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3,5-Dibenzoyl-4-(3-phenoxyphenyl)-1,4-dihydro-2,6-dimethylpyridine (DP7): A new multidrug resistance inhibitor devoid of effects on Langendorff-perfused rat heart

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Abstract

Cardiac effects of 3,5-dibenzoyl-4-(3-phenoxyphenyl)-1,4-dihydro-2,6-dimethylpyridine (DP7), a novel multidrug resistance (MDR) inhibitor, in Langendorff-perfused rat heart have been investigated and compared to that of nifedipine. Nifedipine decreased concentration-dependently ($IC_{50}=8.89\pm1.09\times10^{-8}$ M) left ventricular pressure leaving unaltered coronary perfusion pressure, whereas DP7 did not affect both parameters. Nifedipine did not modify both QRS and QT intervals of electrocardiogram (ECG). Second-degree atrioventricular block or ventricular rhythm occurred in presence of nifedipine, however, in 4 out of 6 hearts. DP7, up to 30 μM, failed to alter ECG parameters. In conclusion, DP7, may represent a lead compound for the development of potent dihydropyridine MDR chemosensitizers devoid of cardiac effects.

Keywords: Dihydropyridines; Electrocardiogram; Langendorff-perfused rat heart

1. Introduction

The overexpression of P-glycoprotein and other drug transporters (ATP-binding cassette) confers a multidrug resistance (MDR) phenotype on cells in various diseases, including many forms of cancer. Development of MDR is one of the main reasons of failure in cancer chemotherapy, as tumour cells, by increasing drug efflux, acquire cross-resistance to many structurally and functionally unrelated anticancer agents, which therefore never achieve effective intracellular concentrations (Fusi et al., 2006). In the past few years, extensive studies have been performed with the aim of developing effective chemosensitizers to overcome MDR of human cancer cells. Potent P-glycoprotein inhibitors have been tested in clinical trials so far, including Ca^{2+} channel blockers such as verapamil and dihydropyridines (Bates et al., 1994; Bellamy, 1996). However, clinical application of these agents has not been extensively pursued to date, owing to their unwanted and sometimes life-threatening cardiovascular side effects such as atria-ventricular block and hypotension. As a consequence, in the last few years, much attention has been focused on congeners of this first generation-MDR inhibitors, in order to develop compounds characterized by appropriate potency and selectivity but also by reduced cardiovascular toxicity.

Recently, novel 3,5-diacetyl- (DP1-DP5) and 3,5-dibenzoyl-1,4-dihydropyridine (DP6-DP11) derivatives were synthesized and screened for their ability to reverse MDR *in vitro* assay systems (Shah et al, 2000; Kawase et al, 2002). Some of them considerably reduced the activity of P-glycoprotein in L5178 Y mouse T-lymphoma cells transfected with MDR1 gene and were therefore envisaged as new candidates for MDR reversion in human cancer treatment. Furthermore, they were assessed on vascular function *in vitro*, by comparing mechanical and

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electrophysiological phenomena, in an attempt to identify, among them, those endowed with the lowest vascular effects.

3,5-Dibenzoyl-4-(3-phenoxyphenyl)-1,4-dihydro-2,6-dimethylpyridine (DP7), at a concentration two orders of magnitude higher than its IC_{50} as a P-glycoprotein inhibitor, was neither an antispasmodic nor a spasmolytic agent and inhibited L-type Ca^{2+} current by a mere 20%: this clearly indicates that the P-glycoprotein inhibitory effect of this compound could be obtained without any effects on blood vessels (Saponara et al., 2004).

However, as dihydropyridine derivative, this compound is expected to be active also on the cardiac system. In order to provide further information on this new MDR inhibitor, its effects on cardiac function have now been investigated in isolated rat heart and compared to those of nifedipine, a well known dihydropyridine L-type Ca^{2+} channel blocker.

2. Materials and methods

2.1. Langendorff-perfused rat heart

Principles of laboratory animal care (NIH publication No. 85–23, revised 1996) were followed. Male Sprague–Dawley rats (150–200 g) were anaesthetized with a mixture of Ketavet® (0.3 mg/kg; Gellini, Italy) and Rompum® (0.08 mg/kg; Bayer, Germany) containing heparin (5000 U/kg), decapitated and bled. The hearts were rapidly explanted and mounted on a Langendorff apparatus for retrograde perfusion *via* the aorta (Langendorff, 1895) at a constant flow rate of 10 ml/min using a peristaltic pump (Alitea C4 Midi, Watson-Marlowe, Sweden). The perfusion medium was a Krebs–Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11.5, Na pyruvate 2 and EDTA 0.5, bubbled with a 95% O_2–5% CO_2 gas mixture (pH 7.4) and kept at 37 °C.

After a 20-min stabilization period, the hearts were paced at the frequency of 5 Hz (8 V amplitude; 0.6 ms duration) through a platinum electrode placed on the right atrium. The hearts were allowed to equilibrate for at least 10 min before drug exposure.

