A Thesis
For the Degree of Master of Veterinary Science

Heparin Monitoring by Activated Partial Thromboplastin Time in Sheep

Department of Veterinary Medicine
Graduate School, Cheju National University

Seokgon Park

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Seokgon Park

(Supervised by professor Kyoungkap Lee)

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This thesis has been examined and approved.

Jongtae cheong

Thesis director, Jongtae Cheong, Prof. of Veterinary Medicine

Young Jae Lee

Youngjae Lee, Prof. of Veterinary Medicine

Kyoung Kap Lee

Kyoungkap Lee, Prof. of Veterinary Medicine

2001. 2.

Department of Veterinary Medicine
GRADUATE SCHOOL
CHEJU NATIONAL UNIVERSITY

초 록

면양에서 APTT를 이용한 heparin monitoring

박 석 곤

제주대학교 대학원 수의학과

항응고제인 헤파린은 동물의 혈관수술에서 문제되는 급성 또는 만성의 혈전 생성을 줄이기 위해 이용된다. 이에 면양에서 적절한 헤파린의 항응고 작용유지를 위해서 정맥과 피하로 헤파린을 투여 후에 APTT 를 측정하였다. 건강한 면양 9 두를 실험에 이용하였으며, 체중에 따라 세 군(A, B, C)으로 나누었다. A 군은 40 kg 미만, B 군은 40-80 kg, 그리고 C 군은 80 kg 이상의 체중으로 각각 나누었다. 각각의 면양에 헤파린을(300 IU/kg) 정맥으로 투여하고, 피하투여는 4 주후에 실시하였다. 헤파린 투여 전과 투여 후 매시간마다 APTT, 혈소판 수 그리고 섬유소원을 각각 측정하였다. 정맥 투여 후 APTT 값은 투여 후 1 시간에서 4 시간째까지 유의적인 증가를 보인 후($p<0.05$), 6 시간째 투여 전의 APTT 값으로 되돌아 왔으며, 피하 투여의 경우 투여 후 2 시간부터 6 시간째까지 유의적인 증가를 보인 후 ($p<0.05$), 10 시간째 투여 전의 APTT 값으로 되돌아 왔다. 정맥투여의 경우 APTT 비율이 치료범위 이하로 떨어지는 시간은 투여 후 5 시간째이며, 피하 투여의 경우는 투여 후 8 시간째이다. 피하 투여 후 A 군은 APTT 비율이 투여 후 4 시간째 가장 높았으며, B 군과 C 군은 투여 후 각각 5 시간째와 3 시간째 가장 높았다. 피하 투여 후 가장 높은 APTT 비율은 C 군이 A, B 군보다 보다 유의적으로 높았다 ($p<0.05$). 헤파린 투여 전의 A, B 군 및 C 군의 혈소판 수는 각각 3,197±365.6, 2,886±78.2 및 1,861±298.0 $10^2/\mu L$ 이었다. 헤파린의 피하와 정맥 투여 후 혈소판 수는 유의적인 차이 없이 계속적인 감소를 나타내었다. 면양의 혈관수술에서, 초

기 헤파린의 정맥 투여 후 APTT 비율을 치료범위로 유지시키기 위해서는 투여 후 4 시간째에 헤파린의 2 차 투여가 이루어져야 하고, 치료범위의 지속시간을 길게 하기 위해서는 피하투여가 권장된다.

중심어: 헤파린, APTT, 혈소판, 면양

Contents

Ⅰ. Introduction

Thromboembolisms, induced by the contact of blood with a foreign surface, is a major problem in the development of an artificial organ, either for extracorporeal circulation or for implantation within the heart and blood vessels (Stanford and Sanford, 1986; Stefan et al., 2000). Heparin is effective in the prevention and treatment of venous thrombosis, pulmonary embolism and the prevention of thrombosis after cardiovascular surgery (Jack and Valentin, 1994). Heparin is poorly absorbed from the gastrointestinal tract, therefore the preferred routes of administration of heparin are continuous IV infusion and SC injection (Jack et al., 1991). If the SC route is selected, the initial dose must be sufficiently high to compensate for the reduced bioavailability of IV bolus heparin administered because an anticoagulant effect from SC heparin is delayed for 1 to 2 hours (Russel et al., 1986; Jack and Valentin, 1994).

