Heparinless cardiopulmonary bypass with argatroban in dogs

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Abstract

Objectives: Systemic heparinization is usually required for cardiopulmonary bypass (CPB). However, problems such as heparin-induced thrombocytopenia, protamine shock, and antithrombin III deficiency exist related to CPB with heparinization. The aim of this study was to evaluate argatroban (ARG) as a substitute for heparin during CPB.

Methods: In the pilot study, blood samples were sequentially obtained from dogs with continuous infusion of ARG at a dose of 10 \( \mu \text{g/kg per min} \) for 2 h without CPB. In the main study, dogs underwent CPB for 2 h with 10 \( \mu \text{g/kg per min} \) or 30 \( \mu \text{g/kg per min} \) of ARG or with heparin with blood samples obtained sequentially. Thrombogenicity in each group was evaluated by observation of the blood-contacting surfaces of the CPB circuits with scanning electron microscopy (SEM). Evidence of thromboembolism in the dogs was also investigated in histological specimens of the kidney and spleen in addition to microscopic observation at autopsy.

Results: In the pilot study, the activated coagulation time (ACT) reached a maximum level dose-dependently after continuous infusion of ARG for 30 min. ACT returned to the baseline value within 60 min after the termination of continuous infusion. In the main study, CPB with 30 \( \mu \text{g/kg per min} \) of ARG achieved thrombin–antithrombin III complex (TAT) level similar to that achieved by CPB with heparin. Platelet count with 30 \( \mu \text{g/kg per min} \) of ARG tended to be higher than that with heparin or 10 \( \mu \text{g/kg per min} \) of ARG. The SEM appearance of blood-contacting surfaces of the CPB circuits after infusion with 30 \( \mu \text{g/kg per min} \) of ARG appeared to be similar to that after infusion with heparin. Depositions on the blood-contacting surfaces of the CPB circuits were also frequently observed with 10 \( \mu \text{g/kg per min} \) of ARG.

Conclusions: Coagulability related to CPB was controlled by the appropriate ARG dosage without the use of heparin in dogs. ARG may be a substitute for heparin in CPB.

Keywords: Argatroban; Cardiopulmonary bypass; Coagulability; Scanning electron microscope

1. Introduction

Heparin is generally needed as an anticoagulant for cardiopulmonary bypass (CPB) in open-heart surgery. However, the use of heparin may cause problems in patients with a reduced antithrombin III (AT-III) level or with protamine allergy. Long-term use of heparin may also result in heparin-induced thrombocytopenia (HIT II) or HIT II-associated thrombosis (HITT) [1,2]. Therefore, it is necessary to find heparin substitutes that can be used in patients with problems related to heparin.

Argatroban (ARG) is a selective thrombin inhibitor that, unlike heparin, binds reversibly to thrombin without the need for AT-III as a cofactor and shows excellent anticoagulant activity [3]. The structural formula of ARG is

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(2R–4R)-4\text{methyl-1-}[N^2-(RS)-3\text{methyl-1,2,3,4-tetrahydro-8-quinolinesulfonyl}-L-arginyl]-2\text{piperidinecarboxylic acid hydrate.}
\]

ARG is a derivative of arginine. Molecular formula is C_{23}H_{36}N_{6}O_{5}S. Its half-life is 15–30 min. It has attracted attention in recent years as a treatment for HIT II and HITT [1,2]. At present, ARG is used clinically in patients with arteriosclerosis obliterans or cerebral thrombosis, as well as during hemodialysis in patients with AT-III deficiency [4]. In the cardiovascular field, its use as an anticoagulant during closed extracorporeal circuit operations such as left-heart bypass and percutaneous pulmonary support (PCPS) has been reported [5], but there are few reports of its clinical use as an alternative to heparin during CPB for open-heart surgery [6].

The present study was designed to assess the efficacy and safety of ARG as a substitute for heparin in dogs with CPB and to find the appropriate dose of ARG resulting in anticoagulant activity comparable to that of heparin in dogs.
2. Materials and methods

2.1. Animal care and preparations

All the animals involved in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised in 1996. All procedures were approved by the Animal Research Committee of the Saga Medical School.

Thirty-six adult mongrel dogs, weighing 20–30 kg, were used. Anesthesia was achieved by intramuscular injection of 10 mg/kg of ketamine hydrochloride followed by insertion of an intravenous line and intravenous injection of 20 mg/kg of thiopental sodium and 0.08 mg/kg of the muscle relaxant, pancuronium bromide. After endotracheal intubation, general anesthesia was maintained by inhalation of isoflurane under mechanical ventilation. The left femoral artery was cannulated with a 6 Fr catheter for sequential blood samplings and continuous pressure monitoring.

