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# Beneficial effects of non-quinazoline $\alpha_1$-adrenolytics on hypertension and altered metabolism in fructose-fed rats. A comparison with prazosin

Monika Kubacka [a,*], Magdalena Kotańska [a], Małgorzata Szafarz [b], Krzysztof Pociecha [b], Anna M. Waszkielewicz [c], Henryk Marona [c], Barbara Filipek [a], Szczepan Mogilski [a]

[a] Department of Pharmacodynamics, Faculty of Pharmacy, Medical College, Jagiellonian University, Medyczna 9, 30-688, Kraków, Poland
[b] Department of Pharmacokinetics and Physical Pharmacy, Faculty of Pharmacy, Medical College, Jagiellonian University, Medyczna 9, 30-688, Kraków, Poland
[c] Department of Bioorganic Chemistry, Chair of Organic Chemistry, Faculty of Pharmacy, Medical College, Jagiellonian University, Medyczna 9, 30-688, Kraków, Poland

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**KEYWORDS**
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Hypertension;
Metabolic syndrome;
NO;
TNF-$\alpha$;
Fructose-fed rats

**Abstract** *Background and aims:* Metabolic syndrome associated with insulin resistance and hypertension is often caused by excessive fructose consumption. Treatment of hypertension in patients with metabolic syndrome is a difficult task as many antihypertensive drugs have adverse effects on the metabolic profile. We investigated if MH-76 and MH-79, non-quinazoline $\alpha_1$-adrenoceptor antagonists with an additional ability to stimulate NO/cGMP/$K^+$ pathway, ameliorates metabolic syndrome in fructose-fed rats. As reference compound prazosin was used.
*Methods and results:* Male rats were divided into 5 groups (n = 8) and studied for 18 weeks: group control: standard diet and drinking water; group Fructose: high-fructose diet (20% fructose in drinking water); groups Fructose + MH-76, Fructose + MH-79, Fructose + prazosin: high-fructose diet with subsequent MH-76, MH-79 (5 mg/kg/day ip) or prazosin (0.2 mg/kg/day ip) treatment 12 weeks later. In addition to their antihypertensive effect, the studied compounds reversed endothelial dysfunction, decreased hyperglycemia and hypertriglyceridemia, as well as prevented abdominal adiposity. Moreover, MH-76 reduced insulin resistance and decreased TNF-$\alpha$ concentration and lipid peroxidation in adipose tissue. Prazosin treatment exerted an antihypertensive effect, reduced hyperglycemia but did not improve endothelial dysfunction, insulin resistance, and abdominal adiposity. The lower efficacy of prazosin may be the result of its short half-time and the lack of described pleiotropic effects.
*Conclusions:* $\alpha_1$–adrenoceptor blockade, endothelial protection, TNF-$\alpha$ suppressing and antioxidant activity together with favorable pharmacokinetic parameters determines high efficacy of MH-76, leading to the effective improvement of hemodynamic and metabolic disturbances in metabolic syndrome. The use of non-quinazoline, multiple-targeted $\alpha_1$-blockers may be an interesting option for treatment of hypertension with metabolic complications.


* Corresponding author.
*E-mail address:* mfkuback@cyf-kr.edu.pl (M. Kubacka).

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## Introduction

The metabolic syndrome is defined as insulin resistance associated with two or more additional symptoms that are accompanied by an increased risk for cardiovascular diseases and type 2 diabetes mellitus. According to WHO the symptoms are: low high-density lipoprotein (HDL) and high triglycerides levels, elevated blood pressure and plasma glucose level, and obesity [1,2]. Many components of the metabolic syndrome exert the sympathostimulating effects. On the other side, sympathetic overactivity is involved in the pathogenesis of the metabolic syndrome, due to an enhancing effect on insulin resistance through vasoconstriction of blood vessels, thus diminishing regional blood flow and tissue glucose delivery [3,4].

$\alpha_1$-adrenoceptors are involved in sympathetic nervous system functions and are responsible for vascular smooth muscle contraction and blood pressure regulation. They also exert several metabolic effects, including modulation of insulin activity and lipoprotein metabolism [5]. $\alpha$−adrenergic stimulation restricts insulin release, resulting in hepatic glucose production as well as diminishes skeletal muscle glucose uptake. These effects participate in the role of catecholamines as opposed to the action of insulin. As such, blockade of the $\alpha$−adrenoceptors may lead to an improvement in the metabolic profile of the hypertensive patients [5]. In fact, $\alpha_1$-adrenoceptors antagonists, in addition to lowering blood pressure, have been shown to have favourable effects on the metabolic profiles of patients with hypertension. These include reduced total cholesterol, increased HDL cholesterol, reduced hyperinsulinemia, and an improvement in glucose tolerance [5–8]. However, in other studies treatment with $\alpha_1$-adrenolytics was not associated with improved glycemic control both in human [9] and laboratory animals [2,10]. Moreover, in Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack *Trial (*ALLHAT) study, a quinazoline based $\alpha_1$-adrenoceptors antagonist doxazosin was associated with higher risk of combined cardiovascular disease events, and since then $\alpha_1$-adrenolytics are no longer recommended as a first line therapy of hypertension [6,9,11]. Recent studies have indicated that quinazoline-based $\alpha_1$-adrenoceptors antagonists induce the apoptosis and necrosis of cardiomyocytes and other cell types, but these effects are independent of $\alpha_1$−adrenoceptor blockade and probably are connected with the presence of quinazoline moiety. Moreover, apoptosis induction was never shown for non-quinazoline based $a_1$-adrenolytics [11,12].

In our previous work we described a synthesis of 1-(2-methoxyphenyl)piperazine derivatives [13], among which **MH-76** (1-[(2,6-dimethylphenoxy)propyl]-4-(2-methoxyphenyl)piperazine hydrochloride) and **MH-79** (1-[(2-chlor-6-methylphenoxy)propyl]-4-(2-methoxyphenyl) piperazine hydrochloride) showed the most favorable $\alpha_1$-adrenoceptor blocking properties and significant hypotensive activity [14]. Moreover, they possess some additional pleiotropic features as they also activated the endothelial NO − cGMP signaling pathway [15].