The anticoagulant activity of heparin is mediated by its binding through a specific pentasaccharide to antithrombin Ⅲ (AT Ⅲ), which results in a marked acceleration of the rate at which AT Ⅲ inactivates thrombin, factor Xa, and other serine proteases in the coagulation cascade (Kevin et al., 1993).

The activated partial thromboplastin time (APTT) and activated clotting time (ACT) are clinically useful for the monitoring of heparin therapy. The APTT is used more frequently for general screening tests for assessing the integrity of the intrinsic and common pathways of coagulation (Stanley and Mary, 1968; Douglas and Cathy, 1978; Barbara and Trevor, 1980). The ACT is theoretically equally as useful as that of the APTT for the routine monitoring of heparin therapy, but appears more useful

in situations in which high serum concentrations of heparin are required (Glenn et al., 1988; Robert et al., 1995). The APTT value can be affected by many variables, such as APTT reagent, instrument, clinical state of the patient, and coumarin use (Sal, 1985; Jonathan et al., 1994). The anticoagulant response to heparin varies widely among patients with thromboembolic disease (Robert et al., 1981; Edward et al., 1992). Therapeutic doses of heparin are cleared by combination of the rapid, saturable mechanism and the slower, nonsaturable dose-independent mechanism of renal clearance (Jack and Valentin 1994). Very high doses of heparin are cleared predominantly through the slower nonsaturable mechanism of clearance (Olsson et al, 1963; Cees et al., 1982). The clinical monitoring of heparin with the patient APTT ratio (APTT heparin/APTT baseline) corresponds to heparin plasma concentration level (Eugenio et al., 1980; Patrick et al., 1993). For these reasons, heparin treatment is usually monitored to maintain the ratio of the patient's APTT to the mean control APTT within a defined range of approximately 1.5 to 2.5, referred to as the therapeutic range (Frank et al., 1985; Barry, 1991; Robert et al., 1995).

But many experiments and studies about heparin monitoring using APTT have been performed within the limits of humans, so we investigated change of APTT value after heparin administration by IV bolus and SC injection for heparin therapy monitoring protocol after vascular surgery in sheep.

Ⅱ. Materials and Methods

1. Experimental animals

Nine sheeps (6 males and 3 females) with a range of 31-107 kg were used for this study. Animals were assigned to three groups (A, B and C), according to their body weight: less than 40 kg, from 40 kg to 80 kg and more than 80 kg, respectively. All animals were treated with IV heparin bolus and SC heparin of 300 IU/kg (Heparin, Choongwae) at intervals of 4 weeks. All animals were performed deworming, physical examinations and complete blood count (CBC) during period of preliminary rearing (2 weeks).

2. Blood processing and APTT measurements

Blood samples were collected by jugular venipuncture before heparin administration and every hour after the administration, until APTT value returned to baseline. Two types of sample tube were used; one contained ethylene-diamine tetracetic acid (EDTA), the other contained 0.105 M buffered sodium citrate 0.2 ml (equivalent to 3.2 %). The blood with EDTA was measured for platelet count by automatic blood cell count (Coulter Electric co., USA). The blood with sodium citrate was centrifuged at 2500 rpm for 10 minutes at room temperature, and the plasma was transferred to a effendorf tube to mesure APTT. Plasma (0.1 ml) was

incubated with 0.1 ml APTT reagent (KUCKJE, Japan) for 3 min. After 0.1 ml 0.025 M calcium chloride was added, and time for clot formation was measured. The APTT was measured on a dual-channel CLOT-2 (*SEAC*, Italy). All APTT were performed in duplicate, and mean value was calculated. If APTT exceeded 200 sec, its value was indicated 200 sec.

