were less sensitive to ADP after misoprostol intake (*Fig. 2*).

Table 1. Influence of Misoprostol therapy on exercise test in patients with peripheral arterial disease (in meters) (mean \pm S.D., n=14)

Pain free walking distance		Maximum walking distance	
Before treatment	Following treatment	Before treatment	Following treatment
133 \pm 96	185 \pm 127**	278 \pm 132	452 \pm 313**

**p<0.01 as compared to the values before treatment

Table 2. Time of pain relief after maximum walking distance (in seconds)

Before treatment	Following treatment
105 \pm 54	75 \pm 43**

**p<0.01 as compared to the values before treatment

Table 3. Influence of Misoprostol therapy on AAPR and blood flow in the lower limbs (mean \pm S.D., n = 14)

	More affected limb		Less affected limb	
	Before treatment	Following treatment	Before treatment	Following treatment
AAPR	0.51 \pm 0.27	0.71 \pm 0.23**	0.89 \pm 0.15	1.03 \pm 0.21**
Blood flow (ml/dl/min)	2.67 \pm 0.48	3.0 \pm 0.4**	2.92 \pm 0.54	3.15 \pm 0.49**

**p<0.01, as compared to the values before treatment

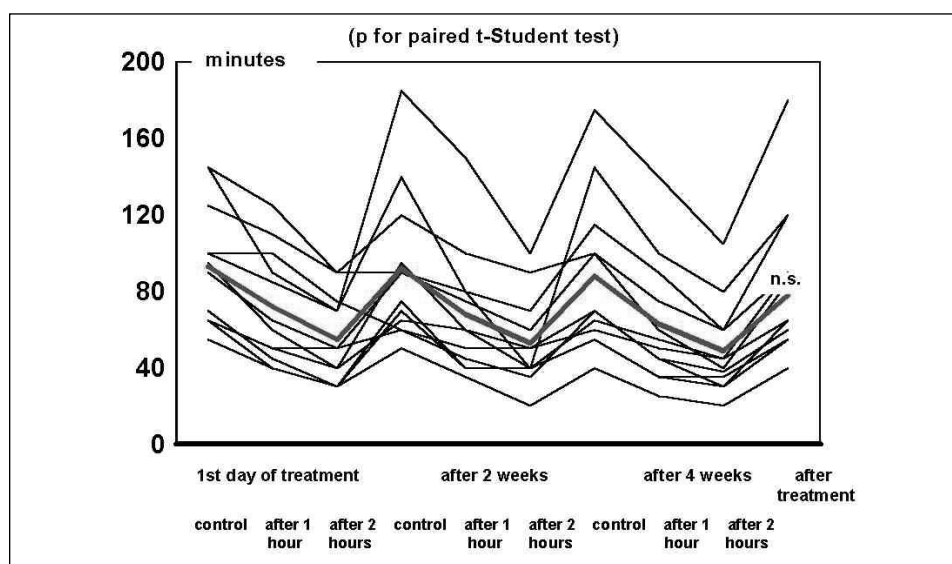


Fig. 1. Euglobulin clot lysis time (ECLT) in patients treated with Misoprostol

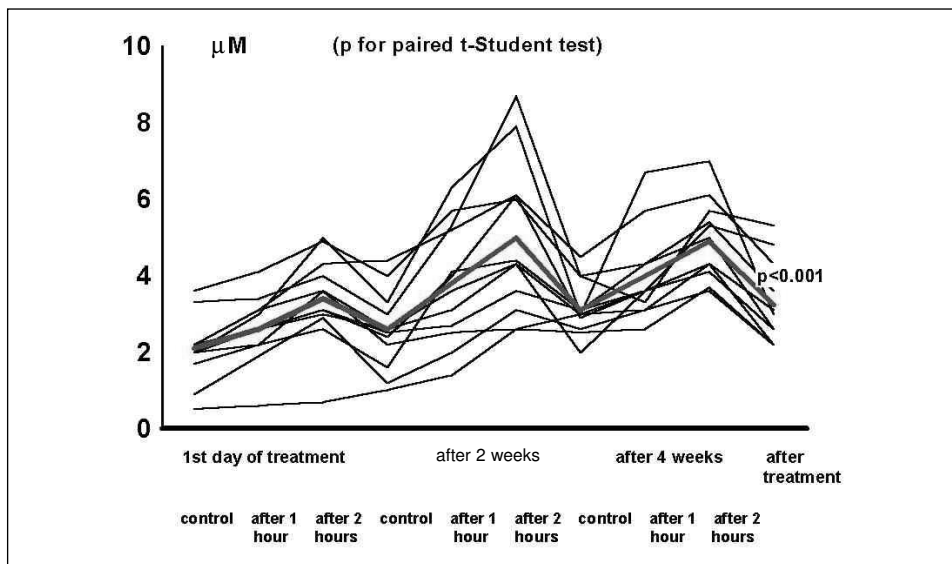


Fig. 2. ADP induced platelet aggregation (EC30) in patients treated with Misoprostol

A rise in platelet aggregate ratio was observed. It indicates that a smaller number of platelets shows a tendency to spontaneous aggregation (Fig 3). All those effects were short-lasting and disappeared within 8-12h after intake of misoprostol.

After intake of misoprostol plasma cAMP levels increased from $3, 14 \pm 0, 86$ to $3, 79 \pm 0, 79$ pmol/ml ($p < 0, 01$) (Fig. 4).