On the basis of our previous studies, we hypothesize that MH-76 and MH-79, non-quinazoline $\alpha_1$-adrenolytics with additional multiple-target properties, may be superior to quinazoline-based $\alpha_1$-adrenolytics exerting more beneficial effects on lipid profile and glucose metabolism in addition to their hemodynamic effect. In order to test our hypothesis, we used a reversal protocol (administration of compounds started 12 weeks after initiation of fructose feeding) to determine whether long term use of MH-76 and MH-79 may treat the evoked hypertension and improve the adverse metabolic profile in fructose-fed rats.

## Methods

### *Animals*

The experiments were carried out using male Wistar rats (Krf: (WI) WU) weighting 180–200 g (pharmacological experiments) or 250–300 g (pharmacokinetic studies). All experimental procedures were conducted in accordance with the guidelines of the National Institutes of Health Animal Care and Use Committee and approved by the Local Ethics Committee on Animal Experimentation (resolution no. 338/2017 and 187/2018) in Krakow, Poland.

### *Drugs and chemicals*

Compounds MH-76 and MH-79 were synthesized in the Department of Bioorganic Chemistry, Chair of Organic Chemistry, Pharmaceutical Faculty, Jagiellonian University [13, Fig. S1].

### *Experimental protocol*

Fructose-fed animals were administered with 20% fructose solution instead of drinking water, whereas the control group was maintained on normal drinking water for 18 weeks. Metabolic syndrome in rats was induced by administering fructose for 12 weeks. To study the effects of the test compounds they were administered during the last 6 weeks of the 18 week experiment in fructose-fed rats. Prazosin (Tocris) was used as a reference compound.

In total 40 male Wistar rats were divided into 5 groups (n = 8) and maintained as follows:

Group 1 - Control group (C). Animals received regular diet and water ad libitum for 18 weeks. After 12 weeks, this group received saline (1 ml/kg *ip* daily) during the last 6 weeks of the 18 week experiment.

Group 2 − Fructose group (F). Group 3 - Fructose and MH-76 (F + MH-76), Group 4 - Fructose and MH-79 (F + MH-79), Group 5 - Fructose and prazosin (F + P). Animals received a regular diet and fructose was administered as 20% solution in drinking water for 18 weeks. After 12 weeks, these groups received saline (1 ml/kg *ip* daily), MH-76, MH-79 (5 mg/kg/day *ip*) or prazosin (0.2 mg/kg/day *ip*) respectively, during the last 6 weeks of the 18 weeks experiment.

20% fructose solution was freshly prepared every day. The regular diet was composed of 65% carbohydrates, 16%

protein, 4% fat and standard vitamins and mineral mix (Metabolic Energy 12 MJ/kg).

Body weight was measured at baseline and throughout the study.

At the end of the experiment, after 16 h fasting but with free access to water all rats were anesthetized with thiopental (75 mg/kg *ip*) and blood was collected, centrifuged for 10 min at 3000 rpm, the heart, liver, and abdominal fat were weighted and all samples were stored at −80 °C until they were assayed. Organ weight indexes were calculated according to the formula: organ weight index = organ weight (mg)/body weight (g). Furthermore, from each animal a segment of the thoracic aorta was removed for evaluation of the endothelial vasodilator function.

### *Measurement of systolic blood pressure*

The systolic blood pressure and heart rate was measured once a week, on weeks 0, 1–18 at the same time of day. A noninvasive tail cuff system (AD Instrument, PowerLab Data Acquisition System) was used. The rats were restrained in restrainers on a heating plate (39–40 °C), (LE 5650/6 Heather Scanner, Panlab) for approximately 30 min. Several blood pressure measurements were obtained from each rat, and the mean of the lowest four values was accepted.

### *Oral glucose tolerance test*

At week 16, all the animals were fasted overnight (16 h) and subjected to an oral glucose tolerance test. A glucose solution 1 g/kg was administered to all the animals via oral gavage. Blood samples were collected from the animals via the tail vein at 0, 30, 60, and 120 min after glucose administration. Glucose concentration was measured using a glucometer Contour TS (Bayer) and appropriate strips. Area under curve was then calculated to estimate glucose tolerance.

### *Biochemical assays*

Fasting glucose concentration was measured after a 16 h fast in blood taken from tail veil puncture using a glucometer Contour TS (Bayer) at week 0, 12 and 18. At week 18 (at the end of experiment) glucose concentration was also measured with standard spectrophotometric test (Biomaxima, S.A. Lublin, Poland).

To determine cholesterol, triglyceride and uric acid levels in the plasma samples standard spectrophotometric tests (Biomaxima S.A. Lublin, Poland) were used.

Plasma insulin level was measured using a sensitive rat insulin kit (Fine Test Biotech). Insulin resistance was assessed using HOMA-IR and was calculated using fasting serum insulin and fasting glucose concentrations at the end of the experimental period according to the following formula: HOMA-IR = [Insulin (U/l) × Blood glucose (mmol/l)]/22.5 [16,17].

The concentrations of adipose tissue TNF-$\alpha$ level were determined with the use of commercially available kits (Fine Test Biotech).

### *Measurements of antioxidant activity*

#### Preparation of tissue homogenates

The frozen adipose tissue and liver were weighed and homogenates were prepared by homogenization of 1 g of the tissue in 4 ml of 0.1M phosphate buffer, pH 7,4 using IKA-ULTRA-TURRAX T8 homogenizer. Tissue homogenates were subsequently used for assay of malondialdehyde level.

#### Determination of lipid peroxidation

The level of malondialdehyde (MDA) as an indicator of lipid peroxidation was estimated using the TBA spectrophotometric assay as described earlier [18]. Briefly, 250 μl of a tissue homogenate was added to 250 μl of distilled water, 500 μl of 15% TCA, and 500 μl of 0.37% TBA. The samples were heated in a boiling water bath for 10 min. After cooling, the samples were centrifuged at 12,000×*g* for 10 min. The absorbance of the supernatant was measured at 535 nm.

