Original Article

The effects of dabigatran etexilate on fracture healing in rats

An experimental study

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Abstract

Background: Deep vein thrombosis leading to pulmonary embolism is one of the major complication after fracture. After a fracture occurs, the coagulation cascade activates thrombin, a protease that finally generates clotting. Dabigatran etexilate reduce clot formation by inhibiting thrombin. Dabigatran etexilate is a widely used drug for thromboprophylaxis. There is no study of the effects of dabigatran etexilate on fracture healing in the literature, so we aimed to evaluate the effects of dabigatran etexilate on fracture healing.

Materials and Methods: Thirty-six female Sprague Dawley rats were divided into 6 groups, each consisting of 6 rats. In all rats, right tibias were used for the fracture model. An oral regimen of dabigatran etexilate suspension in 0.5% hydroxyethylcellulose was administered to the rats. Although the first and second groups received 10 mg/kg daily doses, the third and fourth groups received 50 mg/kg daily doses. The fifth and sixth groups were assigned as sham groups and only hydroxyethylcellulose solution was administered. The first, third and fifth groups were sacrificed on 14th days; whereas the second, fourth and sixth groups were sacrificed on 28th days. Results were evaluated radiologically and histologically.

Results: Radiologically and histologically no statistically significant differences were observed on the 14th day between the first, third and fifth groups; and on the 28th days between the second, fourth and sixth groups.

Conclusion: Radiological and histological evaluations revealed that fracture healing was not affected by dabigatran etexilate. We think that dabigatran etexilate can be used for the prophylaxis of thromboembolism in patients with fractures, but further clinical studies are mandatory.

Key words: Anticoagulant drugs, dabigatran etexilate, fracture healing, thromboembolism
MeSH terms: Anticoagulant, thromboembolism, experimental surgery, thrombolytic agent

Introduction

A fracture is defined as a skeletal injury that results in a break in the physical structure of the bone tissue.[1] The fracture healing has been investigated thoroughly, but there is still much to be learned.[2] One major complication after fractures is deep vein thrombosis (DVT), which can lead to pulmonary embolism. Although there are many mechanical and pharmacological methods for DVT prophylaxis pulmonary embolism is still lethal.[3] New drugs such as orally used direct thrombin inhibitors have been developed because the use of subcutaneous pharmacological agents for DVT prophylaxis have some side-effects and difficulties in practice.[4,5] The anticoagulant dabigatran is one of these drugs. Dabigatran etexilate is a potent small nonpeptide molecule and it inhibits specifically and reversibly both free and clot bound thromb interaction with food hence in by binding to the active site of thromb ion with food hence in molecule.[6] Dabigatran etexilate can be administered without the need for routine coagulation monitoring or dose adjustment.[6] Peak plasma concentrations occur 2 h after oral administration.[6] It is not metabolized by cytochrome P450 isoenzymes and has no potential for drug interaction.[6] Gender, body weight, ethnic origin, obesity and mild-moderate hepatic impairment does not affect its pharmacokinetic profile.[6] The oral form of dabigatran etexilate has gained approval for thromboprophylaxis especially after major orthopedic surgeries.[6]

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Fracture healing is a special healing process in which the associated cellular and biochemical events are nested within a complex system. Inflammatory cells, vascular cells, osteochondral progenitor cells and osteoclasts play an important role at the cellular level of fracture healing. Proinflammatory cytokines, growth factors, proosteogenic factors and angiogenic factors play a role at the molecular level of the bone repair process.[1] After a fracture occurs, the coagulation cascade activates thrombin, which is a protease that finally generates clotting. Unfractionated heparin, low molecular weight heparins (LMWHs), and direct thrombin inhibitors reduce clot formation by inhibiting thrombin. Aspenberg and Pountos *et al*. showed that anticoagulant treatments delay fracture healing and reduce bone formation.[7,8] To the best of our knowledge, there is no study of the effects of dabigatran etexilate on fracture healing in the literature. In this experimental study, we aimed to investigate the effects of dabigatran etexilate on fracture healing.

