Department of Biopharmacy, Medical University of Łódź, Poland

Influence of imatinib at a low dose and sildenafil on pulmonary hypertension in rats

M. JASIŃSKA-STROSCHEIN, J. OWCZAREK, K. CICHA, D. ORSZULAK-MICHALAK

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Magdalena Jasińska-Stroschein, Department of Biopharmacy, Medical University of Łódź, Muszyńskiego 1, 90-151 Łódź, Poland
magdalena.jasinska-stroschein@umed.lodz.pl

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The study investigates whether combination therapy of sildenafil with imatinib at a low dose (20 mg/kg) further ameliorates pulmonary hypertension (PH) in rats. The effects on right ventricle pressure (RVP), and right ventricle hypertrophy (RVH) were assessed in experimental monocrotaline (MCT)-induced pulmonary hypertension. Combined therapy reversed the MCT-induced increase in RVP more than each drug alone and decreased RV hypertrophy (RV/LV + S ratio), significantly. Such additive effects toward improvement of PH may result from both pharmacodynamic and pharmacokinetic drug-drug interactions, however, further studies are required to assess its mechanistic background.

1. Introduction

Imatinib mesylate is widely used for the treatment of chronic myeloid leukemia. It also reveals advantageous effects in pulmonary hypertension (PAH). Chronic acute and oral-50 mg/kg body weight (bw) drug administration reversed PH in experimental models (Nakamura et al. 2012; Schermuly et al. 2005). As reported in recent clinical trials, imatinib improves pulmonary hemody namics and exercise capacity, even in patients with advanced PAH (Ghofrani et al. 2010). However, serious adverse events leading to the discontinuation of the trial were common (Hoeper et al. 2013). This unsatisfactory safety profile has demonstrated the necessity for further studies to assess the risk-benefit profile or optimal dosage of imatinib in PAH. PAH remains a lethal disease; currently approved therapies including phosphodiesterase type 5 (PDE-5) inhibitors are believed to reverse or diminish several pathological abnormalities. The PDE-5 inhibitor sildenafil (Revatio®) was approved as a thrice-daily therapy for patients with WHO II and III functional class. Similarly to imatinib, sildenafil is metabolized *via* CYP3A4 P450 hepatic enzymes (Frumkin 2012). Hence, the potential efficacy (or safety) of PDE-5 and TKI used in combination should be increased/reduced. The aim of the present study was to assess the effects of the combined treatment of a low dose of imatinib (20 mg/kg) given with PDE-5 inhibitor in pulmonary hypertension induced by monocrotaline (MCT) in rats.

2. Investigations and results

Imatinib at 20 mg/kg caused a moderate, but significant decrease in right ventricle pressure (RVP) compared to MCT + vehicle ($P < 0.001$); the observed values were also significantly higher in comparison to Sham ($P < 0.0005$). The mean RVP in rats receiving sildenafil at 25 mg/kg was significantly lower than those of the MCT-treated rats ($P < 0.0005$). 14-day combined administration of imatinib and sildenafil caused a significant decrease in RVP compared to MCT + vehicle ($P < 0.0005$); and to imatinib ($P < 0.0005$) or sildenafil alone ($P < 0.05$). The ratio of right ventricle weight to left ventricle plus septum weight (RV/LV + S) was significantly lower in rats exposed to imatinib compared to MCT + vehicle ($P < 0.05$), but significantly higher than that observed in Sham rats ($p < 0.0005$). Sildenafil decreased RV/LV + S compared to MCT-treated rats ($P < 0.05$). Imatinib given together with sildenafil reduced right ventricle hypertrophy compared to sildenafil ($P < 0.05$), imatinib alone ($P < 0.01$) and MCT + vehicle ($P < 0.005$) (Fig.). Imatinib combined with sildenafil decreased SABP compared to Sham and MCT + vehicle ($P < 0.05$) (Table).