### *Functional studies*

Segments of thoracic aorta obtained from the five experimental groups of rats, cleaned of adherent connective tissue were placed in a Krebs–Henseleit solution, suspended in organ bath chambers (30 ml) and attached to an isometric force displacement transducer (FDT10-A. BIOPAC Systems, Inc., COMMAT Ltd., Turkey and 720MO, DMT, Denmark). All rings were gradually stretched to a basal resting tension of 2 g and the preparations were allowed to equilibrate for 45 min. After the equilibration period, the aortic rings were contracted by phenylephrine (3 μM), and when the contractile response was stabilized the relaxation was evaluated by cumulative addition of carbachol (Sigma–Aldrich) (from $10^{-9}$ to $10^{-4}$ M) or sodium nitroprusside (Sigma–Aldrich) (from $10^{-10}$ to $10^{-7}$ M).

### *Pharmacokinetic study*

Twelve rats was used in pharmacokinetic experiments, and three days prior to the experiment, rats jugular vein was cannulated allowing for the multiple blood sampling from the single animal. The experimental groups consisted of three rats each, and MH-76 and MH-79 compounds dissolved in 0.9% sterile isotonic saline were administered i.p. at the dose of 5 mg/kg or i.v. at the dose of 2.5 mg/kg. Blood samples (approximately 300 μL) were collected to Eppendorf tubes containing heparin at 5, 15, 30, 60, 120, 240, and 360 min after dosing. Plasma was harvested by centrifuging at 3000 r.p.m. for 10 min and stored at −80 °C until bioanalysis. Samples were quantified using validated LC-MS/MS method. After protein precipitation with acetonitrile and centrifugation supernatant was injected onto a LC-MS/MS system. Chromatography was performed on XSelect HSS Cyano 3.5 μm 3 × 75 mm (Waters, Ireland) analytical column with isocratic elution using a mobile phase containing acetonitrile and water with 0.1% of formic acid. The chromatographic run lasted 6 min and detection was performed by an Applied Biosystems MDS Sciex API 2000 triple

quadrupole mass spectrometer set at unit resolution. The mass spectrometer was operated in the selected reactions monitoring mode (SRM), monitoring the transition of the protonated molecular ions to their specific fragments. Two pairs were used for each compound (for MH-76 355/205 *m*/*z* and 355/136 *m*/*z* and for MH-79 375/190 *m*/*z* and 375/136 *m*/*z*) which can result in both quantitation and confirmation simultaneously. Data acquisition and processing were accomplished by the Applied Biosystems Analyst version 1.4.2 software. Pharmacokinetic parameters were calculated employing non-compartmental analysis using Phoenix WinNonlin v. 6.3 software (Pharsight Corporation, CA, USA). For the purpose of comparison with available in the literature pharmacokinetic parameters of prazosin in rats different pharmacokinetic models were also fitted into the experimentally obtained data.

### *Data analysis*

All results are expressed as mean ± S.E.M. Pharmacokinetic parameters are expressed as geometric mean (GM) and associated 90% confidence interval (90%CI) with coefficient of variation (CV%). Statistically significant differences between groups were calculated using either one-way or two-way ANOVA and the post-hoc Bonferroni multiple comparison test. For comparison of two groups non-parametric Mann–Whitney test was used. The criterion for significance was set at $P < 0.05$.

Concentration-response curves were constructed based on the responses to cumulative concentrations of carbachol and analyzed by non-linear curve fitting using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). Relaxations were expressed as a percentage of inhibition of the maximal tension obtained with the contractile agent ($E_{max}$ = 100%) and the maximum response ($E_{max}$) was calculated.

## Results

### *Effect of MH-76, MH-79 and prazosin on systolic blood pressure in fructose-fed rats*

Figure 1 shows changes in systolic blood pressure of control and fructose-fed rats with and without treatments.

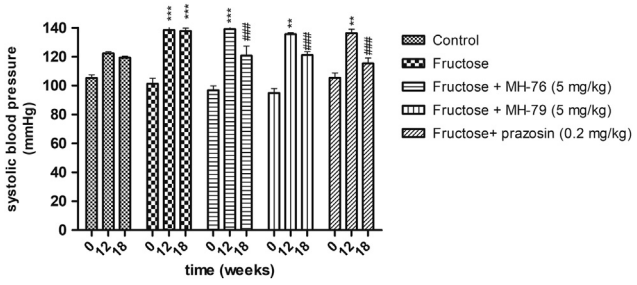


**Figure 1** Effects of MH-76, MH-79 (5 mg/kg ip) and prazosin (0.2 mg/kg ip) on systolic blood pressure in fructose-fed rats. Values are means ± SEM (n = 7–8), $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. fructose-fed rats (two-way ANOVA, post hoc Bonferroni test).

The fructose-fed animals were found to have an elevated systolic blood pressure after 12 weeks of fructose feeding. Subsequent treatment with MH-76, MH-79 and prazosin reversed the rise in systolic blood pressure: in the 18-th week of fructose administration systolic blood pressure was elevated in F group, whereas in F + MH-76, F + MH-79 and F + P groups it did not differ from the control rats.

Fructose feeding as well as fructose-feeding with respective treatments had no effect on heart rate.

### *Effect of MH-76, MH-79 and prazosin on body and organs weight in fructose-fed rats*

Table 1 shows that the final body weight in fructose-fed rats was significantly increased as compared to control rats, and similar pattern was observed in the F + P group. However, the final body weight was significantly decreased following the treatment with MH-76.

The body weight gain also showed similar variations. Treatment with MH-76 prevented excessive body weight gain and F + MH-76 treated rats showed statistically lower body weight gain when compared to F and F + P groups (Table 1).

Similarly, F and F + P treated rats had significantly higher abdominal adiposity than control animals. Treatment with MH-76 and MH-79 reduced the abdominal adiposity markedly (Table 1).

The kidney and heart index was not different in any of the groups compared to the control. However, F + P treated animals presented a markedly heavier liver than control animals (Table 1).