2.2. Pilot study: response to ARG in dogs without CPB

ARG was continuously infused for 2 h in dogs without CPB at a rate of 10 μg/kg per min (n = 6) in the low-dose group, 20 μg/kg per min (n = 6) in the middle-dose group, or 30 μg/kg per min (n = 6) in the high-dose group. Blood samples were sequentially obtained every 30 min before, during, and after continuous infusion to measure activated whole-blood clotting time (ACT) and other coagulation and fibrinolysis parameters.

2.3. Main study: CPB with ARG in dogs

The chests were entered through the bilateral fourth intercostal spaces and the sternum was divided transversely. Continuous infusion of ARG was started 30 min before CPB, and a rate of 10 μg/kg per min (n = 6) or 20 μg/kg per min (n = 6) in the low-dose group (n = 6) was established. In the control group (n = 6), heparin (200 IU/kg or more) was injected intravenously to maintain the ACT over 400 s. After systemic anticoagulation therapy was achieved, a 16 Fr arterial-blood-return cannula was inserted into the ascending aorta and a 28 Fr venous drainage cannula was inserted into the right atrium. Extrapulmonary circulation was performed for 2 h at a flow rate of 60–80 ml/kg per min with a roller pump, a membrane-type gas exchanger (Mera Exelan alpha-HCP, Senko Medical Industry, Inc., Tokyo, Japan), and an arterial filter (Pall Biomedical Inc., New York, USA) simulating clinical CPB.

Blood samples were sequentially obtained before surgery, before CPB, and then every 30 min during CPB, as well as after CPB for 1 h.

After completion of 2 h of extracorporeal circulation, the CPB circuit was immediately rinsed with lactated Ringer’s solution at a perfusion pressure of 120 cmH2O. Then it was fixed using 2.5% glutaraldehyde in 0.1 M cacodylate buffer with 3% sucrose. After fixation was achieved, the specimens were rinsed with 0.1 M cacodylate buffer solution, and then dehydrated through an ethanol series and freeze-dried. The specimens were coated with gold (IB-3 ion coater, Eiko Ltd., Mito, Japan), and then observed by scanning electron microscopy (SEM) (JSM-5200LV, JEOL Ltd., Tokyo, Japan).

After the simulation of CPB, the animal was euthanized with a lethal dose of intravenous thiamylal sodium and potassium after additional systemic heparinization (500 IU/kg) to prevent postmortem thrombus formation. Both kidneys and the spleen were immediately removed with careful macroscopic observations of the main arterial branches where thrombus/embolus may exist. The organs were fixed using 10% formalin. Then, the specimens were stained with hematoxylin–eosin (H and E) and histologically examined for evidence of clot/fibrin deposition as well as the presence of microemboli.

2.4. Hematological analysis

Blood samples were obtained as described above from the femoral artery through a 6 Fr catheter to determine counts of blood cells (CBC) with hemoglobin/hematocrit, activated clotting time (ACT), prothrombin time (PT), activated partial thromboplastin time (APTT), levels of fibrinogen and α-2 plasmin inhibitor (α2PI), and the level of thrombin–antithrombin III complex (TAT). ACT was measured with a Hemoctron 401 whole blood detection system (International Technidyne Co., Edison, NJ). CBC and hemoglobin/hematocrit were determined using an automated analyzer (Coulter Electronics, Harpenden, UK). TAT was measured using time-resolved fluorescence immunoassay. Fibrinogen was measured by the thrombin-time method of Claus. α2PI was measured by a chromogenic substrate method [7]. PT was measured by the clot detection system using the Quick-One method with reagent Simplastin-HTF (Japan bioMerieux, Tokyo, Japan) [8]. APTTs were measured by the clot detection system using the Langdell method with reagent Platelin LS (Japan bioMerieux, Tokyo, Japan) [9]. Blood samples for PT, APTT, TAT, fibrinogen and α2PI were immediately cooled on ice, centrifuged and the plasma was stored at −70°C before being assayed, which was carried out by a biochemical assay laboratory (SRL Tokyo Medical, Tokyo, Japan).

2.5. Statistical analysis

Data are expressed as the mean ± SE. Statistical analyses were performed by analysis of variance (factorial ANOVA); when a significant F value was obtained,
comparisons between groups were done by Schefte’s test as a post hoc test. Differences were considered significant at the level of \( P < 0.05 \).

3. Results

3.1. Pilot study: Response to ARG in dogs without CPB

During administration of ARG, ACT reached a steady state at about 30 min after the start of infusion and was significantly prolonged in a dose-dependent manner. Within about 1 h after ceasing ARG administration, ACT returned to the baseline level without any need for a neutralizing agent (Fig. 1).

