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Short communication

Antiarrhythmic activity of new 2-methoxyphenylpiperazine xanthone derivatives after ischemia/reperfusion in rats

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ABSTRACT

Background: We have previously shown significant prophylactic and therapeutic antiarrhythmic activity in adrenaline-induced arrhythmia, as well as α_1-adrenolytic properties of new derivatives of xanthone. Herein, we investigated their antiarrhythmic activity in the model of ischemia/reperfusion in isolated hearts. Furthermore, we assessed antioxidant activity in biochemical studies.
Methods: Antiarrhythmic activity in the model of ischemia/reperfusion in isolated perfused hearts was performed according to the Langendorff technique. Antioxidant activity was measured by lipid peroxidation level in tissue homogenate and in the FRAP assay.
Results: All studied compounds (**MH-94**, **MH-99** and **MH-105**) showed significant antiarrhythmic activity in the model of ventricular arrhythmias associated with coronary artery occlusion and reperfusion. However, they did not demonstrate antioxidant effect, probably, because of the lack of free hydroxyl group(s) at a key position in the xanthone scaffold.
Conclusions: The present study provides evidences for antiarrhythmic activity of some 2-methoxyphenylpiperazine derivatives of xanthone.

Introduction

Ventricular tachycardia and ventricular fibrillation are the principal reasons of death in patients with myocardial infraction [1]. Since the prevalence of coronary heart disease increases worldwide, the possibility of sudden cardiac death (SCD) is more common. The occurrence of SCD in Europe and USA seems to be similar and there is approximately 1 incidence per 1000 per year [2,3]. On the one hand, ventricular arrhythmias are susceptible to many groups of antiarrhythmic drugs, such as sodium and calcium channel blockers, β-blockers, potassium current blockers. On the other hand, antiarrhythmic drug therapy is often limited by the adverse effects of these drugs, such as bradycardia, tiredness, dizziness or thyroid dysfunction, as well as the risk of causing new life-threatening arrhythmias (proarrhythmic effect). Therefore, the searching for new potential antiarrhythmics that can improve heart function with minimal side effects seems to be reasonable.

Extensive pharmacological studies in the group of xanthone derivatives indicated that they possess beneficial effects on several cardiovascular diseases [4–6]. In the pathogenesis of post-ischemic myocardial dysfunction the generation of oxygen free radicals and lipid peroxides seem to play a crucial role. Earlier studies have shown that xanthones were able to decrease ischemia/reperfusion induced arrhythmias and that this cardioprotective effect was related to the inhibition of lipid peroxides in myocardial tissues. It has been also reported that among oxygenated and prenylated xanthone derivatives new compounds with strong antioxidant activity were found [6,7]. On the other hand, in animal models of arrhythmia, α_1-adrenoceptor, as well as, α_1/α_2-adrenoceptor antagonists, like urapidil, prazosin and phentolamine protected against ischemia/reperfusion changes in isolated rat hearts [8,9]. Moreover, it has been recently reported that the 1,4-substituted piperazine derivatives with α_1-adrenolytic properties displayed significant antiarrhythmic activity in ischemia/reperfusion arrhythmias [10].

Our earlier work has demonstrated significant prophylactic and therapeutic activity in adrenaline-induced arrhythmias, as well as prominent hypotensive activity of several xanthone derivatives

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with 2-methoxyphenylpiperazine moiety [11,12]. Three of them: **MH-94**, **MH-99** and **MH-105** also revealed high affinity for α_1-adrenoceptors in radioligand binding assay. Moreover, we confirmed α_1-adrenolytic activity these agents in the *in vitro* (isolated rat aorta contracted by phenylephrine) and *in vivo* (influence on blood vasopressor response in rats) tests [11]. The obtained results suggest that the antiarrhythmic and hypotensive effects could be related to their α_1-adrenoceptors antagonistic properties.

In our present study we planned to investigate antiarrhythmic activity in the model of ischemia/reperfusion in isolated perfused rat hearts. In order to examine a possible antioxidant activity some *in vitro* biochemical studies were performed.