Heart contractility was measured as left ventricle pressure by means of a steel cannula (Hugo Sachs Elektronic, Germany), inserted into the left ventricle *via* the mitral valve and connected to a pressure transducer (BLPR, WPI, Germany).

Alteration in coronary perfusion pressure, arising from changes in coronary vascular resistance were recorded by a pressure transducer (BLPR, WPI, Germany).

Surface electrocardiogram (ECG) was recorded by means of two steel electrodes, one placed on the apex and the other on the left atrium of the heart. The ECG analysis included the following measurements: PQ interval, i.e. the atrioventricular conduction time; QRS interval, i.e. the intraventricular conduction time; QT interval, i.e. the duration of ventricular depolarization and repolarization (the action potential duration). Cardiovascular parameters (left ventricle pressure and coronary perfusion pressure) and ECG were recorded with a digital PowerLab data acquisition system (PowerLab 8/30; ADInstruments, Castle Hill, Australia) and analysed by using Chart Pro for Windows software (Power Lab; ADInstruments). The sampling rate was 1 kHz.

2.2. Chemicals

Nifedipine was supplied by Sigma Chimica, Italy. DP7 was synthesized as described by Kawase et al. (2002). Nifedipine dissolved directly in ethanol and DP7 dissolved in dimethylsulphoxide (DMSO), were diluted in Krebs–Henseleit solution prior to use. The resulting concentrations of ethanol and DMSO (below 0.03% and 0.1%, respectively) did not affect the responses (data not shown).

2.3. Statistical analysis

Data are reported as means±S.E.M.; *n* (indicated in parentheses) represents the number of rat hearts. Analysis of data were accomplished using GraphPad Prism version 4.03 (GraphPad Software, U.S.A.).

Statistical analyses and significance as measured by one-way or two-way ANOVA (followed by Dunnet's or Bonferroni post test), were obtained using GraphPad InStat version 3.06 (GraphPad Software, U.S.A.) as appropriate. In all comparisons, $P<0.05$ was considered significant.

Left ventricular pressure was calculated by subtracting the left ventricular diastolic pressure from the left ventricular systolic pressure.

3. Results

Under control conditions, left ventricular pressure and coronary perfusion pressure values of 61.9±4.1 mmHg and 60.7±2.8 mmHg ($n=13$), respectively, were obtained. Nifedipine reduced left ventricular pressure in a concentration-dependent manner ($IC_{50}=8.89\pm1.09\pm10^{-8}$ M, $n=6$) leaving unaltered coronary perfusion pressure, whereas DP7 did not affect both cardiovascular parameters (Fig. 1). In untreated hearts PQ, QRS and QT values of 32.6±1.1 ms, 11.7±0.4 ms and 62.0±2.5 ms ($n=10$), respectively, were recorded.

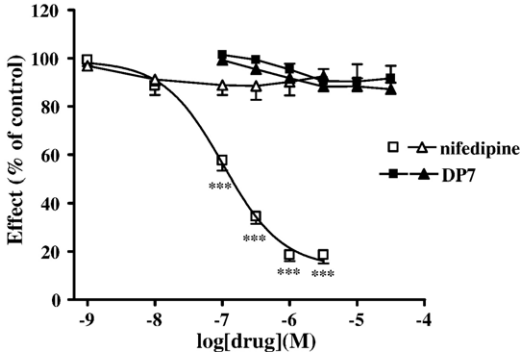

Fig. 1. Effects of nifedipine and DP7 on left ventricular pressure and coronary perfusion pressure in Langendorff-perfused rat hearts. Concentration-effect relationship of nifedipine (open symbols) and DP7 (closed symbols) on left ventricular pressure (squares) and coronary perfusion pressure (triangles). On the ordinate scale, response is reported as % of left ventricular pressure and coronary perfusion pressure measured before addition of drugs. Each value represents mean±S.E.M. ($n=3$–7). Concentration-effect relationship of nifedipine shows a $P<0.001$, ***$P<0.001$ nifedipine *vs.* DP7, two-way ANOVA, Bonferroni post test.

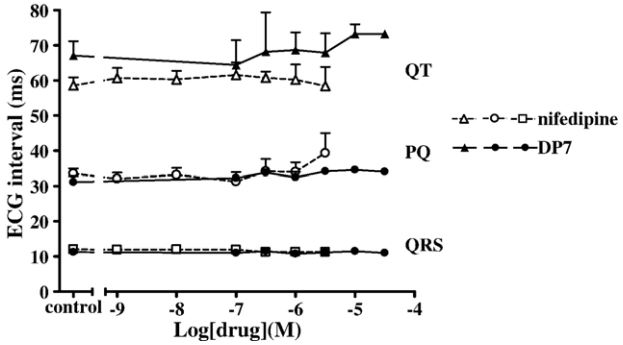

Fig. 2. Effects of nifedipine and DP7 on ECG parameters in Langendorff-perfused rat hearts. Concentration-effect relationships of nifedipine and DP7 on ECG intervals (namely QT, triangles; PQ, circles; QRS, squares) recorded either in the absence (control) or in the presence of various concentrations of nifedipine (open symbols) or DP7 (closed symbols). PQ values of three hearts in which AV block occurred during perfusion with either 1 or 3 μM nifedipine are not included. Each value represents mean±S.E.M. (n=3–6). P>0.05 *vs.* control, one-way ANOVA, Dunnett's post test; P>0.05 nifedipine *vs.* DP7, two-way ANOVA, Bonferroni post test.