### *Effect of MH-76, MH-79 and prazosin on fasting glycemia, insulin resistance and glucose tolerance in fructose-fed rats*

At 12-th week of experiment blood glucose levels in all the fructose-fed rats were significantly higher than those in the control animals (Fig. 2). Further fructose feeding increased fasting glycemia. Treatment with MH-76, MH-79, and prazosin significantly decreased the glucose level in the fructose-fed rats at 18 weeks when compared with fructose-fed rats with no treatment. However, fasting glucose level was still significantly higher in the F + P treated rats than in the control animals. Moreover, in the F + MH-76 group, fasting glucose concentration was significantly lower than in the F + P group at week 18.

The results of the additional glucose measurement with the spectrophotometric method performed at the end of experiment are presented in Table 1. Fasting glucose concentration in F group was markedly higher than in the control animals. Treatment with MH-76, MH-79 and prazosin significantly lowered the glucose level.

A high fructose diet caused the rise of plasma insulin concentration (Table 1) which has been diminished by MH-76 treatment. The level of HOMA-IR index in F group was also higher than in control rats and subsequent treatment with MH-76 caused HOMA-IR levels decrease near the control values. On the other hand, treatment with prazosin did not improve HOMA-IR level (Table 1).

**Table 1** Physiological Parameters of Control, Fructose, Fructose + MH-76, Fructose + MH-79 and Fructose + prazosin − treated rats at the end of the experimental period.

| Parameter | Control | Fructose | Fructose + MH-76 | Fructose + MH-79 | Fructose + prazosin |
|---|---|---|---|---|---|
| **Whole body** | | | | | |
| **Body weight (g)** | | | | | |
| Initial | 199.4 ± 3.3 | 191.5 ± 3.3 | 191.0 ± 4.4 | 195.1 ± 2.8 | 204.9 ± 2.9 |
| Final | 483.6 ± 8.1 | 517.8 ± 10.6* | 469.9 ± 10.6$^{\#\#\wedge}$ | 505.6 ± 13.4 | 530.4 ± 10.9* |
| Gain | 284.1 ± 7.7 | 334.1 ± 6.2* | 281.0 ± 9.9$^{\#\#\wedge}$ | 310.4 ± 12.5 | 331.0 ± 13.1* |
| Abdominal fat weight (mg/g body wt) | 41.11 ± 5.05 | 58.88 ± 6.38*** | 36.21 ± 6.82$^{\#\#\#}$ | 42.97 ± 5.64$^{\#\#\#}$ | 57.67 ± 9.02*** |
| Heart (mg/g body wt) | 2.99 ± 0.09 | 2.89 ± 0.06 | 3.19 ± 0.20 | 3.10 ± 0.20 | 3.02 ± 0.08 |
| Kidney (mg/g body wt) | 2.78 ± 0.03 | 2.71 ± 0.26 | 2.88 ± 0.29 | 2.87 ± 0.32 | 2.67 ± 0.26 |
| Liver (mg/g body wt) | 2.76 ± 0.07 | 3.06 ± 0.02 | 3.03 ± 0.16 | 3.11 ± 0.09 | 3.15 ± 0.09* |
| **Biochemical parameters** | | | | | |
| Glucose (mg/dl) | 100.8 ± 4.7 | 132.3 ± 3.2** | 89.5 ± 5.9$^{\#\#\#}$ | 100.1 ± 5.2$^{\#\#\#}$ | 107.1 ± 4.9$^{\#}$ |
| Insulin (pmol/l) | 113.2 ± 23.1 | 241.7 ± 68.9 | 150.5 ± 44.5 | 183.9 ± 22.3 | 336.0 ± 43.3** |
| HOMA-IR | 1.75 ± 0.2 | 5.29 ± 1.5* | 1.59 ± 0.3$^{\#,\wedge}$ | 3.35 ± 0.5 | 6.51 ± 0.8** |
| Triglycerides (mmol/l) | 1.87 ± 0.19 | 3.66 ± 0.28*** | 1.91 ± 0.18$^{\#\#\#}$ | 2.39 ± 0.23$^{\#}$ | 2.62 ± 0.38 |
| Total Cholesterol (mmol/l) | 2.0 ± 0.23 | 1.57 ± 0.12 | 1.62 ± 0.13 | 1.99 ± 0.10 | 1.76 ± 0.18 |
| HDL Ch (mmol/l) | 0.96 ± 0.24 | 0.66 ± 0.21 | 0.79 ± 0.15 | 1.06 ± 0.37 | 0.80 ± 0.27 |
| Uric acid (mmol/l) | 153.6 ± 13.1 | 154.0 ± 32.4 | 161.3 ± 32.7 | 152.0 ± 20.9 | 156.0 ± 43.4 |
| MDA (adipose tissue, nmol/l) | 0.22 ± 0.03 | 0.36 ± 0.04* | 0.18 ± 0.03$^{\#}$ | 0.28 ± 0.05 | 0.28 ± 0.02 |
| MDA (liver, nmol/l) | 0.60 ± 0.08 | 1.24 ± 0.15** | 0.80 ± 0.13$^{\#}$ | 0.67 ± 0.12$^{\#\#}$ | 0.51 ± 0.04$^{\#\#\#}$ |

*P < 0.05, **P < 0.01, ***P < 0.001 vs control, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001 versus fructose-fed, $^{\wedge}$P < 0.05, $^{\wedge}$P < 0.01, $^{\wedge}$P < 0.001 versus Fructose + prazosin group, n = 7–8, One-way ANOVA, post hoc Bonferroni test.

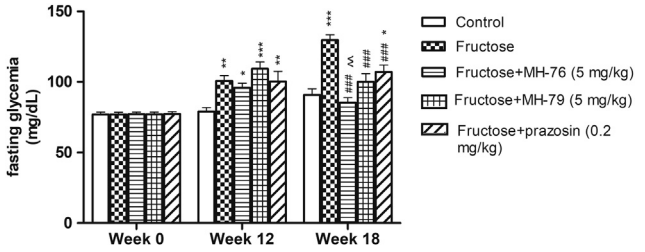


**Figure 2** Effects of MH-76, MH-79 (5 mg/kg ip) and prazosin (0.2 mg/kg ip) on fasting glucose concentration in fructose-fed rats. Values are means ± SEM (n = 7–8), *P < 0.05, **P < 0.01, ***P < 0.001 vs Control, $^{\#\#\#}$P < 0.01 versus Fructose, $^{\wedge}$P < 0.01 versus Fructose + Prazosin group, n = 7–8, (two-way ANOVA, post hoc Bonferroni test).

