Bismuth Subgallate as a Local Hemostatic Agent : Pilot Animal Experiments

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Abstract

Patients on clopidogrel increased bleeding risk after surgery. This drug prolonged bleeding time, increased bleeding volume and induced secondary bleeding because its active metabolite inhibited platelets aggregation and interfered with haemostatic plug stabilization. Conventional methods, such as pressing sterile gauze on the surgery site, showed less effective to stop bleeding in patients on clopidogrel. This research aims to prove the haemostatic effect of bismuth subgallate both on normal and delayed platelet aggregation due to clopidogrel.

Twenty-eight Wistar rats were equally and randomly administered with clopidogrel (10 mg/kgBW) or NaCl 0.9% (saline) via oral gavage. After anesthetizing, we amputated transversely their tail 10 mm from the distal tip. Bleeding after amputation was controlled with pressing gauze soaked in saline or bismuth subgallate solution. After 60 seconds, bleeding assays (bleeding time, bleeding volume, and secondary bleeding) have been observed, recorded, and analysed both in normal and clopidogrel groups.

Clopidogrel groups had significantly longer bleeding time, greater bleeding volume, and had more secondary bleeding rather than saline groups ($p < .05$). Using bismuth subgallate as local haemostatic agent decreased bleeding time and bleeding volume significantly ($p < .05$) both in normal and clopidogrel groups. Conclusions: Bismuth subgallate has a haemostatic effect on both clopidogrel and normal rat tail bleeding models.

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Introduction

Clopidogrel is a first-line antiplatelet drug for patients with cardiovascular disease[1], because inhibited platelet aggregation and reduced platelet plug formation, resulting in prolonged bleeding time, increased bleeding volume, and secondary bleeding[2,3]. Bleeding always happens in oral and maxillofacial surgery due to blood vessel ruptures[4]. Pressing sterile gauze alone on the surgery site seems less effective to stop bleeding in patients on clopidogrel, in contrast adding common local haemostatic agents, such as epinephrine, can stop bleeding effectively[5]. Unfortunately, epinephrine is not recommended to be used as local haemostatic agent for patients who have a cardiovascular disease and taking antiplatelet drugs because it can increase systolic pressure and heart rate, inhibit fibrinolysis, also have life risk adverse reaction up to 11 percent[6].

Blood clot is the main role of wound healing process[31] and there were many competitions to find a new local hemostatic agent during antithrombotic therapy. We were investigating bismuth subgallate as a local haemostatic agent for patients using antiplatelet drugs. Theoretically, it can stop bleeding safely by increasing number of reactive oxygen species (ROS) and tissue factor (TF), activating TF rapidly and enhancing eryptosis locally around the injured blood vessels to form artificial clots[7,8,9]. Kim et al (2010) stopped bleeding from palatal soft tissue graft by applying periodontal dressing containing bismuth subgallate[10]. Other studies conducted by Callanan et al (1995), Hatton et al (2000), and Sharma et al (2007) also prove the effectiveness of bismuth subgallate–adrenaline paste as a local

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haemostatic agent after tonsillectomy[11,12,13].

There have been no studies using bismuth subgallate as local haemostatic agent in rat tail bleeding models.

Materials and methods

Animal Model

Twenty-eight Wistar rats (from Pharmacology and Toxicology Laboratory, Universitas Gadjah Mada, 12 weeks old, male, weight range 200-300 grams) had been adapted and maintained for 2 days in a single animal cage. All rats were given the same food and drink according to the feeding standards for experimental animals of the Pharmacology and Toxicology Laboratory, Universitas Gadjah Mada. This research had received Ethical Clearance from the Research Ethics Commission of the Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta (No. 0101 / EC-FKH / Eks.2019) and had followed the ARRIVE Guideline.

Groups and Experimental Design

This pilot study has been using 2x2 factorial design, which sample size was calculated using Federer formula with correction of loss research subject. We randomly divided the rats into 4 groups, each group has 7 animals (Figure 1). According to Allgoewer et al (2017), seven animals per group is reasonable for continuous and categorical outcome[30]. First group (N) was a group of rats administered with saline and controlled bleeding with sterile gauze soaked in saline solution (NaCl 0.9%); second group (N+BSG) was a group of rats administered with saline and controlled bleeding with sterile gauze soaked in bismuth subgallate 1 g/ml; third group (CPG) was a group of rats administered with clopidogrel (10 mg/kgBW) and controlled bleeding with sterile gauze soaked in saline solution (NaCl 0.9%); and the fourth group (CPG+BSG) was a group of rats administered with clopidogrel (10 mg/kgBW) and controlled bleeding with sterile gauze soaked in bismuth subgallate 1 g/ml.

