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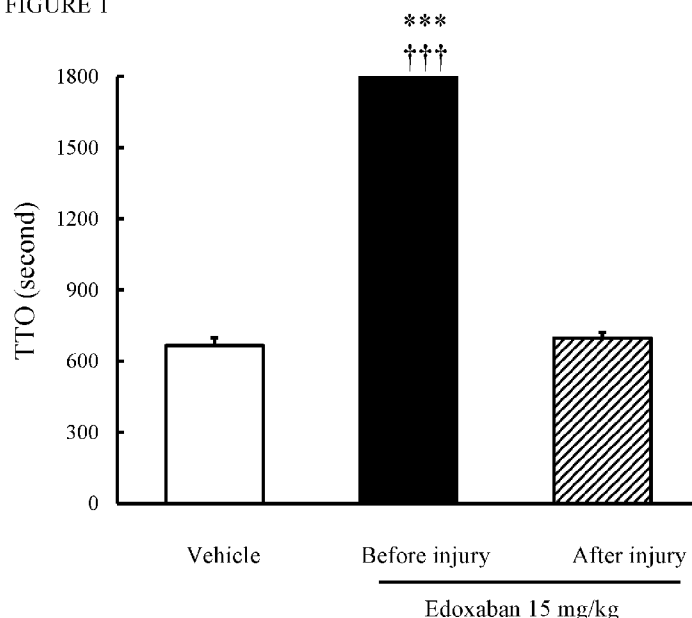
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(54) Title: PHARMACEUTICAL COMPOSITION FOR INHIBITING NEOINTIMA FORMATION OF BLOOD VESSEL

FIGURE 1



(57) Abstract: An object of the present invention is to provide a pharmaceutical composition for inhibiting neointima formation of a blood vessel. The object is attained by a pharmaceutical composition for inhibiting neointima formation of a blood vessel, comprising edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt.

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PHARMACEUTICAL COMPOSITION FOR INHIBITING NEOINTIMA FORMATION OF BLOOD VESSEL

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Application No. 62/407,877, filed October 13, 2016, expressly incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to a pharmaceutical composition for inhibiting neointima formation of a blood vessel, comprising edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt, a method for inhibiting neointima formation of a blood vessel using edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt, etc.

DESCRIPTION OF THE RELATED ART

Edoxaban tosylate hydrate competitively and selectively inhibits activated blood coagulation factor X (hereinafter, referred to as "FXa") in mammals such as humans.

Pharmaceutical compositions containing edoxaban tosylate hydrate are clinically used for the purposes of, for example, prevention of stroke and systemic embolism in adult patients with nonvalvular atrial fibrillation, treatment of deep vein thrombosis (DVT) and pulmonary embolism (PE) and prevention of recurrent DVT and PE, and prevention of venous thromboembolism (VTE) in patients who are undergoing orthopedic surgery of the leg.

The effect of preventing restenosis after angioplasty by FXa inhibitors has been reported in Non Patent Literatures 1 to 3.

However, there has been no report on oral FXa inhibitors, particularly, edoxaban.

[Citation List]

[Non Patent Literature]

[Non Patent Literature 1] Circulation. 1994; 89: 1262-1271

[Non Patent Literature 2] J Am Coll Cardiol 1996; 28: 1849-55

[Non Patent Literature 3] Thromb Res 98 (2000) 175-185

An object of the present invention is to provide a method for inhibiting neointima formation of a blood vessel using edoxaban tosylate hydrate.

SUMMARY OF THE INVENTION

The present invention relates to

(1) a pharmaceutical composition for inhibiting neointima formation of a blood vessel, comprising edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt, and also encompasses aspects described below.

(2) A pharmaceutical composition for preventing artery occlusion or reocclusion after percutaneous transluminal angioplasty for acute coronary syndrome, cerebral infarction, or peripheral arterial disease, comprising edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt.

(3) A pharmaceutical composition for preventing reocclusion after percutaneous transluminal angioplasty, comprising edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt.