Materials and Methods

This study was performed at the surgical research and application center in our university from February to June 2011. The study was approved by our university's animal experiments local ethics committee. National law on the care and use of the laboratory animals was followed.

Thirty six Sprague Dawley type female rats were used. The average weight of the rats was 250 g (range 234–258 g), and all of the rats were 4–5 months old. The rats were put in individual cages with 12 h of light and 12 h of darkness cycles and they were held at 20°C–24°C. They were fed a standard diet of rat chow and water. After 1-week of adaptation time, they were randomly divided into six equal groups.

Feed was withheld for 4 h prior to surgery. 5 mg/kg of xylazin hydrochloride (Rompun; Bayer Healthcare) and 50 mg/kg of ketamine hydrochloride (Ketalar; Pfizer) were injected intraperitoneally as anesthetic agents. If necessary, 15 mg/kg of ketamine hydrochloride was administered for additional anesthesia. After cleaning surgical area, 1 mm diameter Kirschner wire was placed in the medullary cavity of the right tibias. The tibial shaft was exposed via a 1 cm longitudinal skin incision, and the soft tissues were protected. Three holes were drilled in the middle portion of the tibial shaft. The tibia was fractured using a gentle maneuver.[9] The skin was closed by using 4.0 silk sutures. After the operation, the rats were returned to normal feeding and living conditions.

A 0.5% hydroxyethylcellulose (Natrosol) solution was prepared.[10] Drug concentration was set as 1 cc of solution for each application. First dose was administrated just after the operation in all groups. The first four groups were classified as the experimental groups. Ten mg/kg dabigatran etexilate was added into the 0.5% hydroxyethylcellulose solution and given once a day via oral gavage to the first group for 14 days and to the second group for 28 days. Similarly, prepared 50 mg/kg dabigatran etexilate was administered to the third group for 14 days and to the fourth group for 28 days. The fifth and sixth groups were used as sham groups, and 0.5% hydroxyethylcellulose solution was administered to fifth and sixth groups for 14 and 28 days, respectively.

The rats in the first, third, and fifth groups were sacrificed on the 14th day; and the rats in the second, fourth, and sixth groups were sacrificed on the 28th day via cervical dislocation after anesthetic administration. Direct X-rays of all tibias were taken for radiological evaluation. The soft tissues were dissected and specimens were put in 10% formaldehyde solution for histological assessment.

Huo *et al*.'s histological grading system was used for histological assessment[11] and the Lane and Sandhu grading system was used for radiological assessment.[12]

The SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for statistical assessment. In order to compare the quantitative data for the groups, the Kruskal–Wallis test and the Mann–Whitney U-test were used. The data taken after measurements were shown as median (minimum-maximum). Significance was defined as $P < 0.05$; for multiple comparisons, significance was defined as $P < 0.05$/comparison number.

Results

There were no complications related to anesthetics or oral drug administration. Postoperatively, limping was observed for 4–5 days. After this period, the animals were able to use their lower extremities normally. No infections occurred at the surgical site. No rats died during the study.

Radiologically no statistically significant differences was observed between the groups on the 14th and 28th days ($P = 0.770$ and $P = 0.809$). The differences were statistically significant in the 10 mg/kg, 50 mg/kg and sham groups with respect to the sacrification time (14 and 28 days) ($P = 0.030$, $P = 0.014$, and $P = 0.043$ respectively) [Table 1 and Figure 1].

Histologically, no statistically significant differences were observed between the groups on the 14th and 28th days ($P = 0.434$ and $P = 0.116$, respectively). The histological data of 10 mg/kg, 50 mg/kg and sham groups

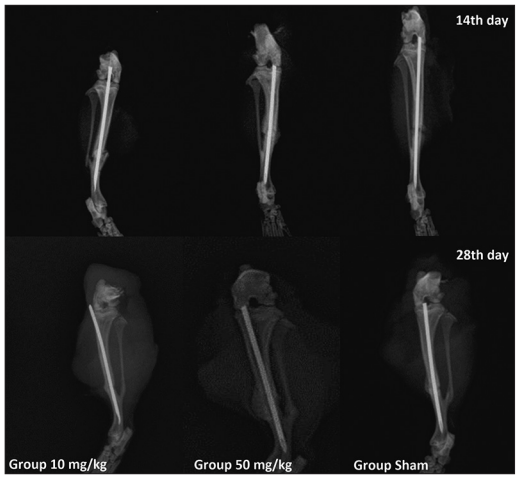

Figure 1: Upper row of images (14th day), the bottom row (28th day): Obvious radiological improvement in fracture line was observed

were assessed at 14th and 28th days and statistically significant differences were found ($P = 0.003$, $P = 0.003$ and $P = 0.003$, respectively) [Table 2 and Figure 2].