An oral glucose tolerance test was performed at week 16 of the experiment; from these measurements the area under the concentration–time curve (AUC) was calculated (Fig. S2). Blood glucose levels following oral glucose loading were markedly higher in all animals receiving fructose at the time points 30 and 60 min after administration. However, at time point equal 120 min there were no significant differences between control rats and fructose-fed rats, and between control and fructose-fed MH-76 treated rats. Moreover at the same time point the glucose concentrations in rats receiving fructose and treated with MH-79 or prazosin were still markedly higher. Calculation of the AUC showed that fructose feeding significantly increased the AUC for glucose irrespective of treatment (Fig. S2B).

### *Effect of MH-76, MH-79 and prazosin on lipid profile and uric acid concentration in fructose-fed rats*

The effects of a high-fructose diet alone and in combination with MH-76, MH-79 or prazosin treatment on serum triglycerides and cholesterol concentration are presented in Table 1. A high-fructose diet significantly increased serum triglycerides with no effect on total cholesterol and HDL cholesterol levels at week 18 compared with the control rats. Treatment with MH-76, MH-79 but not with prazosin significantly improved the levels of serum triglycerides in the fructose-fed rats with MH-76 being the most effective. Fructose feeding had no effect on serum uric acid concentration (Table 1).

### *Effect of MH-76, MH-79 and prazosin on lipid peroxydation*

Fructose feeding markedly increased lipid peroxydation assessed in liver and adipose tissue homogenates by malondialdehyde levels (Table 1). Treatment with all $\alpha_1$-adrenolytics decreased lipid peroxydation in liver, however, in adipose tissue only treatment with MH-76 was effective.

### *Effect of MH-76, MH-79 and prazosin on TNF-α concentration in fructose-fed rats adipose tissue*

Fructose feeding significantly increased the TNF-α concentration in adipose tissue. Treatment with MH-76 but not MH-79 or prazosin significantly decreased the TNF-α level compared with fructose-fed rats (Fig. 3).

### *Effect of MH-76, MH-79 and prazosin on endothelial dysfunction in fructose-fed rats*

The effects of MH-76, MH-79 and prazosin on relaxing responses to carbachol in the fructose-fed rats are shown in Fig. 4A. The maximal aortic relaxation induced by carbachol in the F group was significantly reduced when compared with the C group. The maximal aortic relaxation in the F + MH-76 and F + MH-79 was significantly larger

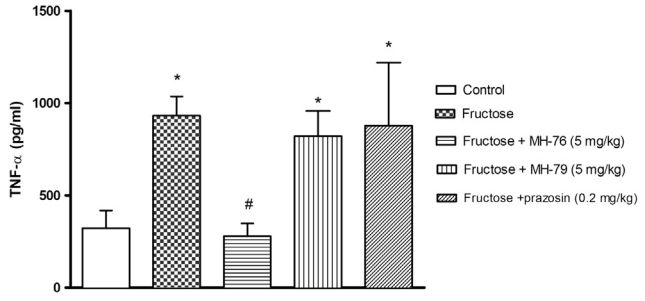


**Figure 3** Effects of MH-76, MH-79 (5 mg/kg ip) and prazosin (0.2 mg/kg ip) on TNF-$\alpha$ concentration in fructose-fed rats adipose tissue. Values are means ± SEM (n = 5–7), *P < 0.05 vs Control, #P < 0.05 versus Fructose group, (One-way ANOVA, post hoc Bonferroni test).

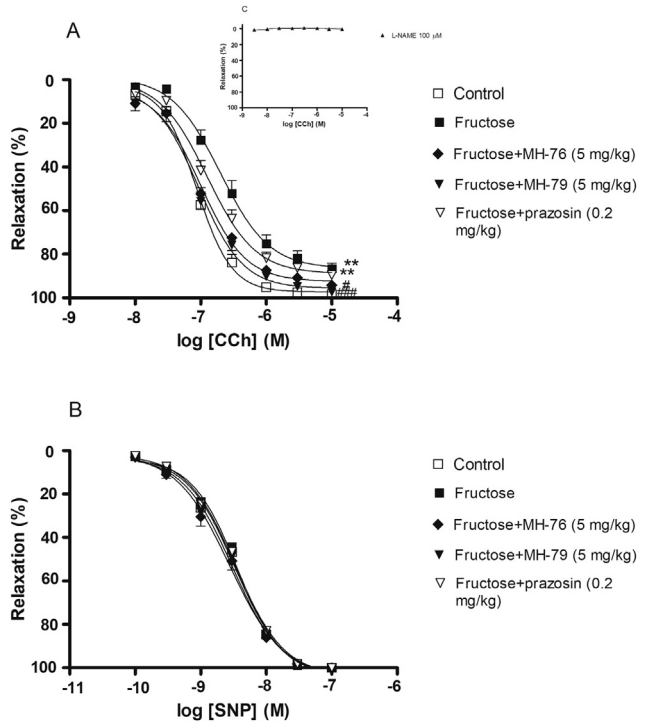


**Figure 4** (A) Endothelium-dependent vascular relaxations in response to carbachol (CCh) and (B) endothelium-independent vascular relaxations in response to sodium nitroprusside (SNP), in thoracic aortic rings from control, fructose-fed, fructose-fed + MH-76–treated, fructose-fed + MH-79–treated and fructose-fed + prazosin–treated rats. (C) Effect of L-NAME (100 μM) pretreatment on vascular relaxations to CCh (**P < 0.01 vs. control; #P < 0.05, ###P < 0.001 vs. fructose-fed rats, One-way ANOVA, post hoc Bonferroni test).

than in the F group. However, prazosin treatment did not restore endothelium-dependent aortic relaxation. On the other hand, relaxation produced by sodium nitroprusside was not significantly different among these groups (Fig. 4B). In rat aorta vasorelaxant effect of carbachol depends mainly on nitric oxide pathway, as pretreatment with L-NAME, a nitric oxide synthase inhibitor, eliminated carbachol induced relaxation (Fig. 4C).

