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Full Length Article

Study on antiplatelet effect of a new thiophenopyridine platelets $P2Y_{12}$ receptor antagonist DV-127

Zhiping Xu[a,1], Jingkai Gu[b,1], Mingyue Gao[a], Na Du[a], Peng Liu[a], Xueyu Xu[b,c], Jiayu Wang[a], Xia Cao[a,*]

[a] Department of Pharmacology, School of Pharmacy, Jilin University, Fujin Road, Changchun 130021, Jilin, China
[b] College of Life Sciences, Jilin University, Qianjin Street, Changchun 130012, Jilin, China
[c] Tasly Academy, Tasly Holding Group Co.,Ltd., Tianjin 300410, China

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ABSTRACT

$P2Y_{12}$ receptor antagonists are a class of drugs that act on platelet $P2Y_{12}$ receptors and inhibit adenosine diphosphate-induced platelet aggregation. As a thienopyridine antiplatelet agent which is approved by the US Food and Drug Administration for the treatment of cardiovascular diseases, currently, clopidogrel was once considered to be the most safe and effective antiplatelet drug in the $P2Y_{12}$ receptor antagonists, however, it has become increasingly clear that clopidogrel does not satisfactorily inhibit the platelets of approximately one-third of patients. This is in part due to clopidogrel is a prodrug and reliance on multiple cytochrome P450 enzymes for conversion into its active metabolite. Prasugrel and ticagrelor reduces the risk of adverse cardiovascular events compared to clopidogrel in acute coronary syndromes patients, however, the cardiovascular benefit of both drugs is counter-balanced by increased rates of spontaneous bleeding. Unlike clopidogrel, which is a prodrug, cangrelor is an active drug not requiring metabolic conversion, despite fewer bleeding events during cardiac surgery, cangrelor carries the risk of potential autoimmune reactions manifesting as breathlessness.

DV-127 was synthesized by using three generations of thienopyridine $P2Y_{12}$ receptor antagonists as research models, using high resolution mass spectrometry, selective deuteration, and 2,7-position replacement groups technologies in order to maximize cardiovascular benefit while minimizing adverse effects on hemostasis. Our results show that although the dose of DV-127 is greatly reduced, it can achieve similar anticoagulant and antiplatelet effects as clopidogrel, and DV-127 can more strongly inhibit the release of α-granules even though the inhibitory effect of dense granules is similar to clopidogrel.

1. Introduction

Platelet $P2Y_{12}$ inhibitors are some of the most commonly used medications worldwide, due to their established benefit in the treatment and prevention of arterial thrombosis, as reviewed by Heptinstall and colleagues [1]. $P2Y_{12}$ receptor antagonists have a strong activation of signal amplification which is manifested not only in the amplification of platelet aggregation signals but also in subsequent multistep signaling including the release of platelet particles and platelet procoagulant activity increased.

Platelet granules release is an important mechanism of its own activation signal amplification, playing an important role in platelet's normal physiological function and mediate the formation of thrombus and vascular inflammation [2,3]. Dense granules, α-granules and lysosomes are three typical granules in platelet cytoplasm [4]. Dense granules mainly contains some small molecule active substances, such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), serotonin (5-HT), calcium ions, etc. Involved in platelet activation, aggregation and vasoconstriction. α-granules mainly contain some large protein such as α-granules membrane protein-140 (GMP-140), β-thromboglobulin (β-TG) and other protein polypeptides, and play an important role in coagulation, inflammation wound healing. Lysosomes contain some hydrolytic enzymes.

Clopidogrel (CLP) can selectively and irreversibly block the platelet membrane ADP receptor $P2Y_{12}$ so that the fibrinogen binding site of the coupled platelet glycoprotein IIb/IIIa receptor cannot be exposed, affecting the binding of fibrinogen so that the platelets cannot further gather together [5]. However, some patients in clinical CLP

* Corresponding author.
E-mail addresses: gujk@jlu.edu.cn (J. Gu), caoxia@jlu.edu.cn (X. Cao).
[1] The authors contributed equally to this manuscript.

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Abbreviations

ATP	adenosine triphosphate
ADP	adenosine diphosphate
5-HT	serotonin
GMP-140	α-granules membrane protein-140
β-TG	β-thromboglobulin
CLP	Clopidogrel
CR	clopidogrel resistance
UPLC-HR-MS	ultra-high performance liquid chromatography-high resolution mass spectrometry
ELISA	enzyme-linked immunosorbent assay
PRP	platelet-rich plasma
PPP	Platelet-poor plasma
CT	coagulation time
BT	bleeding time
AC	adenylyl cyclase
cAMP	cyclic adenosine monophosphate
5-HTT	5-hydroxytryptamine transporter