3. Statistical analyses

The APTT, platelet count from each group were analyzed by Duncan's new multiple range test after ANOVA analysis. Differences with a p-value<0.05 were considered as significant, and all statistical analyses using a SAS (SAS Institute, 1996) program.

Ⅲ. Results

1. APTT

The mean value of the APTT after IV bolus and SC injection, was shown in Figure 1. The APTT increased significantly between 1 to 4 hours after IV bolus injection ($P<0.05$), and between 2 to 6 hours after SC injection ($p<0.05$). The APTT returned to baseline values 6 and 10 hours after the respective treatments. The APTT showed peak 1 and 2 hours after IV bolus injection, and showed peak 3 hour after SC injection.

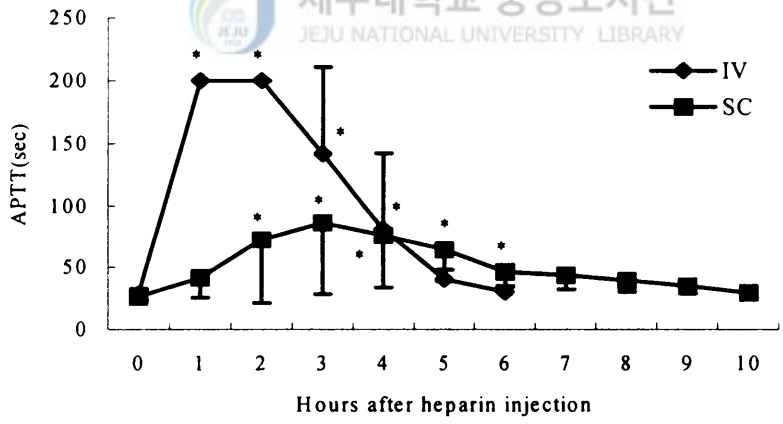

Figure 1. The APTT response to heparin by administrating different routes.
IV: intravenous bolus injection, SC: subcutaneous injection (300 IU/kg)
*; compared to hour 0 in each treatment ($p<0.05$)

The mean APTT among three groups after injection was shown in Figure 2 and Figure 3. The APTT in Group C was consistently higher than in Group A and B after heparin treatment by the two routes. The APTT in Group B and C was significantly higher than that in group A, 3 and 4 hours after IV bolus injection ($p<0.05$). The APTT in Group C was significantly higher than that in Group A and B, 5 hour after IV bolus injection ($p<0.05$).

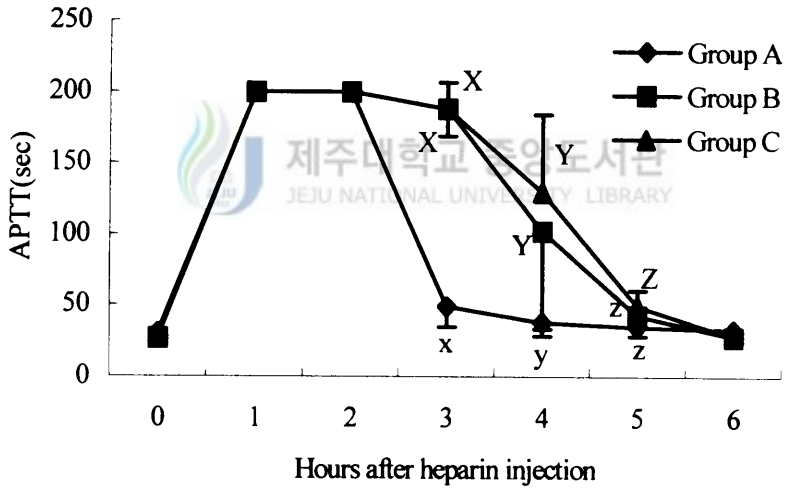

Figure 2. Change of the APTT after IV bolus injection. The APTT in Group B was significantly higher than that in Group A, 3 and 4 hour after injection ($p<0.05$). The APTT in Group C was significantly higher than that in Group B, 5 hour after injection ($p<0.05$). X:x, Y:y, Z :z; significantly differential pairs ($p<0.05$). Group A; <40kg, Group B ; 40 to 80kg, Group C; >80kg.