Clopidogrel and Bismuth Subgallate Preparations

Clopidogrel tablets (75 mg each, CPG, Kalbe Indonesia) ground until they become fine powder using mortar and pestle[14]. Bismuth subgallate preparation in this study was made by mixing 1 g of bismuth subgallate powder into 1 ml of sterile distilled water then stirred until homogeneous using cement spatula and dappen glass[15]. We made clopidogrel and bismuth subgallate preparations at the Pharmacology and Toxicology Laboratory, Gadjah Mada University.

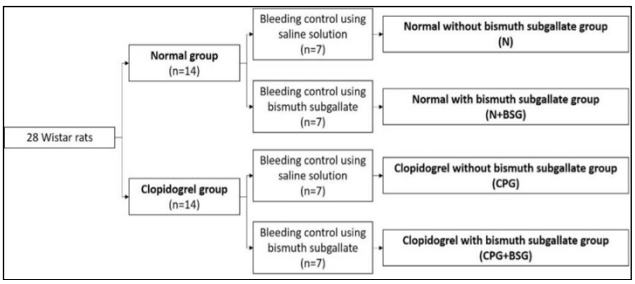

Figure 1. Study groups in experiment.

Rat Tail Bleeding Model

We adopted the method of making a rat tail bleeding model from Sogut et al[15]. Twenty-eight Wistar rats were randomly administered with clopidogrel (10 mg/kgBW) or NaCl 0.9% (saline) via oral gavage 2 hours before surgery. We amputated transversely their tail 10 mm from distal tip using surgical scalpel no. 15 under general anaesthesia (Figure 2).

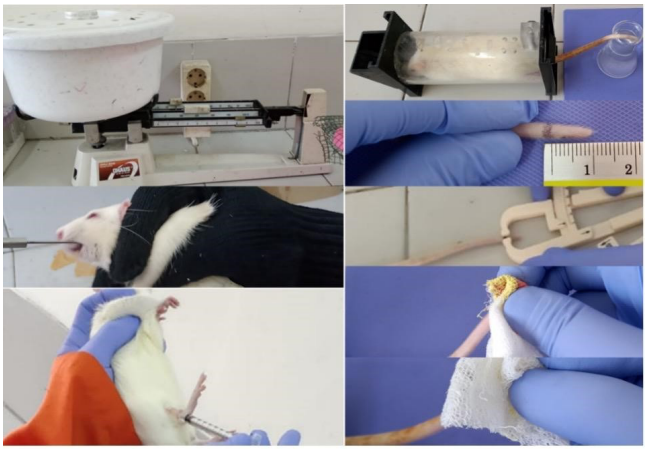

Figure 2. Experimental research method step by step.

Bleeding Control

Bleeding after amputation was controlled by pressing sterile gauze that had been soaked in saline solution (NaCl 0.9%) or bismuth subgallate 1 gr/ml for 60 seconds.

Bleeding Assays

Bleeding assays included bleeding time, bleeding volume, and secondary bleeding. Bleeding time protocols in this study used the Duke Method, which was changed by Sogut et al[15]. Blood from amputated rat tail tip was

dripped onto a sterile gauze every 30 seconds, turned off stopwatch when there was no blood spot. Bleeding volume in this study was measured by the gravimetric method, which sees the weight difference of sterile gauze before and after the procedure using a precision laboratory digital scale[15,16]. Re-bleeding after haemostasis occurred within 20 minutes was recorded as secondary bleeding[15].

Statistical Analysis

Bleeding time (second) and bleeding volume (milligram) were presented as mean + standard deviation. Two Way ANOVA followed by Post Hoc LSD Test was used if data were distributed normally and homogeneity. On the other hand, Kruskal Wallis Test followed by Mann Whitney U Test was used if normal distribution or homogeneity-of-variance assumptions were not satisfied. We analysed secondary bleeding with the Fisher Exact Test.

The confidence level of this study was 95% ($p < 0.05$) and data were processed with IBM SPSS software version 22 on Windows 10 systems (SPSS, Chicago, Illinois).

Results

None of the research subjects died during the study. Mean rat body weight in this study was 262.04+22.53 grams ($p = 0.115$) and average amputated rat tail diameter was around 1.04+0.13 millimetre ($p = 0.119$).