(4) A drug-eluting device for preventing reocclusion after percutaneous transluminal angioplasty, comprising edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt.

(5) A method for inhibiting neointima formation of a blood vessel, comprising administering an effective amount of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt to a patient.

(6) A method for preventing artery occlusion or reocclusion after percutaneous transluminal angioplasty for acute coronary syndrome, cerebral infarction, or peripheral arterial disease, comprising administering an effective amount of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt to a patient.

(7) A method for preventing reocclusion after percutaneous transluminal angioplasty, comprising administering an effective amount of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt to a patient.

(8) A method for preventing reocclusion after percutaneous transluminal angioplasty, comprising indwelling a drug-eluting device containing an effective amount of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt into a blood vessel of a patient.

(9) Edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt for use in inhibiting neointima formation of a blood vessel.

(10) Use of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt for inhibiting neointima formation of a blood vessel.

The present invention exerts an effect of being able to provide a novel pharmaceutical composition that can inhibit neointima formation of a blood vessel. Specifically, the present invention can provide a pharmaceutical composition that is useful in the prevention of artery occlusion or reocclusion after percutaneous transluminal angioplasty for acute coronary syndrome, cerebral infarction, peripheral arterial disease, etc. Particularly, the pharmaceutical composition of the present invention is useful in the prevention of artery occlusion after endovascular treatment such as percutaneous transluminal angioplasty.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 illustrates the effects of edoxaban on injury (time to occlusion, TTO) and shows the time to arrest blood flow for at least 1 minute after initiation of ferric chloride (FeCl_3) injury (TTO) in a mouse model (ApoE KO mice) of neointima formation of the carotid artery. Each value represents mean $\pm$ S.E. of $n=10$. Edoxaban at 15 mg/kg was orally administered to mice 1 hour before or after FeCl_3 -injury in carotid artery. Carotid blood flow was monitored for 30 minutes after the induction of injury. If no arrest of blood flow longer than 1 minute was observed within 30 minutes, time to occlusion (TTO) was defined as 30 minutes. Vehicle: 0.5% methylcellulose 400 (10 mL/kg, *p.o.*). *** $p<0.001$ vs. vehicle group (Student's t-test). ††† $p<0.001$ vs. edoxaban group treated after injury (Student's t-test).

FIGURES 2A-2C show changes in the status of vascular closure and patency caused by ferric chloride injury in a mouse model of neointima formation of the carotid artery. FIGURE 2A shows results for the vehicle (0.5% methylcellulose 400 (10 mL/kg, *p.o.*). FIGURE 2B shows status before injury. FIGURE 2C shows results after injury.

FIGURE 3 illustrates the effects of edoxaban on patency rate of a mouse model (ApoE KO mice) of neointima formation of the carotid artery. Open and solid columns indicate a patent and a closed vessel, respectively. Vehicle: 0.5% methylcellulose 400 (10 mL/kg, *p.o.*). Each value represents mean $\pm$ S.E. of $n=10$. Vehicle: 0.5% methylcellulose 400 (10 mL/kg, *p.o.*). *** $p<0.001$ vs. vehicle group (Student's t-test). ††† $p<0.001$ vs. edoxaban group treated after injury (Student's t-test).

FIGURES 4A-4C compare images of hematoxylin-eosin (HE)-stained sections of the injured carotid artery after administration of a vehicle or edoxaban to a mouse model

(ApoE KO mice) of neointima formation of the carotid artery for 42 days. Each figure is the representative of arteries after treatment of vehicle or edoxaban for 42 days. Vehicle (4A): Vehicle (10 mL/kg, b.i.d., *p.o.*) was treated for 42 days except for Day 1 (10 mL/kg vehicle treatment before injury). Before injury (4B): edoxaban was administered 1 hour before injury at 15 mg/kg, *p.o.* on Day 1 and followed by 10 mg/kg, b.i.d., *p.o.* for 41 days. After injury (4C): edoxaban was administered 1 hour after injury at 15 mg/kg, *p.o.* on Day 1 and followed by 10 mg/kg, b.i.d., *p.o.* for 41 days. Bar: 100 μ m.