intervention will encounter the recurrence of the thrombotic events, and the platelet inhibition rate of some patients cannot achieve the desired goal after the intervention of CLP [6]. There are literatures show that the rate of clopidogrel resistance (CR) is different in different population, the highest rate could reach 44% in China [7,8], and this phenomenon increases the risk of the CLP treatment. Prasugrel is also an irreversible $P2Y_{12}$ receptor antagonist from the thienopyridine class, with a different mechanism for the formation of the active metabolite [9], it achieves stronger inhibition of platelet aggregation, but the superior efficacy versus CLP in moderate- to high-risk patients with ACS undergoing PCI [10,11] is associated with an increased bleeding risk [12]. Ticagrelor, the first reversible $P2Y_{12}$ receptor antagonist, is also more effective than CLP at reducing the risk of adverse cardiovascular events in patients with ACS, but also increases the risk of spontaneous bleeding [13]. Cangrelor is a $P2Y_{12}$ inhibitor FDA approved as of June 2015 as an antiplatelet drug for intravenous application, it does not require metabolic conversion to an active metabolite, this allows cangrelor's immediate effect after infusion and the therapeutic effects can be maintained with continuous infusion, Despite fewer bleeding events during cardiac surgery, cangrelor carries the risk of potential autoimmune reactions manifesting as breathlessness [14].

These disadvantages of platelet $P2Y_{12}$ receptor antagonists including a delayed onset of action [15], a significant variability in response [6], an insufficient antiplatelet activity in some patients [16], and increase the risk of bleeding [12] have led to a large degree of limitations in the clinical use of these drugs, thence three generations of thienopyridine $P2Y_{12}$ receptor antagonists were used as research models, and UPLC-HR-MS/MS, selective deuteration, and 2,7-substituent substitution techniques were used to synthesize a series of thienopyridine derivatives, and after the preliminary work and study, we found that DV-127 has sufficient advantages from the perspective of pharmacokinetics, then whether its pharmacodynamics is also shown as an advantage will be the focus of this study.

Compared with CLP, the molecular structure of DV-127 (Fig. 1) is improved: one is the deuteration of 7-position methyl carboxylate, which can stabilize the structure, that can slow down the rate of inactivation caused by hydrolysis; the other one is the acetoxy is introduced to 2-position of thiophene, can overcome "CR" caused by the genetic polymorphism. Therefore, DV-127 is a potentially superior antiplatelet agent which can maximize cardiovascular benefit while minimizing adverse effects on hemostasis. The detailed synthesis route, pharmacokinetic parameters and metabolic characteristics of DV-127 (compound 10a) can be seen in Significant Improvement of Metabolic Characteristics and Bioactivities of Clopidogrel and Analogs by Selective Deuteration [17].

2. Methods

2.1. Animals

Male wistar rats (SPF) weighing about 240–280 g and male Kunming (KM) mice (SPF) weighing about 18–22 g were purchased from laboratory animal center of Jilin University; The rats and mice were housed at a constant room temperature (22 ± 4 °C) and humidity (50%) under a 12 h light/dark cycle. The animals were allowed free access to standard chow and water. Before the experiment, they were fasted for 12 h. Care of the animals and drug administration were performed under veterinary control according to European Union Directive 2010/63/EU for animal experiments and with approval from the Institutional Animal Care and Use Committee of Jilin University.

2.2. Reagents

Clopidogrel were purchased from Sanofi (Hangzhou) Pharmaceutical Co., Ltd.; DV-127 were synthesized by Drug Metabolism Research Institute Beijing, after HPLC analysis and MS identification, the purity of DV-127 reached 99.5%; Sodium carboxymethyl cellulose were obtained from Sinopharm Chemical ReagentCo., Ltd.; The drugs used in the experiment were all dissolved with 0.5% sodium carboxymethyl cellulose, and the experimental animals were

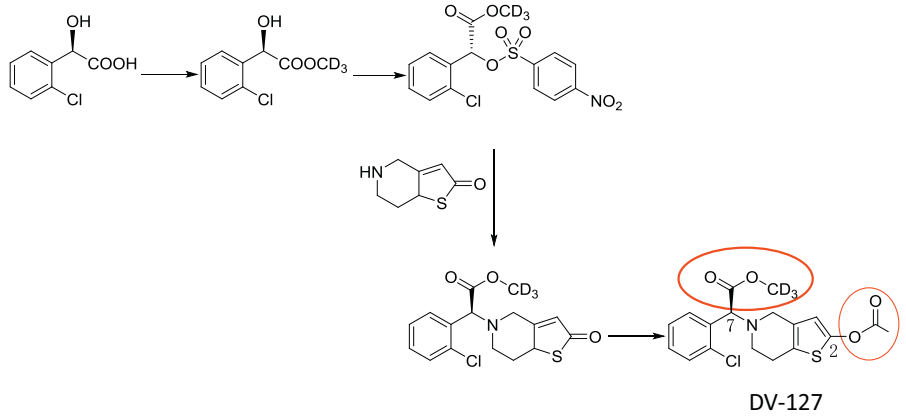

Fig. 1. The structure of DV-127 and its synthetic route.