### *Pharmacokinetic analysis*

Pharmacokinetic parameters calculated by the non-compartmental approach are summarised in Table 2. Both compounds have rather low bioavailability after i.p. administration equal to 15.15% and 26.28% for MH-76 and MH-79 respectively. After i.v. administration compound MH-76 has higher volume of distribution at steady state as compared to MH-79 (3.13 vs 1.53 L/kg) what might indicate its better ability to penetrate to the deep compartments. After i.p. administration compound MH-76 has shorter half-life at the elimination phase (2.9 vs 4.88 h), higher clearance (14.12 vs 6.12 L/h/kg) and lower maximal concentration (433 vs 731.8 μg/ml) as compared to the corresponding values for MH-79. Different pharmacokinetic models were fitted into the experimental data and for both investigated compounds the best fit was achieved using two compartmental model. Such model was also found to be optimal in describing the pharmacokinetic behaviour of prazosin after its i.v. administration to rats [19]. Pharmacokinetic parameters calculated based on this model as well as literature values for prazosin are presented in Table S1.

## Discussion

Prevalence of metabolic syndrome, associated with excessive fructose consumption, has largely increased over the past years mainly as a result of the use of high-fructose corn syrup [20,21]. One of the most common symptoms related to metabolic syndrome is hypertension. Treatment of these patients is especially difficult since such complex vascular and metabolic disease requires not only blood pressure reduction but also improvement of the adverse metabolic profile. Unfortunately, many antihypertensive drugs (e.g. beta-blockers, diuretics) have adverse effects on the metabolic profiles in patients with hypertension. Moreover, treatment of hypertension often requires a combination therapy, since a single drug of any class of antihypertensives usually proves to be ineffective [7,9].

In the present study we investigated the effects of **MH-76** and **MH-79** in fructose-fed rats, two 1-(2-methoxyphenyl)piperazine derivatives with $\alpha_1$-adrenoceptor blocking and hypotensive activity and additional ability to stimulate NO/cGMP/$K^+$ pathway [14,15]. As reference compound prazosin was used, a quinazoline derivative as mainly quinazoline-based $\alpha_1$-adrenolytics have been used as antihypertensive therapy so far. In our study all $\alpha_1$-adrenoceptor antagonists produced hypotensive, hypoglicemic and hypolipemic effects, which may be attributed to the $\alpha_1$-adrenoceptor blockade. However, the beneficial effect on body weight (MH-76), abdominal adiposity (MH-76, MH-79), insulin resistance (MH-76) or endothelial dysfunction (MH-76, MH-79) might be due to the pleiotropic effects of studied compounds.

In our experiment, 12 weeks of fructose administration to rats in drinking water resulted in blood pressure elevation and hyperglycemia which indicated that metabolic syndrome had been generated. Since then we have started administration of MH-76, MH-79 or prazosin concurrently with further fructose administration for subsequent 6 weeks. The dose of 5 mg/kg i.p. of test compounds was chosen based on our previous research

**Table 2** Pharmacokinetic parameters calculated from the concentrations of MH-76 and MH-79 compounds in plasma after single i.v. or i.p. administration at the doses of 2.5 and 5 mg/kg respectively (non-compartmental analysis).

| Pharmacokinetic parameter | MH-76 | | | | MH-79 | | | |
|---|---|---|---|---|---|---|---|---|
| | i.v | | i.p. | | i.v. | | i.p. | |
| | GM(90%CI) | CV% | GM(90%CI) | CV% | GM(90%CI) | CV% | GM(90%CI) | CV% |
| $C_0/C_{max}$ [μg/L] | 2027 (1026-4001) | 38.33 | 433* (354.7–528.6) | 11.42 | 2900 (1351-6229) | 44.87 | 731.8 (605.3–884.8) | 11.59 |
| $t_{max}$ [h] | – | | 0.083 | | – | | 0.083 | |
| $t_{1/2(\lambda z)}$ [h] | 1.68 (1.22–2.31) | 7.13 | 2.9* (2.06–4.09) | 19.63 | 2.09 (1.08–4.02) | 37.64 | 4.88 (4.61–5.16) | 3.36 |
| CL/(CL/F) [L/h/kg] | 2.09 (1.57–2.8) | 17.71 | 14.12* (12.81–15.55) | 5.68 | 1.35 (0.85–2.16) | 28.32 | 6.12 (4.75–7.88) | 14.39 |
| $AUC_{0\text{-inf}}$ [μg·h/L] | 1192 (891.9–1594) | 16.6 | 354.1* (321.6–389.9) | 15.6 | 1843 (1159-2930) | 26.13 | 817.2 (634.1–1053) | 15.6 |
| $V_{SS}$ [L/kg] | 3.13* (2.55–3.85) | 12.1 | – | | 1.53 (0.57–4.13) | 52.55 | – | |
| $V_z/F$ [L/kg] | – | | 59.14 (45.64–76.64) | 17.35 | – | | 43.09 (31.61–58.72) | 17.35 |
| MRT [h] | 1.48 (1.32–1.67) | 6.81 | – | | 1.12 (0.63–2.00) | 30.49 | – | |
| F [%] | 15.15 | | | | 26.28 | | | |

*$P < 0.05$ versus corresponding values for MH-79; $C_{max}$ – maximal concentration; $t_{max}$ – time to reach maximal concentration; $t_{1/2(\lambda z)}$ – half life in the elimination phase, CL – clearance; $AUC_{0\text{-inf}}$ – area under the concentration–time curve extrapolated to infinity; $V_z$ – volume of distribution at the elimination phase; $V_{ss}$ – volume of distribution at steady state, MRT – mean residence time, F-bioavailability.

