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A novel porcine model of thrombotic myocardial infarction with cardiac dysfunction sensitive to dual antiplatelet therapy

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ABSTRACT

Few effective porcine models of myocardial infarction (MI) related to platelet thrombus formation are available. In this study, we established a novel porcine MI model and examined the effect of dual antiplatelet therapy (DAPT) with aspirin and prasugrel, a $P2Y_{12}$ antagonist, using this MI model. Thrombotic MI was photochemically induced using rose bengal. Male miniature pigs were divided into 3 treatment groups: Sham, MI, and DAPT. In the DAPT group, aspirin (10 mg/kg, p.o.) and prasugrel (1 mg/kg, p.o.) were administered 4 h before photo-irradiation. Platelet aggregation, MI volume, and cardiac function were evaluated 24 h after photo-irradiation. Inhibition of ADP-induced platelet aggregation in the DAPT group was about 45%, similar to the effects of DAPT in a clinical setting. No MI was observed in the Sham group, and MI volume was 12.9 ± 2.9% in the left ventricle ($P = 0.0016$) in the MI group. Additionally, an increase in end-systolic volume ($P = 0.0006$), and a decrease in stroke volume ($P = 0.0001$) and ejection fraction ($P < 0.0001$) were observed in the MI group compared to the Sham group without any changes in end-diastolic volume. DAPT significantly decreased MI volume ($P = 0.0006$) and ameliorated cardiac dysfunction compared to the MI group. In conclusion, a novel porcine model of thrombotic MI with cardiac dysfunction was established. In this model, DAPT decreased MI volume and ameliorated of cardiac dysfunction, suggesting that this porcine MI model could be useful for future research on MI and antithrombotic agents.

1. Introduction

Myocardial infarction (MI) is a leading cause of death worldwide (GBD 2016 Causes of Death Collaborators, 2017). Dual antiplatelet therapy (DAPT) with aspirin and a $P2Y_{12}$ antagonist is the first-line treatment for acute coronary syndrome (ACS) (O'Gara et al., 2013; Ibanez et al., 2018). Mice, rats, and dogs have been used as animal models of MI (Kumar et al., 2016). Because the porcine heart has structural and physiological characteristics similar to those of the human heart, porcine models are gaining prominence in cardiac research (Suzuki et al., 2011). Domestic pigs are often used as porcine models, but experiments using domestic pigs present some difficulties, such as handling and large volumes of test substances, because of their large body size. Experimental miniature pigs have body size smaller than that of domestic pigs, and several studies have demonstrated the utility of miniature pigs in a variety of experimental cardiovascular studies (Skyschally et al., 2017; Suzuki et al., 2016; Salameh et al., 2012).

Several ischemia/reperfusion models are often used for MI research; however, platelet thrombus formation is not the main cause for MI in these models (Yang et al., 2006). The microsphere-induced MI model (Ajijola et al., 2017) and permanent coronary artery ligation model (Matthaios et al., 2017), are additional porcine MI models. However, as these models are induced by physical occlusion, platelet thrombus formation may not be involved. In contrast, a few porcine thrombotic MI models, induced by $FeCl_3$ (Dogné et al., 2005) and ethanol (Kim et al., 2011), have been reported to have several limitations. In the $FeCl_3$-induced MI model, all the three layers of the vessel wall (the intima, media, and adventitia) are damaged, disturbing vessel wall integrity (Day et al., 2004). In the ethanol-induced MI model, special tools, such as coronary angiography, are needed. Furthermore, the effects of DAPT have not been evaluated in these models. Thus, the establishment of another miniature porcine model of MI, which is sensitive to DAPT, is desired.

The photochemical reaction of rose bengal with green light generates reactive oxygen species that cause injury to the endothelium. At the injured endothelium site, platelets adhere, activate, and aggregate, followed by thrombus formation (Matsuno et al., 1993). Therefore, photochemically-induced thrombosis (PIT) is a useful method for initiating thrombotic diseases such as stroke (Tomizawa et al., 2015), MI

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(Hirata et al., 1995), and peripheral artery disease (Ohno et al., 2016). In addition, several PIT models have been widely used to evaluate the effects of antithrombotic agents, including antiplatelet agents (Matsuzawa et al., 2012; Momi et al., 2005; Kihara et al., 2001; Kawano et al., 2000; Umemura et al., 1995); however, to the best of our knowledge, a porcine MI model by PIT has not been reported.

In the present study, we established a novel thrombotic MI model with cardiac dysfunction using miniature pigs and evaluated the preventive effect of DAPT with aspirin and prasugrel on MI.

2. Materials and methods

2.1. Materials

Prasugrel hydrochloride (hereafter referred to as prasugrel) was synthesized by Ube Industries, Ltd. (Yamaguchi, Japan). Aspirin was purchased from Cayman Chemical (Ann Arbor, MI, USA). Prasugrel and aspirin were suspended in a 5% w/v solution of gum arabic (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Rose bengal was purchased from Wako Pure Chemical Industries, Ltd. and dissolved in saline. ADP was purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA). Collagen was purchased from LMS Co., Ltd. (Tokyo, Japan).

2.2. Experimental animals

All animal studies were approved by the Institutional Animal Care and Use Committee (New Drug Research Center Inc., permit number: No. 161228A; JOINN Laboratories, permit number: ACU16–1122) and were conducted according to Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Male Bama miniature pigs (3 weeks old) were supplied by Wujiang Tianyu Biological Technology Co., Ltd. (Jiangsu, China) and were housed under constant temperature and humidity in a 12-h light/dark cycle.