In SC injection, the APTT in Group C was significantly higher than that in Group A and B between 1 to 5 hours after injection ($p<0.05$), and the APTT in Group B and C was significantly higher than that in Group A between 6 to 9 hours after injection ($p<0.05$).

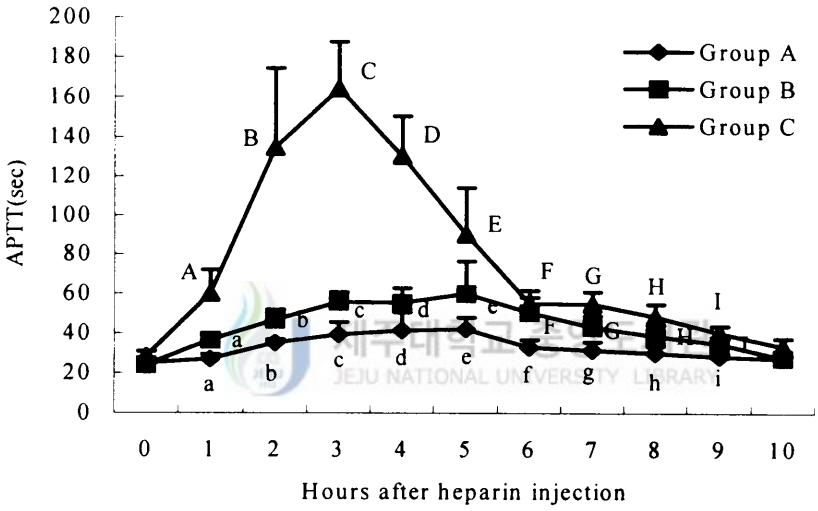

Figure 3. Change of the APTT after SC injection. The APTT in Group B was significantly higher than that in Group A between 6 to 9 hours after injection($p<0.05$). Between 1 to 5 hours after SC injection, the APTT in Group C was significantly higher than that in Group B ($p<0.05$). A:a, B:b, C:c, D:d, E:e, F:f, G:g, H:h, I:i; significantly differential pairs ($p<0.05$).

2. APTT ratio

The APTT ratio (APTT heparin/APTT baseline) obtained on IV bolus and SC heparin injection was shown in Figure 4. The maintaining time of APTT ratio (1.5 to 2.5 times baseline) referred to the therapeutic range after SC injection was longer than that of after IV bolus injection, however the time reached the therapeutic range after SC injection, was longer than that of IV bolus injection.