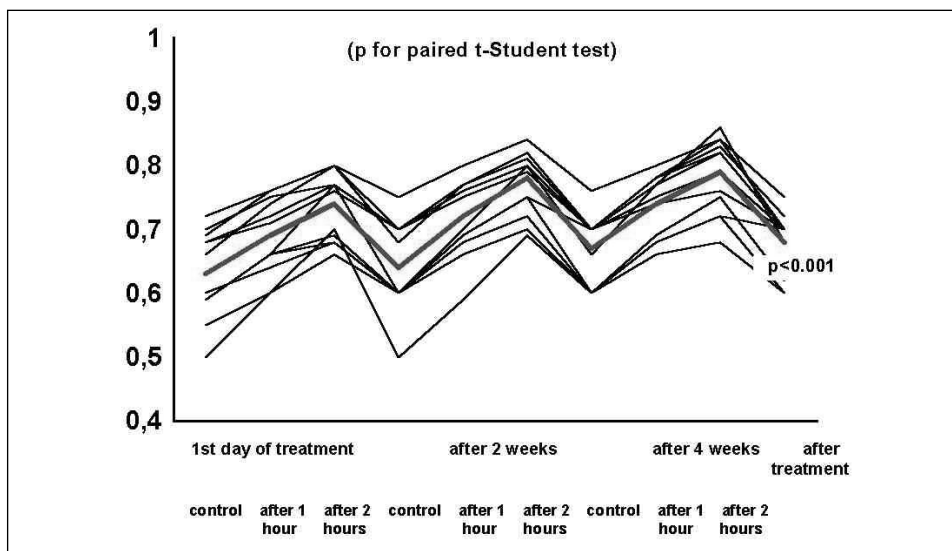


Fig. 3. Platelet aggregate ratio in patients treated with Misoprostol

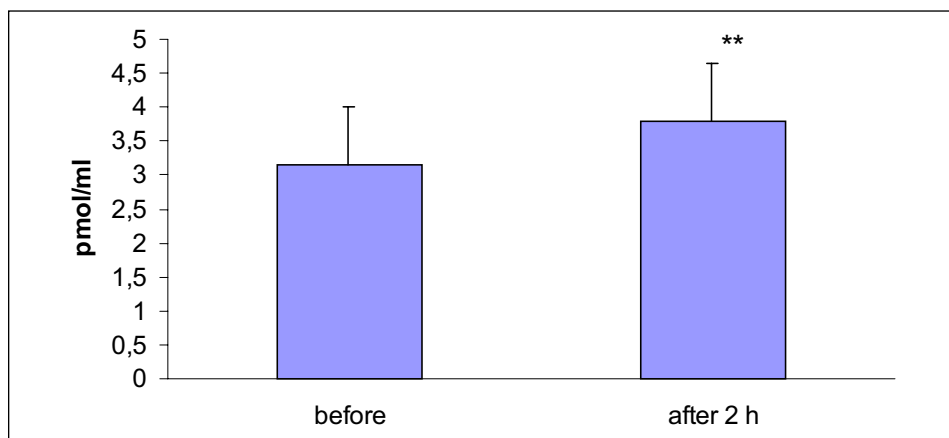


Fig. 4. Influence of misoprostol on cAMP level n=14,** p<0.01

DISCUSSION

Misoprostol is a synthetic 15-deoxy-16 hydroxy-16 methyl analog of natural prostaglandin E_1 – alprostadil. It is sold as methyl ester because the ester is more stable and better for clinical applications. The ester in vivo is absorbed rapidly and transformed to free acid, which is its active metabolite

The efficacy of misoprostol in preventing or healing non steroidal anti-inflammatory drug (NSAID) induced gastric and duodenal injury has been demonstrated clinically (7). It is suggested that misoprostol activates the PGE-receptor in the digestive tract (8). The drug has mucosal protective and gastric antisecretory action. properties in gastrointestinal disorders, it also exerts bronchodilating effects (9). In a double-blind clinical trial in patients with peptic ulcer prostacyclin ordered i. v. in statistically significant manner accelerated the process of healing gastric mucous membrane. Simultaneously in prostacyclin treated patients an increase into gastric juice was noted. Earlier finding suggests that IP_2 receptors are also present in the digestive tract (10, 11).

A few years ago we performed (2) a pilot study with misoprostol in PAD patients. Misoprostol induced therapeutic benefits to PAD patients. We repeated the study with misoprostol in PAD patients. Like in the previous trial, a delayed onset of claudication pain was observed, the time of pain relief after maximum walking distance was shortened. No changes were observed in blood pressure of patients treated with misoprostol, although the treatment resulted in AAPR rise and in an increase in blood flow in the calves. Moreover, misoprostol inhibited the spontaneous aggregation and ADP induced aggregation.

