Cardiac safety profile of sildenafil: chronotropic, inotropic and coronary vasodilator effects in the canine isolated, blood-perfused heart preparations

Nur Jaharat Lubna1,*, Yuji Nakamura1,2,*, Xin Cao1, Takeshi Wada1, Hiroko Izumi-Nakaseko1, Kentaro Ando1 and Atsushi Sugiyama1,2

1Department of Pharmacology, Faculty of Medicine, Toho University, 5-21-16, Omori-nishi, Ota-ku, Tokyo 143-8540, Japan
2Yamanashi Research Center of Clinical Pharmacology, 73-5 Hatta, Isawa-cho, Fuefuki, Yamanashi 406-0023, Japan

(Received April 15, 2016; Accepted August 19, 2016)

ABSTRACT — Sildenafil is a phosphodiesterase type-5 inhibitor. We evaluated the effects of sildenafil on the sinoatrial rate, developed tension of the papillary muscle and coronary blood flow by using the canine isolated, blood-perfused sinoatrial node and papillary muscle preparations. The former preparation had a regular automaticity rate of 106 ± 1 beats/min (n = 4), whereas the latter showed a developed tension of 22 ± 4 mN (n = 4) and a coronary blood flow of 3.9 ± 0.1 mL/min (n = 4). Intracoronary injection of 10, 30 and 100 μg of sildenafil, which would provide about 20 to 200 times higher plasma drug concentrations than its therapeutic level, increased the automaticity rate by 4, 12 and 22%, the developed tension by 19, 55 and 118% and the coronary blood flow by 42, 95 and 142%, respectively. These results indicate that supratherapeutic concentration of sildenafil possesses direct positive chronotropic and inotropic effects together with a coronary vasodilator action, confirming that caution has to be paid on the use of sildenafil for patients with ischemic heart diseases, obstructive hypertrophic cardiomyopathy and/or ventricular arrhythmias. The information on sildenafil reported in this study may help establish a guidance on cardiac safety assessment of newer phosphodiesterase type-5 inhibitors.

Key words: Sildenafil, Automaticity, Contraction, Coronary vasodilator

INTRODUCTION

Sildenafil is an inhibitor of cyclic guanosine monophosphate (GMP)-specific phosphodiesterase type-5 (PDE5), which can increase intracellular cyclic GMP level (Wallis et al., 1999). After its approval for erectile dysfunction (Chew et al., 2000; Goldstein et al., 1998), several reports of sudden death among patients treated with sildenafil have raised some concerns regarding its cardiovascular adverse events (Kloner, 2000). It has been reported that sildenafil did not exert a positive inotropic effect in previous in vitro studies using the human right atrial/left ventricular muscles at concentrations of 0.1 nM to 10 μM (Cremers et al., 2003) or the small sections of trabeculae carneae from canine hearts dissected from the endocardial surface of the right ventricle at concentrations of 10 nM to 10 μM (Wallis et al., 1999). However, intravenous administration of 3 mg/kg of sildenafil caused the positive inotropic effect in anesthetized dogs, in which C max was 3.1 μg/mL (6.4 μM) (Sugiyama et al., 2001). Moreover, sildenafil was shown to significantly increase the net cyclic AMP production rate in the canine ventricular membrane preparation at a concentration of 10 μM, and to decrease the cyclic AMP hydrolyzing speed of PDE extracted from bovine hearts at concentrations of 1-10 μM (Sugiyama et al., 2002). In order to better explain the discrepancies among these in vivo, in vitro and molecular results of sildenafil, moreover to clarify its safety profile, we performed the current experiments by using the canine isolated blood-perfused heart preparations (Sugiyama et al., 1990, 1991).

MATERIALS AND METHODS

Experiments were planned in accordance with the rules and regulations of the Committee for Research at
Yamanashi Research Center of Clinical Pharmacology (#2002-03). Animals were obtained through the Animal Laboratory for Research of University of Yamanashi.