Discussion

The new direct thrombin inhibitors have some advantages. The advantages of dabigatran etexilate are oral administration, no need for monitoring, no reaction with other drugs or foods and few associated side effects.[5,13,14]

The mechanisms that are responsible for the relationship between anticoagulant drugs and fracture healing are not fully understood. Heparin is a widely used drug. It has some side effects, such as bleeding and rarely thrombocytopenia; in addition to these side effects, some studies have shown that heparin can cause osteoporosis with long term usage.[15,16] Heparin is linked to the loss of mechanical strength, slimming in the cortices, reduction in the bone collogen synthesis, reducing bone formation and increasing bone resorption in experimental groups.[17,18] Stinchfield *et al*. concluded that heparin and warfarin have a negative effect on fracture healing.[19] Shaughnessy *et al*. found that heparin stays in the bone for a long time, and its effects are not reversed even 1-month later.[20]

Low molecular weight heparins with fewer side effects are used widely as an alternative to heparin. Street *et al*. observed a histological delay in fracture healing and reduced mechanical strength compared to control group in all groups on using enoxaparin.[21] In contrast, a study by Hak *et al*. revealed that a subcutaneous deltaparin injection in stabilized rat femur fractures showed no differences in terms of fracture healing when compared to the control group.[22] We found similar results as Hak *et al*. with dabigatran etexilate.

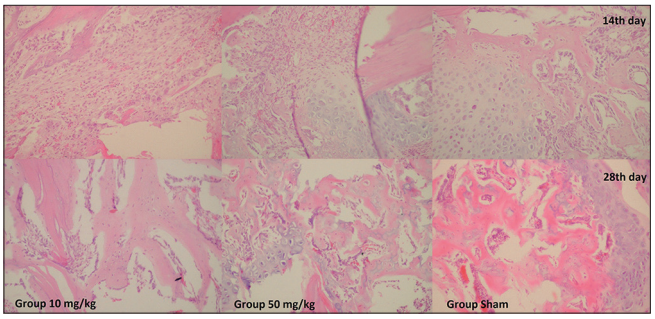

Figure 2: Upper row of histological images (14th day), the bottom row (28th day): There was no statistical difference between groups

Table 1: Lane-Sandhu scoring system

Group	Median (minimum-maximum)		*P*
	Day 14	Day 28	
10 mg/kg	Group 1	Group 2	0.030
	2.0 (1-2)	2.5 (2-3)	
50 mg/kg	Group 3	Group 4	0.014
	2.0 (1-2)	3.0 (2-3)	
Sham	Group 5	Group 6	0.043
	2.0 (1-2)	2.5 (2-3)	
P	0.770	0.809	

Table 2: Histological data: Huo's histological grading system

Group	Median (minimum-maximum)		*P*
	Day 14	Day 28	
10 mg/kg	Group 1	Group 2	0.003
	3.0 (2-4)	7.5 (7-8)	
50 mg/kg	Group 3	Group 4	0.003
	3.0 (2-4)	7.0 (6-8)	
Sham	Group 5	Group 6	0.003
	3.5 (3-5)	8.0 (7-8)	
P	0.434	0.116	

We didn't compare dabigatran etexilate with heparin and LMWHs. Biomechanical tests were not performed. The number of rats were limited for each group. These were the weakest points of our study.

As a conclusion, radiological and histological evaluations revealed that fracture healing was not effected by dabigatran etexilate. We believe that, dabigatran etexilate can be used for the prophylaxis of thromboembolism in patients with fractures, but further clinical studies are mandatory to establish facts.

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