Bleeding Time

We found the shortest bleeding time in the normal group with bismuth subgallate (N+BSG) (157.14+29.58 seconds), followed by the clopidogrel group with bismuth subgallate (CPG+BSG) (213.14+23.51 seconds), normal group without bismuth subgallate (N) (451.29+21, 17 seconds), and the clopidogrel group without bismuth subgallate (CPG) (1116.57+24.78 seconds). The Post Hoc LSD test showed bleeding time in each group significantly different (Figure 3).

Bleeding Volume

The clopidogrel group without bismuth subgallate (CPG) had the most bleeding volume (160.29+28.77 milligrams), followed by the normal group without bismuth subgallate (N) (121.43+18.57 milligrams), clopidogrel with bismuth subgallate (CPG+BSG) (37.86+19.83 milligrams), and normal with bismuth subgallate (N+BSG) (4.83+2.23 milligrams). The comparative analysis using Mann Whitney U Test showed bleeding volume of each group significantly different (Figure 4).

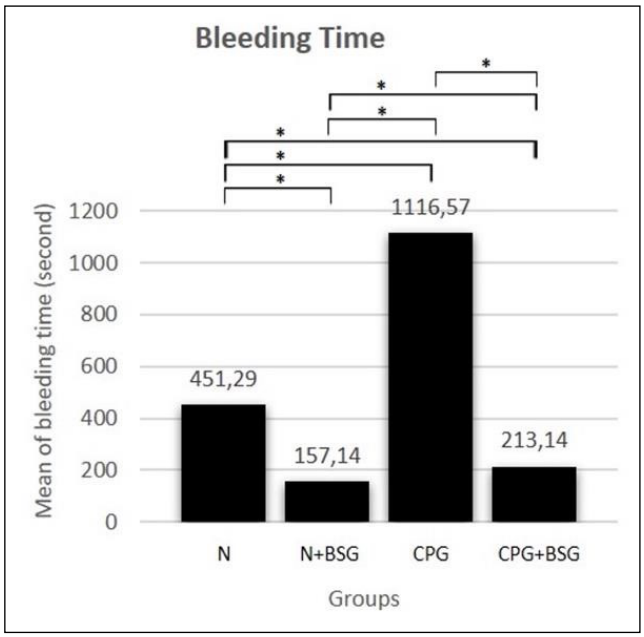

Figure 3. Bleeding time comparison between study groups. The sign (*) means the result of Post Hoc LSD Test between groups is significantly different (α = 95%).

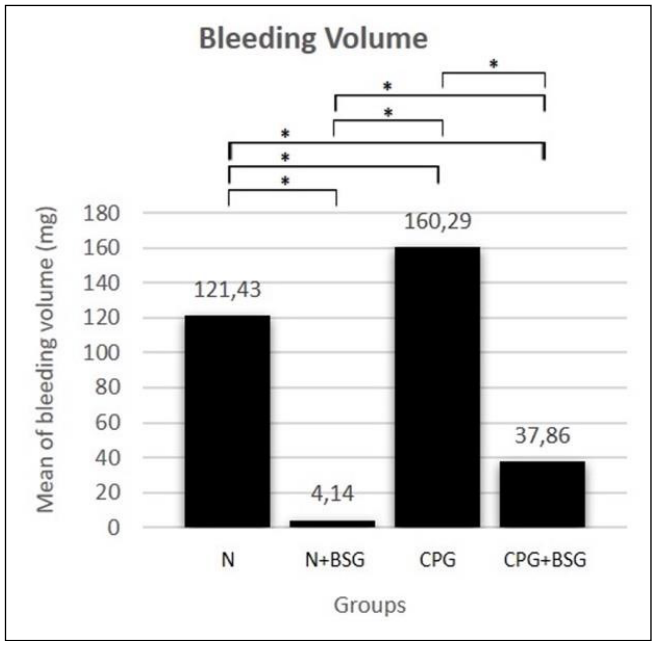

Figure 4. Comparison of bleeding volume between study groups. The sign (*) defines the different result of Mann Whitney U Test each group is significant (α = 95%).

Secondary Bleeding

Secondary bleeding occurred in all samples of the CPG group (100%) and 1 sample of the N group (14 %), but in the N+BSG group and the CPG+BSG group there was no secondary bleeding (0%). Fisher Exact Test showed that secondary bleeding found in the clopidogrel group without bismuth subgallate (CPG) differed significantly from the normal group without bismuth subgallate (N) (p = 0.005), and with the clopidogrel group with bismuth subgallate (CPG+BSG) (p = 0.001) (Figure 5).