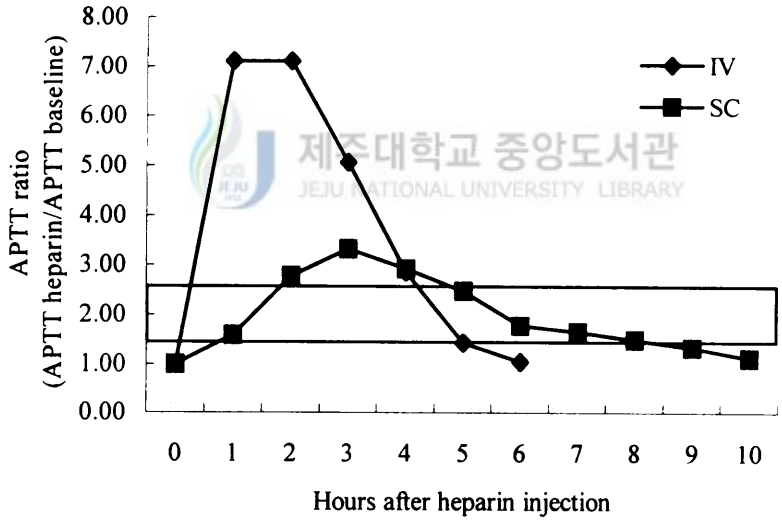

Figure 4. The change of the APTT ratio after IV bolus and SC injection. The APTT ratio became subtherapeutic range 5 and 8 hours after IV bolus and SC injection, respectively. Closed area (□) indicated the therapeutic range.

The highest APTT ratio after SC heparin injection was shown in Figure 5. The highest APTT ratio after SC heparin injection in Group A, B and C was 1.6±0.37, 2.4±0.62 and 5.7±0.69, respectively. The highest APTT ratio in Group C was significantly higher than that in Group A and B (p<0.05).

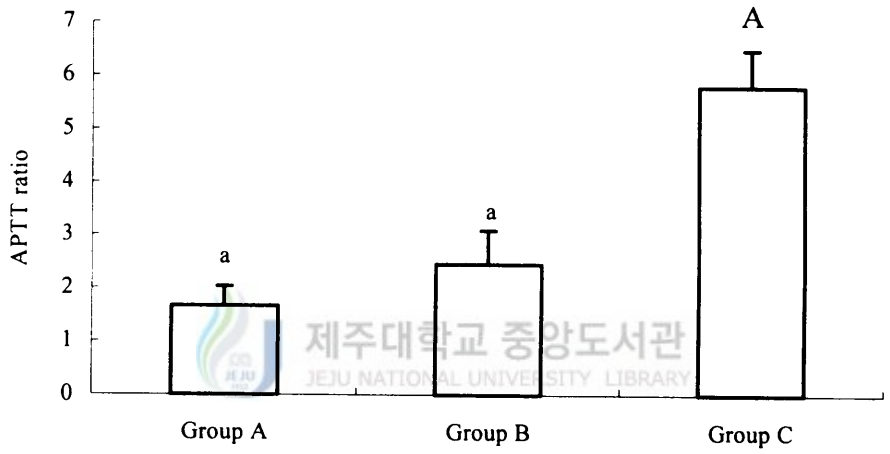

Figure 5. The difference of the highest APTT ratio between the groups after SC injection. The highest APTT ratio in Group A and B was significantly lower than that in Group C (p<0.05). A:a; significantly differential pairs (p<0.05)

3. Platelet count

The mean platelet count after IV bolus and SC heparin injection was shown in the Figure 6. The mean platelet count of Group A, B and C before injection was 3,197±365.6, 2,886±78.2, and 1,861±298.0 $10^2/\mu L$, respectively. The mean platelet count in Group A and B before injection significantly higher than that in Group C ($p<0.05$).

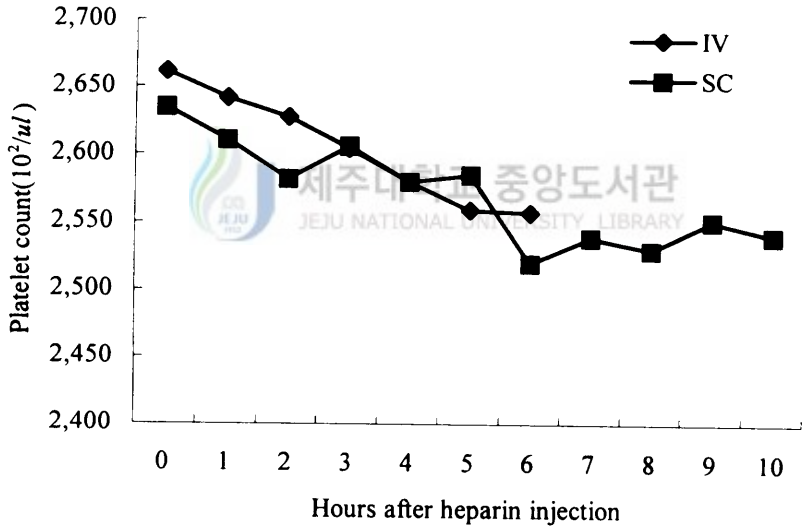

Figure 6. Influence of heparin administrated by two routes on the mean platelet count. The mean platelet count after IV bolus and SC injection gradually decreased without significant variation.