**Production of the canine isolated, blood-perfused heart preparations**

Experiments were performed by using the canine isolated sinoatrial node and papillary muscle preparations cross-circulated with heparinized arterial blood of the blood-donor dog as shown in Fig. 1 (Sugiyama *et al.*, 1990, 1991).

**Isolated heart preparations**

The preparation was obtained from a beagle dog (CSK Research Park, Nagano, Japan) of either sex, weighing approximately 10 kg (n = 4). The dog was anesthetized with pentobarbital sodium (30 mg/kg, i.v.), given heparin calcium (500 U/kg, i.v.), and exsanguinated. The heart was excised and plunged into cold Tyrode’s solution kept at about 4°C. The sinoatrial node preparation consisted of the entire right atrium. The sinus node artery was cannulated through the right coronary artery. Bipolar recording electrodes were attached on the atrial epicardium close to the sinus nodal region. The papillary muscle preparation consisted of the anterior papillary muscle of the right ventricle attached to the interventricular septum. The anterior septal artery, a sole nutrient artery of the preparation, was directly cannulated. Bipolar stimulating electrodes were attached onto the His-bundle region.

**A blood-donor dog**

Mongrel dogs (Kitayama Labes, Yoshiki Farm, Gifu, Japan) of either sex, weighing 17-20 kg, were used as blood-donor ones (n = 4). The dog was anesthetized with pentobarbital sodium (30 mg/kg, i.v.), and supplemented with 4-5 mg/kg/hr. After intubation, the dog was artificially ventilated with room air (SN-480-3, Shinano, Tokyo, Japan). The systemic blood pressure and surface lead II electrocardiogram were monitored with using a polygraph system (RM-6000, Nihon Kohden, Co., Tokyo, Japan). At the start of cross-circulation, heparin calcium (500 U/kg, i.v.) was given which was followed by an additional dose of 200 U/kg/hr.

**Cross-circulation**

The preparations were placed in a double-wall glass jacket maintained at 38°C by circulating warm water and were perfused with arterial blood from the carotid artery of the blood-donor dog. Perfusion pressure was kept at 120 mmHg with a peristaltic pump (7553-00, Cole-Parmer, Chicago, IL, USA) and Starling’s pneumatic resistance placed parallel to the perfusion circuit. Venous blood from the preparations and excess blood passing through the pneumatic resistance were collected in a blood reservoir and returned to the jugular vein of the blood-donor dog.

---

**Fig. 1.** Schematic circulating diagram of the canine isolated, blood-perfused sinoatrial (SA) node and papillary muscle (PM) preparations.
Parameters

The spontaneously beating rate of the sinoatrial node preparation (i.e., sinoatrial rate) was measured with a heart rate counter (AT-601G, Nihon Kohden) triggered by the atrial electrogram. The papillary muscle preparation was electrically driven through the stimulating electrodes at a cycle length of 500 ms using a stimulator (SEN-7203, Nihon Kohden) with an isolation unit (SS-201J, Nihon Kohden). The stimulation pulses were rectangular in shape, 1-2 V in amplitude (about 20% above the threshold voltage), and of 5-ms duration. The developed tension of the papillary muscle under a resting tension of 20 mN was measured isometrically by using a force displacement transducer (DRM-200S, Dia Medical System Co., Ltd., Tokyo, Japan) with an amplifier (DRM-T20, Dia Medical). The coronary blood flow through the nutrient arteries of each preparation was continuously monitored with an electromagnetic flowmeter (MFV-3200, Nihon Kohden).

Experimental protocol

Once the preparations were stabilized, sildenafil in doses of 10-100 μg or vehicle saline were injected into each nutrient artery with a small micro-syringe in volumes of 10-100 μL over 4 sec. Physiological recordings were performed for 10 min after each dose. Since a relatively small amount of a drug was administered to the preparations compared with that required for an in vivo animal experiment, and the effluent blood through each preparation was discarded immediately after the drug injection to eliminate the impact on the blood-donor dogs, the effects of multiple drug doses were studied in the same preparation.