FIGURES 5A-5C compare images of Elastica van Gieson (EVG)-stained sections of the injured carotid artery after administration of a vehicle or edoxaban to a mouse model (ApoE KO mice) of neointima formation of the carotid artery for 42 days following ferric chloride injury. Image analysis values (intimial area, media area, and I/M ratio) are shown in FIGURES 6-8, respectively, and Table 3. Each figure is representative of arteries after treatment of vehicle or edoxaban for 42 days. Vehicle (5A): Vehicle (10 mL/kg, b.i.d., *p.o.*) was treated for 42 days except for Day 1 (10 mL/kg vehicle treatment before injury). Before injury (5B): edoxaban was administered 1 hour before injury at 15 mg/kg, *p.o.* on Day 1 and followed by 10 mg/kg, b.i.d., *p.o.* for 41 days. After injury (5C): edoxaban was administered 1 hour after injury at 15 mg/kg, *p.o.* on Day 1 and followed by 10 mg/kg, b.i.d., *p.o.* for 41 days. Bar: 100 μ m.

FIGURE 6 shows the effects of edoxaban on a morphometric parameter (intima area) in ApoE KO mice. Each value represents mean $\pm$ S.E. of n=10 in vehicle group and in edoxaban group treated before injury, or n=9 in edoxaban group treated after injury. Vehicle: 0.5% methylcellulose 400 (10 mL/kg, *p.o.*). *** p <0.001 vs. vehicle group (Student's t-test). †† p <0.01 vs. edoxaban group treated after injury (Student's t-test).

FIGURE 7 shows the effects of edoxaban on a morphometric parameter (media area) in ApoE KO mice. Each value represents mean $\pm$ S.E. of n=10 in vehicle group and in edoxaban group treated before injury, or n=9 in edoxaban group treated after injury. Vehicle: 0.5% methylcellulose 400 (10 mL/kg, *p.o.*). ** p <0.01 vs. vehicle group (Student's t-test).

FIGURE 8 shows the effects of edoxaban on a morphometric parameter (I/M ratio) in ApoE KO mice. Each value represents mean $\pm$ S.E. of n=10 in vehicle group and in edoxaban group treated before injury, or n=9 in edoxaban group treated after

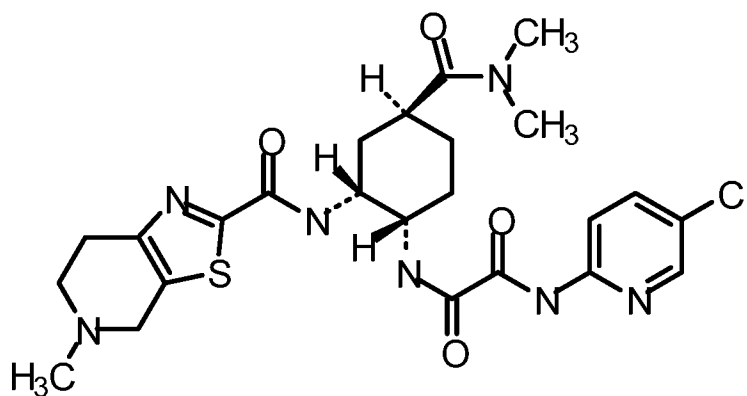
injury. Vehicle: 0.5% methylcellulose 400 (10 mL/kg, *p.o.*). ** $p < 0.01$ vs. vehicle group (Student's t-test).

