Effects of Ticlopidine on Platelet Aggregation in Beagles with Unstable Angina

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Abstract: Aim To study the antiplatelet aggregation of ticlopidine (TC). Methods Using coronary artery Folts model in beagles, the efficacy of TC on platelet aggregation was studied. Results (1)TC16, 32 mg·kg\(^{-1}\) dramatically inhibited the frequency of CFRs dose-dependently, and the times of CFRs decreased by 27%, 56%, 95%, 100% respectively at 1-4 h than that in control group’s after TC 32 mg·kg\(^{-1}\) administration. CFRs were abolished and did not return when AD was infused after TC 32 mg·kg\(^{-1}\) administration. (2)The time for CFRs absolute disappearance of TC in dose of 32 mg·kg\(^{-1}\) was shorter than that in control group. (3)The platelet aggregation induced by ADP was inhibited obviously after TC 16,32 mg·kg\(^{-1}\) administration, and the aggregation in TC 32 mg·kg\(^{-1}\) didn’t return after AD administration. Conclusion TC had dose-dependent antiplatelet effects in beagles, suggesting TC has reliable therapeutical efficacy on angina pectoris.

Keywords: Ticlopidine, Antiplatelet Aggregation, Unstable Angina Pectoris

1. Introduction

More and more evidence demonstrated that platelet plays an important role in atherosclerosis, and arterial thrombus formation. Antiplatelet treatment became one of the most important techniques for cardio- and cerebro-vascular disease. Unlike aspirin (ASA), Ticlopidine (TC) was an inhibitor of platelet aggregation acted on some agonists such as ADP, adrenalin (AD), arachidonic acid (AA), thrombin (Th), collagen (CG), 5-HT et al, specifically inhibited ADP-induced platelet aggregation without affecting prostaglandin metabolism. It was extensively applied in unstable angina pectoris (UAP), acute myocardial infarction (AMI), transient ischemic attack (TIA), stroke in clinics [1, 2], but still no experimental studies showed that it was effective in models of UAP. So by using Folts’ model in beagles, we studied the antiplatelet efficacy of TC.

2. Materials and Methods

2.1. Reagents and Drugs

TC and ASA were provided by Tianjin Institute of Pharmaceutical Research, ADP were purchased from Sigma (USA).

2.2. Animals

Beagles, weight 7-12 kg, male and female were used, provided by Sin’gang Laboratory Animal Farm (Shanghai, China).

2.3. Instruments

RM6300 polygraph physiological recorder, MFV-3200 electromagnetic blood flowmeter, were manufactured by Nihon Kohden Corporation (Tokyo, Japan) . MP100A—CE polychannel biological signal collection system, provided by BIOPAC Systems Inc (California, USA) . SC-3 electrical respirator, Shanghai Medical Equipment Factory, SPA- III automatic equilibrium platelet aggregometer, made by Keda Test Instrument Factory (Shanghai).

2.4. Methods

2.4.1. Surgical Preparation

The surgical preparation has been described in detail in previous publications [3, 4, 5]. Beagles of either sex were
anaesthetized with sodium pentobarbital 30 mg·kg⁻¹ by intravenous injection, supplemental barbiturate anesthesia (3 mg·kg⁻¹) was administered at 60 min intervals to maintain anaesthesia state. Respiration was supported mechanically via trachea, the chest opened at the left fourth intercostals space, made a hammock to hold the heart, and the left circumflex coronary artery was dissected out 2-3 cm long approximately, and an electromagnetical flow probe (dimension 2-3 mm) was placed around the artery conjuncted electromagnetical MFV-3200 flowmeter to measure coronary blood flow. Mean artery blood pressure (MABP) was measured via a polyethylene cannula inserted into right femoral artery and connected via a normal saline –filled tube to a TP-400T pressure transducer. Venous access was via the right femoral vein, with a 0.9% saline drip administered continuously to maintain blood volume. ECG (lead-II) was measured continuously.

Analogue flow, pressure and ECG signals were amplified via RM6300 polygraph physiological recorder and fed into a MP100A—CE polychannel biological signal collection system for analogue-to-digital converting with collecting frequency 300 HZ. The digital output was then displayed, recorded and processed on a computer running Acknownowledge version 7.2 software (BIOPAC Systems Inc.).