Ⅳ. Discussion

The anticoagulant effect of heparin is mediated primarily by acceleration and potentiation of the action of antithrombinⅢ (ATⅢ), following conformational change resulting from the binding of heparin to ATⅢ (Robert et al., 1995). Heparin binds to ATⅢ and catalyzes inactivation of factor Ⅱ, Ⅸ, Ⅹ are major mechanisms for the anticoagulant effect. ATⅢ is a slow coagulation inhibitor without heparin, but heparin binds to ATⅢ through unique pentasaccharide and induced a conformational change to the ATⅢ that converts from a slow to a very rapid inhibitor. Heparin binds to heparin cofactorⅡ and catalyzes inactivation of factorⅡ, but anticoagulant effect requires very high concentrations of heparin (Jack and Valentin, 1994). Heparin binds to platelets, inhibits platelet function and contributes to the hemorrhagic effects of heparin (Edwin et al., 1980). The side effects of heparin are hemorrhage, thrombocytopenia and osteoporosis (Jack and Valentin, 1994). Resistance to a dose heparin occurs only when there is either a deficiency in the quantity of plasma ATⅢ or a lack of ATⅢ in a heparin-binding site. The absence or near absence of ATⅢ renders heparin useless for antithrombotic therapy, because the antithrombotic action of heparin depends on the plasma ATⅢ concentration and heparin dosage vary accordingly (Ewa and John, 1977). Richard et al. (1994) reported that frequent APTT test are required during the course of treatment to maximize an appropriate anticoagulant effect because of the complex pharmacokinetics and pharmacodynamics of heparin.

Jack and Valentin (1994) reported that the half-life of heparin after IV administration is about 90 minutes (range, 0.5-2.5 hours) and the anticoagulant

effects of SC heparin are delyed for approximately 1 hour and peak levels occurs approximately 3 hour. The APTT after IV bolus injection showed peak 1 and 2 hours, and the APTT after SC injection showed peak 3 hour. This result was corroborated by Jack and Valentin (1994). And this result demonstrates that appropriate selection of administrating route is needed for the efficient induction of therapeutic range.

Sal (1984) reported that the clinical monitoring of heparin with the patient APTT ratio (APTT heparin/APTT baseline) corresponded better to plasma heparin levels. Robert et al. (1995) reported that a ratio of 1.5-2.5 times was more than the APTT control is defined therapeutic range of heparin, and corresponds to heparin plasma concentrations of 0.2-0.4 IU/ml. The APTT ratio entered the sub-therapeutic range 5 and 8 hours after IV bolus and SC injection, respectively. The APTT ratio was maintained in the therapeutic range for about 1 and 4 hours after IV bolus and SC injection, respectively. So a small volume must be administrated frequently for maintenance of therapeutic anticoagulation.

Magda and James (1988), Barry (1991) and Schiele et al. (1995) reported that heparin prolongs the bleeding time, and the mechanism of this effect is not known, but it may relate to a direct effect of heparin on platelet function and mild thrombocytopenia rapidly occur after single dose injection. In this study, the mean platelet count in Group A, B and C before the injection was in inverse proportion to the body weight and age the subject.