3. Discussion

The main finding of the study is that combination therapy is more effective in reversing MCT-induced increase in RVP and right ventricle hypertrophy (RVH) than each drug alone. Similarly, in our previous study RVP and RVH values achieved at least the same level as imatinib at higher dose, i.e. 50 mg/kg bw given in monotherapy when imatinib at 20 mg/kg was combined with statin (Jasińska-Stroschein et al. 2015). The beneficial impact of imatinib on PAH is based on preventing cell proliferation and eliciting pulmonary vasodilatation. Inhibition of the platelet-derived growth factor (PDGF) receptor is considered to be one of the possible molecular mechanisms by which it can act (Klein et al. 2008). In contrast, sildenafil inhibits pulmonary vascular remodeling *via* the eNOS-NO-cGMP pathway (Zhao et al. 2001). Interestingly, previous results obtained by Li et al. (2007) seem to suggest that a mechanistic linkage between sildenafil and imatinib exists, as sildenafil was demonstrated to inhibit PDGF-induced effects, i.e. proliferation of porcine pulmonary artery smooth muscle cells. It was connected with inactivation of extracellular signal-regulated kinase (ERK1/2) and induction of mitogen-activated protein kinase phosphatase-1 (MKP-1): the negative regulator of MAPK signaling (Lin et al. 2003). This effect in turn was suggested to be medi-

Table: Heart rate (HR), systolic; mean and diastolic blood pressure (SABP, MABP, DABP) as mean ± SD

Animals	HR (bpm)	SABP (mmHg)	MABP (mmHg)	DABP(mmHg)
Sham	371.6 (± 27.0)	100.4 (±7 .8)	96.4 (± 6.5)	91.9 (± 5.5)
MCT + Vehicle	405.3 (± 26.8)	100.5 (± 8.8)	94.7 (± 6.5)	87.8 (± 5.1)
MCT + SILDENAFIL	411.6 (± 54.3)	94.1 (± 9.5)	90.8 (± 8.3)	88.3 (± 7.2)
MCT + IMATINIB	421. 0 (± 25.6)	94.1 (± 6.2)	89.1 (± 5.1)	87.0 (± 4.3)
MCT + SILDENAFIL + IMATINIB	395.6 (± 35.4)	89.8 (± 3.3)[a, b]	87.4 (± 3.1)	85.1 (± 2.6)

Sham *(N = 9)*; MCT + vehicle *(N = 9)*; MCT + sildenafil *(N = 8)*; MCT + imatinib at 20 mg/kg *(N = 10)*; MCT + sildenafil + imatinib at 20 mg/kg *(N = 9)*. a -$P < 0.05$ *vs* Sham; b -$P < 0.05$ *vs* MCT + vehicle.

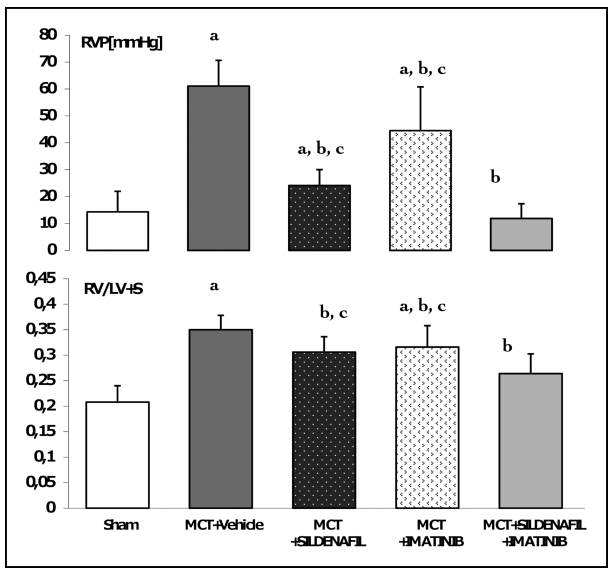

Fig.: Right ventricular pressure (RVP) and right ventricular hypertrophy expressed as the ratio of the right ventricular weight to left ventricle plus septum weight (RV/LV + S). Sham *(N = 9)*; MCT + vehicle *(N = 9)*; MCT + sildenafil *(N = 8)*; MCT + imatinib at 20 mg/kg *(N = 10)*; MCT + sildenafil + imatinib at 20 mg/kg *(N = 9)*. The histograms indicate mean ± SD. a -$P < 0.05$ *vs* Sham; b -$P < 0.05$ *vs* MCT + vehicle; c -$P < 0.05$ *vs* combination.