Nifedipine did not modify either the intraventricular conduction time or the cardiac action potential duration, whereas in 3 heart preparations out of the total 6 examined it prolonged the atrioventricular conduction time. In 2 experiments a Wenckebach-type second degree atrioventricular block occurred in the presence of either 1 or 3 μM nifedipine, whereas a Mobitz II second degree atrioventricular block occurred in the presence of 1 μM nifedipine in one out of 6 hearts. Additionally, in another heart, a ventricular rhythm was recorded in the presence of 1 μM nifedipine. In the range of the concentrations tested (0.1–30 μM), DP7, failed to alter ECG parameters (Fig. 2).

4. Discussion

The present findings clearly demonstrate that, in the isolated rat heart, DP7 failed to alter myocardial contractility, coronary resistance and the ECG parameters monitored.

The mechanisms involved in excitation–contraction coupling in the heart differ from those in the vasculature in that a great portion of the inward current is carried by Na^+ through the fast channel and by Ca^{2+} through the slow channel. In the sinoatrial and atrioventricular nodes, depolarization is largely dependent on the movement of Ca^{2+} through the slow channel. In this study, nifedipine, a well known L-type Ca^{2+} channel blocker, induced a concentration-dependent negative inotropic effect leaving unaltered the coronary perfusion pressure. Furthermore, in 4 hearts it slowed the atrioventricular conduction and depressed the sinus node activity to such an extent that either atrioventricular block or ventricular rhythm occurred, as already reported by Stark et al. (1988).

Recently, we have shown that the dihydropyridine derivative DP7 is devoid of vascular activity in contrast to that observed with nifedipine (Saponara et al., 2004). In the present work we have compared a possible cardiac negative inotropism of DP7 to that of nifedipine and found that only the latter was capable to reduce heart contractility. This lack of inhibition of heart contractility by DP7 could be due to the replacement in DP7 of the 3,5-dicarbomethoxy groups of nifedipine with 3,5-dibenzoyl groups. This is in agreement with what previously reported by Loev et al. (1974) and Janis and Triggle (1983), who showed that replacement of 3,5-dicarbomethoxy groups by other electron-withdrawing substituents, markedly reduces the Ca^{2+} blocking activity of the resulting compounds. Additionally, according to both Loev et al. (1974) and Rodenkirchen et al. (1979), the position of the substituent in the 4-phenyl ring may be critical: *para* and *meta* (DP7) substitutions invariably decrease the spasmolytic and negative inotropic activities, whereas the *ortho* substitution – as in the case of nifedipine – increases these properties owing to its steric hindrance. As previously reported (Rodenkirchen et al., 1979), the lipophilic properties of the ester substituents at 3-and 5-position in the 1,4-dihydropyridine ring plays a key role in the pharmacological activity of the resulting compounds since their Ca^{2+} antagonistic activity decreases as lipofilicity of their substituents increases, as it is the case of DP7.

So far no evidence for voltage gated Ca^{2+} channel has been found in several MDR cell line (e.g. CEM/VLB_{100}, P388-doxorubicin-resistant, Chinese hamster ovary, C6 glioblastoma cells) (Ford and Hait, 1990) thus supporting the hypothesis that DP7 inhibits MDR independently of the changes in inward Ca^{2+} channels.

DP7 has been shown to inhibit P-glycoprotein in L5178 Y mouse T-lymphoma cells transfected with MDR1 gene with an IC_{50} value of 3.02×10^{-7} M (Saponara et al., 2004). Therefore, we can conclude that DP7, at concentrations two orders of magnitude higher than its IC_{50} as a P-glycoprotein inhibitor, is devoid of cardiac as well as vascular effects *in vitro*, indicating clearly that P-glycoprotein inhibitory effects are quite separate from its activity on the cardiovascular system, thus limiting its potential side effects.

Since Langendorff isolated heart is artificially perfused, areflexive and deprived of normal humoral background it does not represent the real physiological situation. Therefore, the present results strengthen the need for further pharmacological and toxicological studies *in vivo.*

In conclusion, DP7 represents a lead compound for the design of novel, safe and potent MDR chemosensitizers which may help to perform effective chemotherapeutic interventions in cancer patients.

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