3.2. Main study: CPB with ARG in dogs

CPB was successfully performed for 2 h in all three groups without any circuit occlusion compared to the ACTs of dogs receiving the same ARG dose but without CPB during the pilot study; the ACTs of dogs receiving low and high ARG doses in the main study were slightly prolonged during CPB and their recoveries were delayed after the discontinuation of ARG administration (Fig. 2). ARG significantly prolonged PT (Fig. 3A). On the other hand, heparin significantly prolonged APTT (Fig. 3B). The level of TAT in the high-dose ARG group was comparable to that in the heparin group. Percent recovery of platelet count in the high-dose ARG group tended to be higher than that in the heparin group. The level of TAT at 2 h after the beginning of CPB in the low-dose ARG group was significantly increased compared with the other two groups. In the low-dose ARG group, percent recovery of platelet count tended to be lower than that of the other two groups (Fig. 4). No significant differences were detected in fibrinolysis parameters such as fibrinogen and \( \alpha 2 \)PI among the three groups (Fig. 5).

![Fig. 1. ACT response to ARG in dogs without CPB. Continuous drip infusion of ARG increased ACT dose-dependently and ACT was normalized within 60 min after termination of ARG infusion without pharmacologic reversion (* \( P < 0.01 \) as compared with baseline). ACT, activated clotting time; CPB, cardiopulmonary bypass; ARG, argatroban.](image1)

![Fig. 2. ACT response to ARG in dogs with CPB. In the heparin group, ACT was maintained at more than 400 s during CPB (* \( P < 0.01 \) as compared with high- and low-ARG groups). ACT, activated clotting time; CPB, cardiopulmonary bypass; High ARG, CPB with high-dose argatroban (30 \( \mu g/kg \) per min); Low ARG, CPB with low-dose argatroban (10 \( \mu g/kg \) per min); Heparin, CPB with heparin (2 mg/kg).](image2)

![Fig. 3. Prothrombin time (PT) (A) and activated partial thromboplastin time (APTT) (B) of dogs with CPB. High-dose ARG significantly increased PT in contrast with heparin (* \( P < 0.01 \) ); on the other hand, heparin increased APTT significantly more than high-dose ARG (* \( P < 0.01 \) ). High ARG, CPB with high-dose argatroban (30 \( \mu g/kg \) per min); Low ARG, CPB with low-dose argatroban (10 \( \mu g/kg \) per min); Heparin, CPB with heparin (2 mg/kg).](image3)
After rinsing, the CPB circuit (especially in the venous reservoir) of the low-dose ARG group appeared to have more thrombi than that in the heparin group. Level of TAT in the high ARG group was comparable with that in the heparin group. Level of TAT at 2 h after the beginning of CPB in the low-dose ARG group was significantly increased compared with the other two groups (*P < 0.05). Percent recovery of platelet count in the high ARG group tended to be higher than that in the heparin group (P = 0.12). In the low-dose ARG group, percent recovery of platelet count tended to be lower compared with the other two groups (P = 0.13). High ARG, CPB with high-dose argatroban (30 μg/kg per min); Low ARG, CPB with low-dose argatroban (10 μg/kg per min); Heparin, CPB with heparin (2 mg/kg).

At autopsy, no thrombus was detected macroscopically in the main arterial branches of the kidneys or spleens of all three groups. In the histological specimens, no thrombus or embolus was found in the kidneys or spleens of all three groups (Fig. 7).

4. Discussion

Heparin is widely used as an anticoagulant for CPB during open-heart surgery. The ACT is used to evaluate the anticoagulant effect of heparin, and clotting is controlled when ACT is 400 s or more during CPB [10]. However, various problems with heparin can occur. For example, there is concern about performing heparinization in patients with a history of HIT II and HITT, AT-III deficiency, or protamine allergy. In HIT II and HITT patients, antiplatelet

Fig. 4. Thrombin–antithrombin complex (TAT) (A) and percent recovery of platelet counts (B) in dogs with CPB. Level of TAT in the high ARG group was comparable with that in the heparin group. Level of TAT at 2 h after the beginning of CPB in the low-dose ARG group was significantly increased compared with the other two groups (*P < 0.05). Percent recovery of platelet count in the high ARG group tended to be higher than that in the heparin group (P = 0.12). In the low-dose ARG group, percent recovery of platelet count tended to be lower compared with the other two groups (P = 0.13). High ARG, CPB with high-dose argatroban (30 μg/kg per min); Low ARG, CPB with low-dose argatroban (10 μg/kg per min); Heparin, CPB with heparin (2 mg/kg).