There was correlation between the APTT and the platelet count. The difference in APTT response for heparin among the groups may be due to a platelet count. Therefore, in order to success fully monitor heparin therapy after vascular surgery in sheep, heparin sensitivity test and platelet count must be carried out before heparin

injection because the anticoagulant response to heparin varies widely among patients. These results produced elementary data for monitoring in sheep using APTT, suggest that heparin must be administrated by the SC route at 4 hour, intervals in order to remain in the therapeutic range, after an initial IV bolus dose. And we must consider whether other drug (protamine, calcium et al.) or blood infusion will effect the heparin's anticoagulant action.

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Abstract

Heparin Monitoring by Activated Partial Thromboplastin Time in Sheep

Seokgon Park

Department of Veterinary Medicine

Graduate School, Cheju National University, Cheju 690-756, Korea

Heparin anticoagulation is utilized during and after vascular surgery in animals to reduce the risk of acute or chronic thromboembolic problems. In this study, we examined variation in APTT (activated partial thromboplastin time) after the intravenous bolus (IV bolus) and subcutaneous (SC) heparin injection in order to monitor heparin therapy in sheep. Nine healthy sheep were assigned to 3 groups (A, B, and C) according to their body weights: less than 40 kg, 40 to 80 kg and more than 80 kg, respectively. All animals were treated with heparin (300 IU/kg BW) through two routes, and the APTT, fibrinogen and platelet count were measured before and every hour after treatment. The APTT increased significantly between 1 to 4 hours after IV bolus injection and between 2 to 6 hours after SC injection ($p<0.05$). The APTT returned to baseline values 6 and 10 hours after the respective treatments. The APTT in Group C was consistently higher than in Group A and B

after heparin treatment by the two routes. The APTT ratio entered the sub-therapeutic range 5 and 8 hours after IV bolus and SC injection, respectively. The APTT ratio was maintained in the therapeutic range for about 1 and 4 hours after IV bolus and SC injection, respectively. The highest APTT ratio in Group C after SC injection of heparin was significantly higher than that in Group A and B ($p<0.05$). The mean platelet counts in Group A, B and C before the injection were 3,197±365.6, 2,886±78.2, and 1,861±298.0 $10^2/\mu L$, respectively. The mean platelet count gradually decreased without significant variation after IV bolus and SC injection. These results produced elementary data for monitoring in sheep using APTT, suggest that heparin must be administrated by the SC route at 4 hour, intervals in order to remain in the therapeutic range, after an initial IV bolus dose.

Key words: heparin, APTT, platelet count, sheep,

감사의 글

짧은 기간이었지만 저의 인생에 있어서 중요한 시간이 될 저의 대학원 생활을 마치면서 많은 분들에게 감사의 글을 올립니다.

맹목적인 정성과 사랑으로 키워주신 부모님과 2년동안 저에게 많은 격려와 조언으로 가르쳐주신 이경갑 교수님, 바쁘신 와중에도 보다 나은 논문을 위해서 애써주신 정종태 교수님, 이영재 교수님, 그리고 대학이라는 사회속에서 훌륭하게 생활 할 수 있게 끔 가르침을 주신 수의학과 모든 교수님들게 깊은 감사의 말씀을 올립니다. 논문교정을 위해서 많은 시간과 조언을 해주신 제주농업시험장의 강승률 연구사님, 축산기술연구소 대관령지소의 서정효 수의사에게도 감사의 뜻을 전합니다. 그리고 같이 밤샘작업을 하며 실험을 도왔던 진아, 건태, 일룡에게도 고마움을 전하며 어렵고 힘들 때 많은 관심과 사랑으로 위로 해주었던 동생 희숙, 후배 진혁, 대엽, 그리고 사랑하는 경희에게 진심어린 감사의 뜻을 전합니다.

많이 부족하지만 이렇게 많은 분들의 관심과 격려를 잊지않고 항상 최선을 다하는 사람이 될 것을 약속 드리며 모든 분들의 앞날에 건강과 행운이 항상 함께 하기를 진심으로 바랍니다.