[22] and a pilot study as an effective and well-tolerated one. It is worth mentioning that prazosin and test compounds were administrated in the equivalent doses regarding their affinity for $\alpha_1$-adrenoceptors. The doses of MH-76 and MH-79 were respectively higher than the dose of prazosin, due to the difference in their affinity for the $\alpha_1$-adrenoceptors ($K_i$ prazosin = 0.1 nM, $K_i$ MH-76, MH-79 = around 2 nM) [13].

At 18 weeks, fructose-fed rats presented a significantly higher weight gain and, finally, higher body weight accompanied with markedly increased abdominal adiposity. Treatment with MH-76 proved to be particularly advantageous and rats receiving fructose and MH-76 were characterized by a smaller increase in body weight, lower final body weight and lack of abdominal obesity. Treatment with MH-79 significantly reduced only abdominal obesity. On the other hand, the animals receiving fructose and prazosin did not differ in terms of body weight and amount of abdominal adipose tissue from fructose-fed rats. The early observations of the effects of quinazoline $\alpha_1$-adrenolytics on body weight showed that prazosin as well as doxazosin treatment caused weight gain in hypertensive humans [23,24]. In our study prazosin did not prevent excessive body weight and abdominal adiposity.

Increased adiposity and hyperinsulinemia-induced stimulation of the sympathetic nervous system is a possible mechanism that connects insulin resistance with hypertension. Hyperinsulinemia leads to sympathetic over-activity and $\alpha_1$-adrenoceptor activation, which results in increased vascular tone and elevated blood pressure [10,20]. These changes may enhance insulin resistance through vasoconstriction and subsequent reduction of glucose delivery. Fructose-fed rats presented mild hypertension, hyperglycemia, insulin resistance and impaired glucose tolerance. We showed that blockade of $\alpha_1$-adrenergic receptors by both MH-76, MH-79 and prazosin resulted in a decrease of blood pressure and an improvement in fasting glycaemia. Presumably, $\alpha_1$-blockers, as a result of their vasodilatory effect, facilitate the passage of glucose from the blood into metabolically active tissues. Interestingly, none of the compounds markedly improved glucose tolerance (Fig. S2) even if they reduced fasting hyperglycemia. This may be explained by the assumption that their positive effect on carbohydrate metabolism is due to their peripheral effects, but they do not directly influence insulin secretion.

In humans and rats, fructose is known to be more lipogenic than glucose or sucrose, resulting in an increase of hepatic triglyceride synthesis [20,21]. Fructose also stimulates intrahepatic de novo lipogenesis, due to its unregulated by feedback mechanism, metabolism to triose-phosphates, which are precursors of fatty acid synthesis. Additionally, fructose enhances the expression of main enzymes involved in lipogenesis [20]. All these mechanisms combined lead to increase in fasting plasma triglycerides, a phenomenon that has been also observed in our studies. Regarding the effects of $\alpha_1$-adrenoceptor blockers on triglycerides in metabolic syndrome in rodents and humans the results are not consistent. Some studies demonstrated that chronic treatment with $\alpha_1$-adrenolytics decreased triglyceride concentration in rats [25] and hypertensive humans [6,26], whereas others reported no [2] or even a negative effect of $\alpha_1$-adrenoceptor blockade [10] on hypertiglyceridemia. In our study, elevated triglycerides were advantageously reduced by MH-76 and MH-79 while a positive effect of prazosin did not reach statistical significance. Thus, it confirms the beneficial effects of studied compounds and $\alpha_1$-adrenoceptor blockade on lipid metabolism. Lack of prazosin efficacy in lowering the triglyceride levels might be the result of its shorter half-life, therefore basic pharmacokinetic parameters of the test substances were determined, i.e. half-life, clearance and volume of distribution after their intravenous and intraperitoneal administration and compared with the corresponding values known from literature for prazosin [19]. Indeed it was found that the half-life of MH-76 and MH-79 is almost three times longer as compared to prazosin (2.4 vs 0.92 h; Table S1). Fast fructose phosphorylation in the liver stimulates ATP hydrolysis resulting in increasing AMP concentration what further facilitates hepatic oxidative

damage and lipid peroxidation [20]. All tested $\alpha_1$-blockers inhibited the process of lipid peroxidation in the liver, showing protective, antioxidant effect. It is known that accumulated AMP may cause the enhanced uric acid synthesis [20], however, in our studies there was no increase in plasma uric acid concentration in fructose-fed rats.

In our study, fructose feeding caused the significant increase in TNF-$\alpha$ concentration as well as the concentration of the lipid peroxidation marker (MDA) in adipose tissue. Treatment with MH-76 was the only one that lowered TNF-$\alpha$ levels and decreased lipid peroxidation in adipose tissue. The reason for the much higher efficacy of MH-76 compared to MH-79 and prazosin may be of the pharmacodynamic nature, but it may also result from physicochemical/pharmacokinetic properties of MH-76. Pharmacokinetic parameter best describing the ability of compound to penetrate into deep compartments such as adipose tissue is volume of distribution ($V_d$). As seen in Table 2 and S1 volume of distribution at steady state ($V_{ss}$) of MH-76 is significantly higher as compared to MH-79 which might explain its superior activity in adipose tissue, however the same reasoning is not valid for prazosin since $V_{ss}$ of this compound in rats is quite high [19]. In this case possible explanation might lie elsewhere, therefore the octanol/water partitioning coefficients were calculated for both MH-76 and MH-79 using Marvin-ChemAxon software. Since the logP value for MH-76 is almost 3 times higher than for prazosin (4.5 vs 1.45), prazosin may to a lesser extent penetrate into adipose tissue than MH-76, despite a similar or even higher volume of distribution.