Fig. 5. Fibrinolysis parameters showed no differences among the three groups. (A) Percent recoveries of fibrinogen; (B) percent recoveries of alpha-2PI. High ARG, CPB with high-dose argatroban (30 μg/kg per min); Low ARG, CPB with low-dose argatroban (10 μg/kg per min); Heparin, CPB with heparin (2 mg/kg).

Fig. 6. Scanning electron micrographs of the arterial filters. The amount of deposition detected on the arterial filters in the high ARG group was not more than that of the heparin group. In contrast, more deposition was observed in the low ARG group than in the heparin and high ARG groups. High ARG, CPB with high-dose argatroban (30 μg/kg per min); Low ARG, CPB with low-dose argatroban (10 μg/kg per min); Heparin, CPB with heparin (2 mg/kg).
antibodies are activated by heparin, causing abnormal aggregation of platelets, and this serious condition has been attracting attention recently. Therefore, alternative anticoagulants to replace heparin are needed.

ARG is a selective thrombin inhibitor that binds reversibly to thrombin without the need for AT-III to show its anticoagulant activity, unlike heparin [3]. ARG also prevents a decrease of the platelet count by inhibiting thrombin-induced platelet aggregation. In Japan, it is used clinically in patients undergoing treatment for the acute cerebral thrombosis or arteriosclerosis obliterans, as well as for hemodialysis in patients with AT-III deficiency [4,11,12]. Moreover ARG has attracted attention as a drug for the treatment of HIT II and HITT. In the United States, a large-scale clinical trial of ARG treatment for HIT II and HITT is ongoing [13].

In the cardiovascular field, Kawada et al. first reported the use of ARG as an anticoagulant during left-heart bypass in surgery for aortic aneurysm [5], but there has been only one limited report of its clinical use as an anticoagulant during CPB in open-heart surgery [6]. Sakai et al. previously reported CPB using ARG for 1 h in a canine model, suggesting the possibility of clinical application [14]. Matsukura et al. and Endo et al. have also reported similar findings [15,16]. It is obvious that further experimental studies on CPB with ARG should be done before ARG is used clinically in the setting of cardiac surgery with CPB.

In the present study, CPB was uneventfully carried out for 2 h in all dogs treated with ARG, as well as heparin. In the low-dose ARG group, however, increased TAT and decreased platelet counts were observed. Moreover, blood clots were macroscopically detected in the venous blood reservoir after rinsing, which demonstrated that low-dose ARG did not work well as an anticoagulant in the dogs on CPB. On the other hand, the high-dose ARG worked adequately (similar to heparin) in dogs on CPB, with platelet counts being preserved, TAT maintained at a low level, and the CPB circuit kept clean without remarkable thrombus formation. In dogs, intravenous continuous infusion of ARG at 30 μg/kg per min may be recommended to maintain safe CPB, with ACT stabilized between 250 and 300 s, which suggests that ARG could work well as a substitute for heparin even in humans on CPB. The clinically appropriate method to administer ARG for CPB should be investigated.

Anticoagulants that have been suggested to replace heparin for CPB include low molecular weight heparin, danaparoid, ancrod, lepirudin, and active site-blocked factor IXa [17–21]. Low molecular weight heparin and danaparoid are reported to show cross-reactivity with HIT II antibodies, so problems related to heparin cannot be completely solved with their use. The fibrinolytic agent ancrod does not inhibit thrombin, which is a significant problem for patients with HIT II and HITT. Lepirubin, like ARG, inhibits thrombin, but there is a concern of renal dysfunction related to its use. Use of active site-blocked factor IXa has been reported in an animal model of CPB, but its anticoagulant action has not been widely evaluated and it has not been applied clinically. On the basis of these concerns, ARG would seem to be a reasonable substitute for heparin.

In the present study, some limitations should be considered. First of all, since cardiac surgery was not done during CPB in the present study, no blood was sucked into the reservoir in the operating field. Therefore, there might be much less thrombogenicity in the present study than in actual cardiac surgery. The usages of administering ARG might be more effective than continuous infusion.
Initial bolus injection of ARG, which was not studied in the present study, may achieve adequate anticoagulability immediately that might be useful especially in emergent surgery. Differences between human and dog coagulation systems should be considered. Accordingly, an experimental study using an animal model with a coagulation system that is more like the human coagulation system may be necessary.

In conclusion, ARG worked well as a substitute for heparin in the canine CPB model. ARG may be one of the safe alternatives to heparin in patients with risk of heparin-related side effects, even in the setting of cardiac surgery with CPB.

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References