Drugs

Sildenafil was extracted in saline from a commercial source (Viagra™, Pfizer Pharmaceuticals Inc., Tokyo, Japan) and dissolved in saline in concentration of 1 mg/mL. Pentobarbital sodium (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and heparin calcium (Sawai Pharmaceutical Co., Ltd., Osaka, Japan) were purchased.

Data analysis

The data were presented as the mean ± S.E. The statistical significances of the injection-induced percent changes between sildenafil and corresponding volume of saline were evaluated by two-way repeated-measures analysis of variance (ANOVA). A p value < 0.05 was considered to be statistically significant.

RESULTS

One hour after the start of cross-circulation, the sinoatrial node preparation showed spontaneous regular automaticity of 106 ± 1 beats/min (n = 4), whereas the papillary muscle preparation induced a developed tension of 22 ± 4 mN and coronary blood flow of 3.9 ± 0.1 mL/min (n = 4). Typical traces showing the positive chronotropic and inotropic effects, and coronary vasodilator action of sildenafil are depicted in Fig. 2, and their dose-
response curves are shown in Fig. 3 (n = 4). Intracoronary injection of 10, 30 and 100 μg of sildenafil increased the sinoatrial automaticity rate by 4, 12 and 22%, the developed tension of the papillary muscle by 19, 55 and 118%, and the coronary blood flow by 42, 95 and 142%, respectively, whereas vehicle saline hardly altered these variables. Significant difference was detected between the sildenafil and vehicle saline injections.

**DISCUSSION**

In order to better explain the discrepancies among the previous reports about the inotropic action of sildenafil (Cremers et al., 2003; Sugiyama et al., 2002; Wallis et al., 1999), moreover to clarify its safety profile, we assessed its chronotropic, inotropic and coronary vasodilator effects by using the canine isolated, blood-perfused heart preparations for the first time. As clearly shown in the results, sildenafil exerted the positive chronotropic, inotropic and coronary vasodilator effects in a dose-dependent manner, which are essentially in accordance with previous in vitro and in vivo studies (Kaumann et al., 2009; Sakuma et al., 2002; Sugiyama et al., 2001) except that the positive inotropic effect has not been observed in the in vitro studies (Cremers et al., 2003; Wallis et al., 1999).

**Rationale of the doses of sildenafil for the positive inotropic effect**

According to our previous experience of the blood-perfused heart preparations (Sugiyama et al., 1990, 1991), the effects of intracoronary administration of 1 μg of a drug on each variable could reflect those at plasma drug concentration of 1 μg/mL in vivo. Thus, currently used intracoronary doses of 10-100 μg of sildenafil may have provided plasma concentrations of 10-100 μg/mL (21-211 μM). Since clinically known therapeutic plasma concentrations of sildenafil have been reported to be 0.127-0.560 μg/mL (Zusman et al., 1999), our study is considered to assess 20 to 200 times higher concentrations than its therapeutic range.

Sildenafil in doses of 10-100 μg, which may reflect plasma concentrations of 10-100 μg/mL (21-211 μM) as described above, showed the positive inotropic effect in this study. On the other hand, such inotropic effect was not detected in the in vitro studies at concentrations of 0.1 nM to 10 μM (Cremers et al., 2003) or 10 nM to 10 μM (Wallis et al., 1999), suggesting that the difference in the drug concentrations among the studies could explain the discrepancies of their results. The difference in the experimental methods may also provide the reason for the discrepancies of the results; namely, superfusion method was used in the previous studies (Cremers et al., 2003; Wallis et al., 1999).