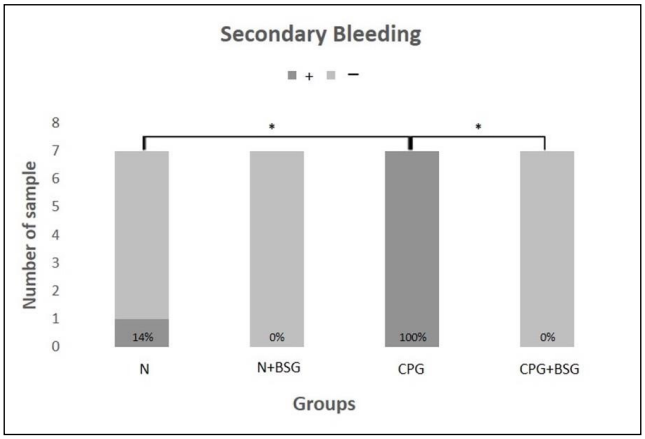

Figure 5. Frequency of secondary bleeding data for each study group. The sign (*) means the result of Fisher Exact Test between groups is significantly different (α = 95%).

Discussion

Clopidogrel prescription has increased up to 77% over the past 10 years because of the high incidence of cardiovascular disease, especially ischemia because of atherothrombosis[17,18]. One side effect of clopidogrel is bleeding during and after surgery. Handscel et al (2011) and Doganay et al (2018) reported massive bleeding after tooth extraction (4.5%) and odontectomy (25.7%) in the antiplatelet therapy[19,20].

Bismuth subgallate (BSG) is one of the natural local haemostatic agents and has been proven to stop bleeding after tonsillectomy[14,16]. Rat tail bleeding model is the most ideal method for observing bleeding after administration of antiplatelet drugs[21,22], although it does not fully represent bleeding in the oral cavity. Saliva contains a plasminogen activator and oral movement, such as chewing and talking, can make bleeding last longer[23,24]. Clinical observations relating to bleeding in the oral cavity still need to be developed further.

It showed clopidogrel extend bleeding time compared to other groups. Bismuth subgallate, both in normal and clopidogrel groups, decreases bleeding time when compared to the group not given bismuth subgallate. Kim et al (2010) also showed shorter bleeding time after bismuth subgallate applied on post-palate excision wounds[10].

Bleeding volume in the clopidogrel group without bismuth subgallate was higher than the normal group of rats without bismuth subgallate. We found similar results in the studies of Saito et al (2016) who measured the volume of bleeding with haemoglobin levels[22]. Bismuth subgallate can decrease bleeding volume, both in the clopidogrel and normal groups, when compared to rats that were not given bismuth subgallate. Research by Callanan et al (1995), Hatton et al (2000) and Sharma et al (2007) also showed a significant reduction in bleeding volume in tonsillectomy patients without systemic abnormalities given topical bismuth subgallate[11,12,13].

All samples of clopidogrel groups without bismuth subgallate in this study experienced secondary bleeding (100%), but we did not find it in the groups which were given bismuth subgallate, both in normal and clopidogrel rats (0%). Excessive tail movement might cause secondary bleeding in a normal group sample (14%) when the rat was conscious of the effects of anaesthesia. Research Liu et al (2012) found secondary bleeding in rats given clopidogrel as much as 100%, while 25% of them were excluded because bleeding did not stop after 20 minutes[21].

Clopidogrel in this study could increase bleeding time, bleeding volume, and secondary bleeding because this drug can decrease platelet aggregation and inhibit thrombus formation. This drug active metabolites (clopi-H4) binds to the P2Y12 receptor and inhibits ADP to activate the receptor. Platelets could not bind each other and failed to aggregate[25].

Bismuth subgallate can reduce bleeding time and bleeding volume both in normal and clopidogrel rats, because bismuth ions bind to metallothionein receptors on the surfaces of TF-bearing cells and erythrocytes[26,27,28]. These binding increased eryptosis and Reactive Oxygen Species (ROS) formation. Eryptosis

formed artificial clots and sped up initial haemostatic plug formation[7,9]. ROS formation increased TF expression and activation, which was required in thrombin and fibrin formation[29]. Bismuth subgallate also prevents secondary bleeding because it has built a stable blood clot from initial haemostatic plug and fibrin, even though clopidogrel inhibits platelet aggregation.

Conclusions

This study concludes that bismuth subgallate sped up formation and stabilized haemostatic plug both on normal and clopidogrel-inhibited platelet aggregation.

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Declaration of Interest

The authors report no conflict of interest.

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