In our study, contrary to chronic prazosin treatment which did not improve insulin resistance treatment with MH-76 was the most effective in reducing hyperinsulinemia and insulin resistance. Research carried out by Tran [10] indicated that prazosin had no effect on insulin sensitivity, similarly studies performed by Zhou [2] revealed that phentolamine, non-selective $\alpha$-adrenoceptor antagonist without quinazoline moiety, also did not influence insulin sensitivity. It may be concluded that different mechanisms, unrelated to $\alpha_1$-blockade together with more favorable pharmacokinetic properties such as longer half-life, high $V_{ss}$ and lipophilic character promoting penetration into adipose tissue might be responsible for the beneficial effect of MH-76 on insulin resistance [19]. We showed that treatment with MH-76 lowered TNF-$\alpha$ concentration in adipose tissue which is strictly connected with inflammation and insulin resistance [1,21,27]. When the visceral adipocytes became hypertrophic and dysfunctional due to fructose feeding they release pro-inflammatory cytokines such TNF-$\alpha$ and reactive oxygen species. At the same time adipocytes are subjected to necrosis, which activates macrophages infiltration. The infiltrating macrophages, pro-inflammatory cytokines and reactive oxygen species contribute to further adipose tissue dysfunction and to a local and systemic low-grade inflammation [1,21,27]. Increased production of TNF-$\alpha$ in adipocytes inhibits insulin signaling, as TNF-$\alpha$ interferes with the phosphorylation of the insulin receptor, which leads to impaired suppression of hepatic glucose production, impaired glucose uptake function, and hyperglycemia [28,29]. Thus, the demonstrated ability of compound MH-76 to normalize TNF-$\alpha$ concentration and lipid peroxidation in adipose tissue could partly be the mechanism of its action to suppress insulin resistance.

Insulin resistance has been also suspected to contribute to the development of elevated blood pressure by triggering endothelial dysfunction and reducing endothelial NO bioavailability [2,30]. Insulin induces vasodilation by the release of NO but in the setting of insulin resistance this mechanism is impaired. Insulin resistance alters the PI3K-Akt downstream signaling in insulin action, which leads to decreased eNOS activity and reduced insulin-mediated relaxation and decreased glucose uptake. However, the second insulin pathway (Ras/Raf/MAP kinase) is not altered, which leads to unopposed ET-1 production [30]. Hyperglycemia also alters endothelial function by the formation of advanced glycation end products, which generate reactive oxygen species [1,30]. Increased ROS production, in turn, reduces NO bioavailabity by accelerated inactivation of NO by $O^{2-}$. Endothelial dysfunction further contributes to insulin resistance by diminishing blood flow and impairing transcapillary passing of insulin to metabolically active tissues [1,30]. Moreover, endothelial dysfunction contributes to visceral adiposity since reduction in NO bioavailability may impair energy homeostasis and inadequate energy production from adipose tissue resulting in excessive fat storage and visceral adiposity. Visceral adipose tissue, in turn, secretes pro-inflammatory cytokines and free fatty acids which aggravate endothelial dysfunction and insulin resistance [30]. TNF-$\alpha$ was also described to contribute to endothelial dysfunction by inhibiting the protective action of insulin on the endothelium and by triggering endothelial ET-1 production [1]. There are some studies describing a positive effect of TNF-$\alpha$ blockade on insulin resistance [29,31] and endothelial dysfunction [32] in rats with metabolic syndrome.

Studies on aortic rings from fructose-fed rats showed decreased relaxation responses to carbachol, suggesting vascular endothelial dysfunction. Impaired endothelial vascular relaxation was reversed by MH-76 and MH-79 treatment but not by prazosin which is consistent with our previous observations [22]. Furthermore, we reported that the mechanism of action of MH-76 and MH-79 involve not only $\alpha_1$-adrenoceptor blocking activity but also the activation of the endothelial NO-cGMP signaling pathway, whereas prazosin did not influence NO generation [15]. Prazosin did not have any effect on endothelial activity also in our *in vitro* studies, whereas MH-76 and MH-79 activated NO/sCG/cGMP pathway [15]. This ability of the studied compounds to activate NO production may, in part, explain why they reverted the fructose induced endothelial dysfunction. MH-76, probably due to the additional ability to reduce TNF-$\alpha$ concentration and lipid peroxidation in adipose tissue, was shown to be the most effective in reversing fructose induced insulin resistance. On the other hand, the lack of endothelial activity may explain why prazosin was not effective.

In our study, treatment with MH-76 was the most effective in reducing abdominal adiposity, the insulin resistance and hyperglycemia, and hypertriglyceridemia. Its superior effect on altered metabolism in fructose-fed rats, especially insulin-resistance is due to its ability to antagonize $\alpha_1$-adrenergic receptors, favorable physicochemical and pharmacokinetic parameters, endothelial protection, decreasing TNF-$\alpha$ generation and lipid peroxidation in adipose tissue. MH-79 did not show the ability to reduce TNF-$\alpha$ concentration in adipose tissue, and despite the endothelial protection effect, it was less beneficial; it decreased abdominal obesity, hyperglycemia and hypertriglyceridemia but did not normalize insulin resistance to the same extent as MH-76, what might be associated with MH-76 larger volume of distribution and better, compared to MH-79 ability to reach the deep tissue compartments. Additional explanation might be connected to unusually low bioavailability of MH-76 after i.p. administration probably being the result of its extensive metabolism since metabolites might be pharmacologically active and usually have much longer half-life than parent compound. However, the exact mechanism of this observation needs further studies.

Our major conclusion is that fructose induced hypertension, endothelial dysfunction, abdominal adiposity, and altered metabolism in rats were alleviated by MH-76, the non-quinazoline $\alpha_1$-adrenoceptor antagonist. On the other hand, prazosin treatment exerted an antihypertensive effect, reduced hyperglycemia but did not improve endothelial dysfunction, insulin resistance, and abdominal adiposity. The lower efficacy of prazosin may be the result of its short half-time and the lack of described pleiotropic effects. $\alpha_1$—adrenoceptor blockade, endothelial protection, TNF-$\alpha$ suppressing and antioxidant activity together with favorable pharmacokinetic parameters determines high efficacy of MH-76, leading to the effective improvement of hemodynamic and metabolic disturbances in metabolic syndrome. The use of non-quinazoline, multiple-targeted $\alpha_1$-blockers may be an interesting option for treatment of hypertension with metabolic complications.

## Conflicts of interest

The Authors declare that they have no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2019.04.003.

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