European Journal of Pharmacology 798 (2017) 72–76

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

# Additive antithrombotic effect of ASP6537, a selective cyclooxygenase (COX)-1 inhibitor, in combination with clopidogrel in guinea pigs

Chinatsu Sakata[a,*], Ken-ichi Suzuki[b], Yoshiaki Morita[b], Tomihisa Kawasaki[a]

[a] Project Management, Development, Astellas Pharma Inc., 2-5-1 Nihonbashi Honcho, Chuo-ku, Tokyo 103-8411, Japan
[b] Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan

ARTICLE INFO

*Chemical compound studied in this article:*
ASP6537 (PubChem CID: 9922739), Aspirin (PubChem CID: 2244)
Clopidogrel (PubChem CID: 60606)

*Keywords:*
Platelet
Cyclooxygenase-1
$P2Y_{12}$ receptor
Aspirin
Clopidogrel
Thrombosis

ABSTRACT

Clopidogrel (Plavix®, Sanofi-Aventis), the adenosine diphosphate $P2Y_{12}$ receptor antagonist, is reported to be effective in the prevention of cardiovascular events and is often used in combination with aspirin, particularly in high-risk patients. ASP6537 is a reversible cyclooxygenase (COX)-1 inhibitor that is under investigation as an anti-platelet agent.

First, we investigated the reversibility of the antiplatelet effect of ASP6537 and its interaction with ibuprofen to compare the usability of ASP6537 with that of aspirin. We then evaluated the antithrombotic effect of ASP6537 in combination with clopidogrel using a $FeCl_3$-induced thrombosis model in guinea pigs.

ASP6537 exerted reversible antiplatelet activity, and no pharmacodynamic interaction with ibuprofen was noted. When administered as monotherapy, ASP6537 exerted a significant antithrombotic effect at ≥3 mg/kg, while aspirin inhibited thrombosis at 100 mg/kg. ASP6537 exerted significant additive effects in combination with clopidogrel, and the minimum antithrombotic dose was reduced by concomitant administration of clopidogrel.

Our study showed that ASP6537 did not interact with ibuprofen and has clear additive effects in combination with clopidogrel. ASP6537 may therefore represent a promising antiplatelet agent for use in clinical settings in combination with clopidogrel.

## 1. Introduction

Platelet adhesion and aggregation play important roles in the pathogenesis of thrombosis. Currently, the most common antiplatelet agents used in the treatment of acute coronary syndrome (ACS) are acetylsalicylic acid (aspirin) and $P2Y_{12}$ inhibitors (clopidogrel and prasugrel) (Lopes, 2011). Aspirin exerts its antiplatelet effect via irreversible inhibition of platelet cyclooxygenase (COX)−1 and is generally effective in the treatment of ACS (Awtry and Loscalzo, 2000). However, aspirin alone is insufficient in preventing ischemic events in high-risk patients (Collaboration, 2002; Lopes, 2011; Patrono et al., 2005). In addition, several drawbacks associated with its use have been reported, such as the need for 7−10 days' withdrawal before surgery due to its irreversibility (Ferrandis et al., 2009; Jimenez et al., 1992), pharmacodynamic interactions with other nonsteroidal anti-inflammatory drugs (NSAIDs) (Anzellotti et al., 2011; Catella-Lawson et al., 2001; Gaziano and Gibson, 2006), and gastrointestinal complications (Garcia Rodriguez and Hernandez-Diaz, 2004; Tomisato et al., 2004).

$P2Y_{12}$ receptor inhibitors are reported to be effective in the prevention of myocardial infarction or transient ischemic attacks and are the gold standard in anti-platelet therapy (Patrono et al., 2005). Clopidogrel (Plavix®, Sanofi-Aventis) is the most widely used $P2Y_{12}$ receptor inhibitor and is often used in combination with aspirin, particularly in high-risk ACS patients (Garcia Rodriguez and Hernandez-Diaz, 2004). Dual antiplatelet therapy with clopidogrel plus aspirin has been shown to reduce the risk of ischemic events in patients with coronary artery disease, ischemic cerebrovascular disease, peripheral arterial disease, and in those at high risk of atherothrombotic disease (Bhatt et al., 2007; Connolly et al., 2009; Markus et al., 2005; Squizzato et al., 2011). However, the beneficial effects of concomitant use of aspirin and clopidogrel are countered by a higher risk of major bleeding (Connolly et al., 2009; Diener et al., 2004; Zhou et al., 2012).