Antiplatelet effects were the most significant 2 hours after ingestion of misoprostol. Misoprostol therapy resulted also in shortening of euglobulin clot lysis time. As it was shown earlier, misoprostol evoked fibrinolytic effect was short lasting and disappeared within 8-12 h after intake of misoprostol. The drug,

as prostacyclin and alprostadil, showed platelet suppression and fibrinolytic activity by stimulating the plasma levels of tissue plasminogen activator (2). In this study we additionally investigated the plasma cAMP levels. Misoprostol 2h after ingestion caused a rise in cAMP levels. It is speculated that misoprostol acts via the same receptor like prostacyclin and alprostadil, but there is a discrepancy among observations in humans and experimental animals.

In rats experiment with arterial blood superfused collagen strip misoprostol given orally 30-100ug/kg evoked a thrombolytic effect preceded by a short thrombogenic action (3).

A stronger thrombolytic effect without thrombogenic phase was exerted by prostacyclin, iloprost (analog of PGI₂) (12, 13) and alprostadil (PGE₁) in the same type of experiments (3). Antithrombotic action of PGE₁ is owed to PGE₀. Misoprostol cannot be transformed to PGE₀ and remaining in its native form, it may act on EP₂ receptor typical for PGE₂. PGE₂ by turn is rather activating platelets than counteracting their aggregation. In human we do not observe a thrombogenic action. Our results suggest that misoprostol rather is acting on IP₂. Maybe that the action of misoprostol in humans on EP₂ is weak, transient and dominated by activation of IP₂ receptor. So, both clinical evaluations with misoprostol suggest that the drug may be used in the therapy of PAD patients, replacing other vasoactive eicosanoids.

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