### 2.4.2. Stenosis

When the status remained stable up to 30 min after surgical preparations, cycle flow reductions (CFRs) were made using the coronary artery Folts’ models in dogs. Distal to the probe, the artery was occluded by holding a silk for 20 second, there is a sudden restoration of blood flow when the artery was loosened again, that is “hyperaemic phenomenon of reaction (HPR)”, when the HPR disappeared, about 4mm long artery was clamped two times and 5 seconds for one time with a special surgical clamp to produce intimal and medial damage. A plastic cylinder of remodulation in inside diameter was placed around the damaged artery. The HPR was no happened when to produce a 70±5% reductions in arterial diameter [6], platelet aggregation periodically in the area of the stenosed artery was performed to get platelet-rich plasma (PRP), the remains were further centrifuged at 1000 g·min⁻¹ for 10 min at room temperature to obtain platelet -poor plasma (PPP). The platelet counts of PRP were adjusted to 300,000 cells per microliter by dilution with PPP. PRP 200 microliters preincubated for 10 min at 37°C were added into colorimetry pool, and tracing pen was adjusted to 0% with PRP, 100% with PPP, the platelet aggregation was studied using ADP(final concentration, 2 mmol·L⁻¹).

### 2.4.4. Criterion for CFRs, and Time for CFRs Disappearance

When the coronary blood flow declined to 30 percent of baseline, CFRS happened. We made 95 % confidence of the frequency of control group’s CFRS as reference, if the times of drugs’ CFRS were lower than that of reference’s, the drug showed efficiency. The zero time of CFRS was made as criterion for absolute inhibition, and the time from drug administration to the last appearance of CFRS was made as the time for CFRS disappearance.

### 2.4.5. Ex Vivo Platelet Aggregation Studies

At predeterminated time, 4.5 mL of whole blood were drawn into a syringe containing 0.5 mL of 3.8% sodium citrate as an anticoagulant, blood was centrifuged at 150 g·min⁻¹ for 10 min at room temperature in order to get platelet-rich plasma (PRP), the remains were further centrifuged at 1000 g·min⁻¹ for 10 min at room temperature to obtain platelet -poor plasma (PPP). The platelet counts of PRP were adjusted to 300,000 cells per microliter by dilution with PPP. PRP 200 microliters preincubated for 10 min at 37°C were added into colorimetry pool, and tracing pen was adjusted to 0% with PRP, 100% with PPP, the platelet aggregation was studied using ADP(final concentration, 2 mmol·L⁻¹).

### 2.4.6. Statistical Methods

Data are presented as mean ±SD and were analyzed using one-way analysis of variance (ANOVA) followed with a post-hoc test (least significance difference). Enumeration data was expressed as percentage, analysed by Chi-square Fisher’s exact test.

### 3. Results

#### 3.1. Effect of TC on CFRs

The frequency of CFRS in control group remained baseline in control period (averaged 10.5±2.1/1 h) and 4 hrs after distilled water administration. TC 16, 32 mg·kg⁻¹ dramatically inhibited the frequency of CFRS dose-dependently, and the frequency of CFRS decreased by 87%, 100% respectively at 1-4 h than that in control group’s. CFRS were abolished and did not return when AD was infused after TC 32 mg·kg⁻¹ administration (Table 1). The time for CFRS absolute disappearance of TC in dose of 32 mg·kg⁻¹ was shorter than that in control group (Table 2).
platelet thrombus caused coronary blood flow to decline to zero. The platelet thrombus was dislodged and dissolved with release (such as ADP, AD, 5-HT, AA, Th, PF4) exposed, which led platelet be adhesive, aggregate, and adhere to vessels. When endothelium cell was damaged, the CG, Vwf exposed CG lying on subendothelium surface of injured formation process was all been from the platelets adhesion to adhere to 

The unstable angina pectoris was mostly related to cracking of atherosclerosis clotty [9], and platelet aggregation and releasing were important in coronary arterial thrombus formation [10]. Regardless the thrombus in heart or in artery and veins, its formation process was all been from the platelets adhesion to exposed CG lying on subendothelium surface of injured vessels. When endothelium cell was damaged, the CG, Vwf exposed, which led platelet be adhesive, aggregate, and release (such as ADP, AD, 5-HT, AA, Th, PF et al). The activated platelet not only related to coagulation cascade process, but also released vascular activator, cellular factor, and growth factor, which associated with pathological conditions of unstable angina pectoris, acute myocardial infarction, transient ischemic attack, stroke and play role on acute or chronic thrombus reocclusion and restenosis after interventional therapy [11].