ASP6537 is a highly selective COX-1 inhibitor synthesized by Astellas Pharma Inc. (Imanishi et al., 2011; Sakata et al., 2013) that is under investigation for possible clinical development as an anti-platelet agent. Here, we investigated the reversibility of the antiplatelet

* Corresponding author.
*E-mail address:* chinatsu.sakata@astellas.com (C. Sakata).

http://dx.doi.org/10.1016/j.ejphar.2017.01.015
Received 2 August 2016; Received in revised form 13 January 2017; Accepted 13 January 2017
Available online 14 January 2017
0014-2999/ 

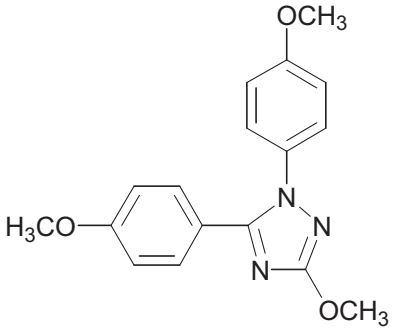


**Fig. 1.** Chemical structure of ASP6537.

action of ASP6537 and its pharmacodynamic interaction with ibuprofen using guinea pigs to determine if ASP6537 suffers from the same drawbacks as aspirin. We then examined the *in vivo* antithrombotic effect of ASP6537 alone or in combination with clopidogrel in a guinea pig model of $FeCl_3$-induced arterial thrombosis to determine whether ASP6537 has an additive effect when combined with clopidogrel.

## 2. Materials and methods

### 2.1. Test drugs and reagents

ASP6537 (Fig. 1) was synthesized at the Chemical Laboratories of Astellas Pharma Inc. (Tsukuba, Japan). Aspirin and ibuprofen sodium salt were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Clopidogrel sulfate was purchased from Apin Chemicals, Ltd. (Oxfordshire, UK). The test drugs were dissolved or suspended in 0.5% methylcellulose solution prior to use. All compounds were administered at a volume of 5 ml/kg by oral gavage, and all vehicle-treated groups were administered 0.5% methylcellulose solution at a volume of 5 ml/kg by oral gavage. Type I collagen from equine tendon was purchased from Moriya Co. (Collagen reagent Horm®; Tokyo, Japan). Adenosine 5′-diphosphate (ADP) was obtained from MC Medical (Tokyo, Japan). All other reagents were obtained commercially.

### 2.2. Animals

Male Hartley guinea pigs weighing 304–581 g (SLC Japan, Inc., Tokyo, Japan) were used in this study. All animals were housed under conventional conditions with controlled temperature, humidity, and light (12-h light–dark cycle) with free access to food and water until the day before the experiments, and were then fasted overnight. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc.

### 2.3. Ex vivo *platelet aggregation study*

For the evaluation of ASP6537 and aspirin, guinea pigs were divided into nine groups (n=5 per group). ASP6537 or aspirin was administered 1 h before blood sampling. For the evaluation of clopidogrel, guinea pigs were allocated to twelve groups (n=4 per group). Clopidogrel was administered 2 h beforehand. Under diethyl ether anesthesia, blood was collected from the vena cava in syringes containing 3.2% trisodium citrate solution (10% of the final volume). Platelet-rich plasma (PRP) was prepared by centrifuging at 189×*g* for 7 min at room temperature. Platelet-poor plasma (PPP) was obtained by centrifuging at PRP 1406×*g* for 10 min. Platelet counts were measured with an automatic cell counter (MEK-6258, Nihon Kohden, Tokyo, Japan). The platelet count was adjusted to $3\times10^5$/μl with PPP. Platelet aggregation in PRP was induced by 0.5 μg/ml of collagen or 1 μM of ADP. An aggregometer (MCM Hema Tracer 212; MC medical, Tokyo, Japan) was used to measure the maximum extent of platelet aggregation by recording the increase in light transmission through a stirred suspension maintained at 37 °C for 10 min.