ated by cGMP signaling pathway. However, a strict evaluation of the proposed mechanistic background of such pharmacodynamic drug-drug interaction is needed. Another mechanistic explanation of the obtained results may relate to pharmacokinetic interactions between sildenafil and imatinib. Both drugs are substrates for CYP3A4. Although no published data exists, sildenafil could theoretically increase imatinib levels and *vice versa*. Rats do not possess CYP3A4, but its activity might be adopted by other enzymes, such as CYP2C11, 3A and 2D3 (Ishigami et al. 2002). Thereby we cannot exclude that the additive effects of the drug combination observed in the present study on a rat model are simply the consequence of a greater exposure to both agents. As the study limitation: the pharmacokinetic interaction between sildenafil and imatinib needs to be investigated in future studies.

The safety profile and hence, the further usage, of TKI in PAH remains in question, despite clinical trials demonstrating that imatinib administration is associated with further improvements in hemodynamics or exercise capacity. In addition, some authors emphasize the need for further studies concerning the long-term safety of imatinib administration in PAH, assessing its benefit-risk ratio or determining the minimum efficacious dose (Hoeper et al. 2013). The best exercise capacity was observed while imatinib was added to combination of endothelin receptor antagonist (ERA) and PDE-5 inhibitor. However, no further studies concerning the significance of this observation have been performed, and no clinical data regarding direct comparisons of sildenafil with imatinib therapy *vs* sildenafil alone has been acquired. Nevertheless, if confirmed, our results indicate that due to drug-drug interaction, the imatinib dose may be reduced when co-administered with sildenafil to improve the TKI safety profile.

4. Experimental

The experiments were performed on 45 outbred Wistar male rats randomly allocated into five groups: 1)Sham: healthy rats receiving 1.0% methylcellulose (MC), 1 ml/kg, i.g. 2)MCT + vehicle: 1.0% MC, 1 ml/kg, i.g. daily for 14 days after MCT injection; 3)MCT-induced rats receiving sildenafil; 4)MCT-induced rats receiving imatinib; 5)MCT-induced rats receiving sildenafil plus imatinib. The first dose of imatinib (20 mg/kg, i.g. bw) (Jasińska-Stroschein et al. 2015), sildenafil (25 mg/kg, i.g. bw) (Guilluy et al. 2005) or their combinations were given 14 days after monocrotaline injection for the next 14 days. Drugs: imatinib mesylate (HBCChem Inc., USA) and sildenafil were administered by oral gavage, suspended in 1.0% methylcellulose (MC). Monocrotaline (Fluorochem Ltd, UK) solution (Schermuly et al. 2004), was administered as a single, subcutaneous (s.c.) injection (60 mg/kg) in a volume of 3 ml/kg. The whole experimental protocol lasted 29 days. The surgery was performed 24 h after the administration of the last drug dose. The animals were anaesthetized with an initial intraperitoneal (i.p.) dose of pentobarbital sodium at 30 mg/kg bw. Anesthesia was maintained by additional bolus doses of pentobarbital sodium (10 mg/kg bw) as needed. Lidocaine was used for local infiltration of the surgical sites. The registration of hemodynamic parameters (mean RVP, SABP, MABP, DABP) in rats was performed by using a Hugo Sachs Electronik Haemodyn (Harvard Apparatus GmbH, March, Germany), as described previously (Jasińska-Stroschein et al. 2013). At the end of the study, after animal euthanasia, the heart was excised. Next, the right ventricle, right atrium and left ventricle plus septum were separated and weighed. The ratio of the right ventricle weight to left ventricle plus septum weight (RV/(LV + S)) was calculated as an index of right ventricular hypertrophy. All the procedures were approved by the Ethics Committee of the Medical University of Lodz, Poland (84/ŁB695/2013). The statistical evaluation was performed using the analysis of variance (ANOVA) and post-hoc comparisons were performed using the Student-Newman-Keuls test (STATISTICA 10.0). All parameters were considered statistically significantly different if $P < 0.05$.

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