Male miniature pigs were classified into 3 groups: the Sham operation group (Sham group, n = 6), myocardial infarction group (MI group, n = 8), and DAPT myocardial infarction group treated with aspirin (10 mg/kg, p.o.) and prasugrel (1 mg/kg, p.o.) (MI/DAPT group, n = 8). Aspirin and prasugrel were administered sequentially. The DAPT doses were selected as inhibition of ADP-induced platelet aggregation achieving about 45–70% (Ohno et al., 2015), which is reported as inhibition of platelet aggregation with DAPT in clinical setting (Yokoi et al., 2012; Jernberg et al., 2006; Storey et al., 2007; Gurbel et al., 2009).

2.3. Photochemically-induced thrombotic myocardial infarction

Animals were anesthetized using intramuscular injections of 10 mg/kg of ketamine (Fujian Gutian Pharma Co., Ltd., Fujian, China). After intratracheal intubation, breathing was regulated with a gas mixture of oxygen and 0.5–2% isoflurane to maintain anesthesia. Prior to operation, 0.01 mg/kg of buprenorphine (Otsuka Pharmaceutical Co., Ltd., Osaka, Japan) was intramuscularly injected for pain relief.

The thoracic cavity was opened and heart was exposed. Blood flow was measured using a pulse-Doppler blood flow meter probe (MA0.7PSB, Transonic Systems Inc., Ithaca, NY, USA), set at the downstream of the left circumflex (LCx) from the photo-irradiation site. A green light (532 nm) probe was set at LCx using a Xenon lamp (GL532TA-100FC, Shanghai Laser & Optics Century Co., Ltd., Shanghai, China). At 4 h after the administration of aspirin plus prasugrel, photo-irradiation (6,000,000 lx) was initiated and continued for 30 min. Just after the initiation of photo-irradiation, 20 mg/kg of rose bengal was intravenously infused for 6 min. Animals in the Sham group were not irradiated and were left untreated for 30 min. Blood flow was measured up to 3 h after the initiation of photo-irradiation, and time to first occlusion (TTO) and patency rate (PR) were calculated. After blood flow measurements were completed, the chest was closed, and 100,000 Units/body of penicillin (CSPC Zhongnuo Pharmaceutical Co., Ltd., Hebei, China) were intramuscularly injected.

2.4. Electrocardiogram monitoring

Electrocardiogram (ECG) electrodes of a Blood-Pressure/ECG transmitter (TL11M2-D70-PCT, Data Sciences International, MN, USA) were subcutaneously placed on the right lateral thoracic and left lateral abdomen. ST elevation during 1 h after the initiation of photo-irradiation was monitored, and abnormal Q-wave rate at 24 h after the initiation of photo-irradiation was calculated from 100 beats on an ECG.

2.5. Measurement of cardiac functions

At 24 h after starting photo-irradiation, the animals were anesthetized using intramuscular injections of 10 mg/kg of ketamine and intravenous injections of 30 mg/kg of sodium pentobarbital (Beijing Oriental Things Fine Chemical Co., Ltd., Beijing, China). Left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), stroke volume (SV), and left ventricular ejection fraction (LVEF) were measured by the B-mode method using echocardiography (S8 Exp, Shenzhen SonoScape medical biomedical technology Co., Ltd., Shenzhen, China) and probe (5P2, Shenzhen SonoScape medical biomedical technology Co., Ltd.).

2.6. Determination of myocardial infarction volumes

After cardiac function measurements were completed, the animals were euthanized and the heart was retrieved, and 4-mm thick tissue slices were prepared from the left ventricle, along the short axis, from the base to the apex, and the tissue slices were stained with 1% 2,3,5-triphenyl-tetrazolium chloride (TTC; Sigma-Aldrich Co., LLC.)/5% formamide solution and scanned for determining myocardial infarct volume. The infarct areas were identified as areas that remained unstained by TTC. The cardiac infarct area was measured using image analysis software (ImageJ 1.44 P, National Institutes of Health, Bethesda, MD, USA). Cardiac infarct volumes (mm^3) were calculated as the product of the infarct area of each section multiplied by the section thickness (4 mm).

2.7. Platelet aggregation measurements

At the time of heart retrieval, blood samples were collected from the abdominal vena cava, using 3.2% sodium citrate. Platelet aggregation in platelet-rich plasma was monitored for 5 min after the application of ADP (5 and 20 μmol/l) or collagen (2, 5, and 12.5 μg/ml) and recorded as the maximum platelet aggregation using a plasma aggregation analyzer (LBY-NJ4, Precil Instrument Co., Ltd., Beijing, China).

2.8. Statistical analysis

All values, except for ST elevation rate, are expressed as the mean ± standard error of the mean. Unpaired two-tailed Student's *t*-test was used to compare Sham vs. MI groups, and MI vs. MI/DAPT groups. The Mann–Whitney *U*-test was used if variance was zero. ST elevation rate (%) was analyzed using Fisher's exact test. Significance levels were set at 5%. Statistical analyses were performed using the SAS System (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Antithrombotic effects on LCx blood flow

The effects of DAPT on LCx blood flow were evaluated for 3 h after photo-irradiation was initiated. Individual blood flow patterns are illustrated in Fig. 1, and TTO and PR are shown in Table 1. No occlusion