This paper adopted the Folts’ model according to this principle, when the lesion coronary artery arrived to critical stenosis for a while, the platelet was to be adhesive, aggregate, release, and the cascade of thrombus and thrombin formation which turn fibrinogen into fibrin was started. The occlusive platelet thrombus caused coronary blood flow to decline to zero. The platelet thrombus was dislodged and dissolved with pressure gradient building up across the stenosed lumen. The CFRs observed in the dog were thought to be correlated well with acute ischemic syndromes in humans [12].

Twinty seven beagles were used for Folts’ model in this research, but 3 beagles did not show CFRs phenomenon, which perhaps was related to lowly platelets count in blood, further more, the times of CFRs affected strongly by animal’s body temperature. With strict conditions, the frequency of CFRs in this research averaged 10.0 ± 1.7(n=24), and the times of CFRs affected strongly by animal’s body temperature. With strict conditions, the frequency of CFRs in this research averaged 10.0 ± 1.7(n=24), and remained stability in whole experiment.

4. Discussion

The unstable angina pectoris was mostly related to cracking of atherosclerosis clotty [9], and platelet aggregation and releasing were important in coronary arterial thrombus formation [10].

Regardless the thrombus in heart or in artery and veins, its formation process was all been from the platelets adhesion to exposed CG lying on subendothelium surface of injured vessels. When endothelium cell was damaged, the CG, Vwf exposed, which led platelet be adhesive, aggregate, and release (such as ADP, AD, 5-HT, AA, Th, PF et al). The activated platelet not only related to coagulation cascade process, but also released vascular activator, cellular factor, and growth factor, which associated with pathological conditions of unstable angina pectoris, acute myocardial infarction, transient ischemic attack, stroke and play role on acute or chronic thrombus reocclusion and restenosis after interventional therapy [11].

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As antiplatelet agent, aspirin showed some disadvantages such as allergic reaction, nausea, vomiting, hallucinations, irritation of stomach and intestines, the harmful to liver and kidney, bleed, inhibiting synthesizes of the PGII, et al), therefore those disadvantages urged people to look for the more superior treatment agents.

TC together with clopidogrel was one sort of thienopyridine, which could inhibit the platelet aggregation induced by a lot of agonists, especially on aggregation induced by ADP, and its action was independent of PGII synthesis [13]. TC inhibited the the first phase of platelet aggregation which was different from other agonists. The research in this paper demonstrated that TC had obvious inhibition of CRFs in canine models, and inhibited platelet aggregation induced by ADP, those data supplied that TC possess a strong potent antiplatlet activity.

The mechanism of TC on inhibition of platelet aggregation
was unknown for a long time. Up to day, We known two subtypes of ADP receptor lying on surface of platelet, one type had something to do with platelets transmogrification and Ca\(^{2+}\) channel, the other was related to protein G complex, which decreased the level of cAMP by influenced the signals transmitting in cells, cAMP promoted the Ca\(^{2+}\) in compact piping system to flow into the plasma, and made the GPIIb-IIIa complex receptor uncovered and linked to fibrinogen which prompted the aggregation among platelets. TC mainly exert it’s action by the second type of ADP receptor [14, 15], though TC was not a antagonist to ADP, or inhibited the syntheses of ADP receptor, but TC perhaps disturbed the function of receptor. Otherwise, TC could inhibit the using of fibrinogen receptors, and decrease the level of cAMP induced by prostaglandin E1 [16].

5. Conclusion

In this study, we established angina model in beagles using Folts methods, and the efficacy of ticlopidine on platelet aggregation was studied. TC had dose-dependent antiplatelet effects in beagles, suggesting TC has reliable therapeutical efficacy on angina pectoris.

References