![Dose-response curves showing the positive chronotropic, inotropic and coronary vasodilator effects of sildenafil. Open symbols indicate the effects of sildenafil, while closed ones show those of vehicle saline of respective doses. Data was shown as the mean ± S.E. *p < 0.01 between the changes by sildenafil and vehicle alone.](image-url)
Cardiac effects of sildenafil

2003; Wallis et al., 1999), whereas the coronary blood-perfusion technique was employed in this study, the latter of which may be more physiological and could have higher sensitivity (Sugiyama et al., 1990, 1991). In addition, the in vivo positive inotropic effect of sildenafil in the previous study needs a comment, which was observed at lower plasma concentrations of 1-3 μg/mL (2.1-6.3 μM) (Sugiyama et al., 2001), that could be largely attributed to sympathetically mediated reflex resulting from its potent vasodilator action.

Mechanism of the positive chronotropic and inotropic effects of sildenafil

PDE3 is expressed in various tissues including myocardium, vascular smooth muscle, penile tissue and adipose tissue, which hydrolyzes cyclic AMP (Bischoff, 2004). Under physiological conditions, PDE3 has been proved to play an important role in regulating the automaticity and contractility primarily through the modulation of the cyclic AMP/protein kinase A signaling (Beca et al., 2011; Knight and Yan, 2012). In our previous study, we have shown that sildenafil can significantly increase net production rate of cyclic AMP in a canine ventricular membrane preparation (Sugiyama et al., 2002), which may explain currently observed positive chronotropic and inotropic effects. The following 2 potential intracellular mechanisms can be speculated. Firstly, sildenafil inhibits the PDE5 activity in the heart (Chrysant, 2013), leading to the increase of cyclic GMP level, which suppresses the PDE3 activity to increase the cyclic AMP level. Secondly, sildenafil might directly inhibit the PDE3 activity, resulting in the increase of the cyclic AMP level. The latter hypothesis may be less plausible because of much higher selectivity of sildenafil for PDE5 than for PDE3. Further study needs to be performed to clarify precise intracellular mechanisms including the cross-talk between cyclic GMP and cyclic AMP-dependent signal transduction pathways.

Mechanism of coronary vasodilator effect

PDE5 is widely distributed in many organs, including the corpus cavernosum, arteries and veins, myocardium, skeletal muscles and platelets (Chrysant, 2013; Wallis et al., 1999). Inhibition of PDE5 by sildenafil can increase the cyclic GMP level in the coronary artery, which will accelerate the dephosphorylation of myosin light chains and prevent the interaction of myosin with actin, causing the relaxation of smooth muscle of coronary artery (Katzung, 2015).

Conclusion

Sildenafil can exert the positive chronotropic, inotropic and vasodilator effects at about 20 to 200 times higher concentrations than its therapeutic range. Caution has to be paid on the use of high dose of sildenafil for patients with ischemic heart diseases, obstructive hypertrophic cardiomyopathy and/or ventricular arrhythmias because of cyclic AMP-dependent cardiostimulatory effects. In addition, since PDE5 and/or PDE3 in the heart might be also inhibited by high concentrations of PDE5 inhibitors other than sildenafil, there may be some concerns for their potential to induce cardiostimulatory actions like sildenafil. On the other hand, since PDE5 is widely distributed in vascular smooth muscle, increased tissue levels of cyclic GMP by PDE5 inhibitors other than sildenafil should cause the smooth muscle relaxation leading to the coronary vasodilation like sildenafil (Wallis et al., 1999). The information on sildenafil reported in this study may help establish a guidance on cardiac safety assessment of newer PDE5 inhibitors.

ACKNOWLEDGMENTS

This study was supported in part by JSPS KAKENHI (#16K08559); the Research Promotion Grant from Toho University Graduate School of Medicine; and Toho University Joint Research Fund. We thank Ms. Misako Nakatani and Mrs. Yuri Ichikawa for their technical assistance.

Conflict of interest——The authors declare that there is no conflict of interest.

REFERENCES

Katzung, B.G. (2015): Vasodilators & the treatment of angina pec-