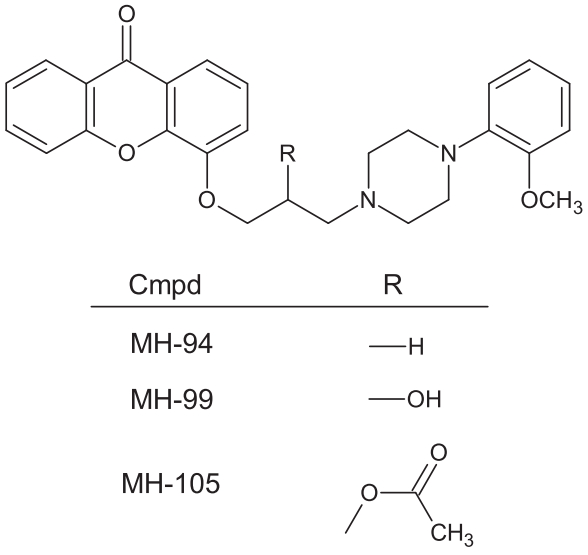
Materials and methods

Animals and experimental conditions

The studies were carried out on normotensive male Wistar rats weighing 180–200 g (KRF: WI (WU)). The animals were kept in plastic cages at room temperature of 20 ± 4 °C, under a 12/12 h light/dark cycle (light on from 7 a.m. to 7 p.m.). Standard food (standard laboratory pellets) and tap water were freely available before experiments. The control and study groups consisted of 6–8 animals each. The study was performed according to the Animal Care and Use Committee guidelines and was approved by the Local Ethics Committee of the Jagiellonian University in Kraków.

Drugs

The examined compounds: **MH-94** (4-(3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride), **MH-99** ((*R*,*S*)-4-(2-hydroxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride) and **MH-105** ((*R*,*S*)-4-(2-acetoxy-3-(4-(2-methoxyphenyl)piperazine-1-yl)propoxy)-9*H*-xanthen-9-one hydrochloride) were synthesized at the Department of Bioorganic Chemistry, Jagiellonian University Medical College (Scheme 1). The synthesis and preliminary pharmacological studies of these compounds were described earlier [12]. The following drugs were used: quinidine, urapidil (Sigma–Aldrich, Germany), heparin sodium (Polfa, Poland), thiopental sodium (Biochemie Gmbh, Austria). Other chemicals used were purchased from Polskie Odczynniki Chemiczne (Poland).



Scheme 1. Schematic structure of the studied 2-methoxyphenylpiperazine derivatives of xanthone.

Ischemia/reperfusion

Antiarrhythmic activity in the model of ischemia/reperfusion in isolated perfused hearts was performed according to the modified Langendorff technique as we precisely described previously [8,13]. After a 20 min stabilization period, a clip was placed on the left coronary artery for 15 min to induce acute myocardial ischemia. Then the clip was removed and the occurrence of ventricular premature beats (VBs), ventricular tachycardia (VT) and ventricular fibrillation (VF) during reperfusion period were monitored for 30 min. The arrhythmia severity index was calculated in order to quantify the arrhythmias [14]. The tested compounds were put into the perfusion solution 15 min before coronary artery ligation and the concentration was sustained for the rest of the perfusion period. The following grades were attributed: the occurrence of up to 10 ventricular extrasystoles during 30 min of reperfusion – 1, more than 10 – 2, ventricular tachycardia – 3, ventricular fibrillation – 4.

In separate series of experiments the effect of the tested compounds, at the concentrations of 10^{-7}–10^{-5} M on electrocardiogram (ECG), was assessed after 20 min of initial equilibration period.

Antioxidant activity

The ferric reducing antioxidant power (FRAP) assay

Antioxidant activity was examined using the FRAP assay as described previously [13]. This method is based on the reduction of ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous (Fe^{2+}-TPTZ) form at low pH by antioxidant agents. The reduced ferrous forms, which have an intense blue color, with absorption maximum at 593 nm were measured [15]. The results were obtained as the increase in absorbance of the test sample compared to the sample containing the solvent alone. Trolox (synthetic vitamin E analog) at the concentrations range from 10^{-5} to 3×10^{-4} M was used as a reference compound. The tested compounds were added at the concentration of 10^{-4} M.

Influence on lipid peroxidation

Lipid peroxidation level was determined in rat brain homogenate as we precisely described previously [8,10]. The membrane lipid peroxide content was quantified by the formation of thiobarbituric acid reactive substances (TBARS). The absorbance of the supernatant was measured at 532 nm. The amount of TBARS was determined using a standard curve of malonaldehyde bis (dimethyl acetal).

Data analysis

All results, presented in the tables as mean ± SEM. Statistically significant differences between groups were calculated using one-way analysis of variance (ANOVA), followed by Dunnett's *post hoc* test or repeated measures ANOVA. Values of $p < 0.05$ were considered to be statistically significant.

Results

Antiarrhythmic activity in the model of ischemia/reperfusion in isolated perfused rat heart

In the control group the incidence of ventricular premature beats and ventricular tachycardia was 100%, whereas ventricular fibrillation occurred in 16.7% hearts. Table 1 shows that all tested