### 2.4. Reversible inhibition of platelet aggregation by ASP6537

Guinea pigs were divided into three groups (n=4 per group). A dose of 30 mg/kg ASP6537 or 100 mg/kg aspirin was administered 1 h before blood sampling. Guinea pig PRP was prepared as described above. Platelet aggregation in PRP was induced by 0.5 μg/ml of collagen, after which aggregation was measured as described above. The PRP was then adjusted to pH 6.7 with 10 mM citric acid and 50 mM EDTA and centrifuged at 625×*g* for 15 min in order to obtain washed platelets. The sedimented platelets were suspended in HEPES–Tyrode's buffer (3.8 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 2.9 mM $NaH_2PO_4$, 5.6 mM dextrose, pH 6.7) containing 0.35% bovine serum albumin, and then centrifuged again after the addition of 50 mM EDTA. The sedimented platelets were then resuspended in PPP containing no drugs to adjust the platelet count to $3\times10^5$/μl. Platelet aggregation after the washing procedure was induced by 1 μg/ml of collagen, and the maximum extent of platelet aggregation was measured as described above.

### 2.5. Effect of pretreatment with ibuprofen on the antiplatelet effects of ASP6537 in guinea pigs

Guinea pigs were divided into six groups (n=5 per group). Ibuprofen (30 mg/kg) or vehicle was orally administered 1 h before administration of vehicle, ASP6537 (30 mg/kg) or aspirin (100 mg/kg). After an interval of 8 h since the last test drug administration, PRP and PPP were prepared as described above. Platelet aggregation in the PRP was induced by collagen (0.5 μg/ml). The maximum extent of platelet aggregation was measured as described above.

### 2.6. $FeCl_3$-induced arterial thrombosis model in guinea pigs

Guinea pigs were divided into fourteen groups (n=5 per group). The experimental protocol is shown in Fig. 2. The animals were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) 15 min before thrombus induction. Arterial thrombosis was induced using a modified rat $FeCl_3$-induced thrombosis model method (Gaddam et al., 2002; Kurz et al., 1990). After the abdomen was opened surgically, approximately 1 cm of abdominal aorta was detached from the surrounding tissues, and a piece of filter paper (5 mm×4 mm, Advantec; Tokyo, Japan) instilled with ferric chloride ($FeCl_3$, 10% [w/v] dissolved in distilled water) was applied to the external surface of the vessel for 10 min and

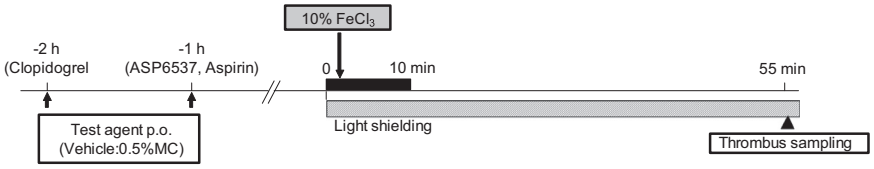


**Fig. 2.** Experimental protocol for the guinea pig models of $FeCl_3$-induced arterial thrombosis. ASP6537 or aspirin was given orally using a gastric tube 1 h before the start of $FeCl_3$ stimulation. Clopidogrel was also given orally 2 h before stimulation. $FeCl_3$-soaked filter paper (5 mm×4 mm) was applied to the abdominal aorta for 10 min and then removed; 55 min after the initiation of $FeCl_3$ stimulation, the thrombus that had formed was removed to measure the thrombus protein content.