FIGURE 9 is an illustration of a representative drug-eluting device coated with an edoxaban composition of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The term "edoxaban" used herein means N-(5-chloropyridin-2-yl)-N'-[(1S,2R,4S)-4-(dimethylcarbamoyl)-2-(5-methyl-4,5,6,7-tetrahydro[1,3]thiazolo[5,4-c]pyridine-2-carboxamido)cyclohexyl]oxamide represented by the following formula:

[Formula 1]

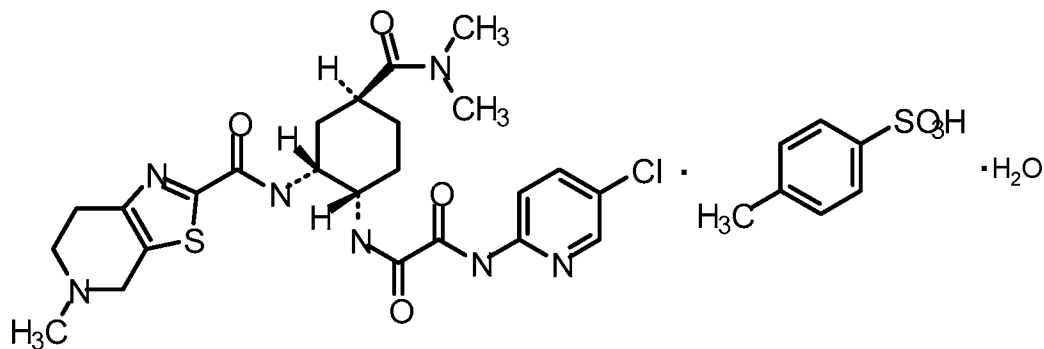


unless otherwise specified.

Examples of the salt of edoxaban include hydrochloride, sulfate, hydrobromide, citrate, hydroiodide, phosphate, nitrate, benzoate, methanesulfonate, benzenesulfonate, 2-hydroxyethanesulfonate, tosylate, acetate, propanoate, oxalate, malonate, succinate, glutarate, adipate, tartrate, maleate, fumarate, malate, and mandelate. The salt of edoxaban is preferably hydrochloride, tartrate, or tosylate, more preferably tosylate.

Edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt is preferably edoxaban tosylate hydrate represented by the following formula:

[Formula 2]



Edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt can be produced according to the description of U.S. Patent No. 7,365,205.

The pharmaceutical composition of the present invention can be administered systemically or locally through an oral or parenteral route to a mammal (e.g., a human, a horse, cattle, or a pig, preferably a human).

Examples of the form of the pharmaceutical composition for an oral route include tablets, powders, granules, capsules, suspensions, emulsions, syrups, and elixirs. The pharmaceutical composition in such a form can be produced according to a routine method using an additive optionally selected from excipients, binders, disintegrants, lubricants, swelling agents, swelling aids, coating agents, plasticizers, stabilizers, antiseptics, antioxidants, colorants, solubilizers, suspending agents, emulsifiers, sweeteners, preservatives, buffers, diluents, wetting agents, and the like usually used.

Examples of the form of the pharmaceutical composition for a parenteral route include injections, ointments, gels, creams, poultices, patches, aerosols, inhalants, sprays, eye drops, nasal drops, suppositories, and drug-eluting devices carrying edoxaban supported on the surface of a stent or a balloon catheter made of a metal or polymer material. The pharmaceutical composition in such a form can be produced according to a routine method using an additive optionally selected from stabilizers, antiseptics, solubilizers, humectants, preservatives, antioxidants, flavoring agents, gelling agents, neutralizing agents, buffers, tonicity agents, surfactants, colorants, buffering agents, thickeners, wetting agents, fillers, absorption promoters, suspending agents, binders, and the like usually used.

Representative drug-eluting devices carrying edoxaban supported on at least a portion of their surfaces include stents or balloon catheters. FIGURE 9 is an illustration of a portion of a representative drug-eluting device of the invention coated with edoxaban or an edoxaban composition of the invention. Referring to FIGURE 9, representative device 100 (portion) has a surface 110 coated with edoxaban or an edoxaban composition 120.

The dose of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt contained in the pharmaceutical composition of the present invention differs depending on symptoms, age, body weight, etc. The dose for oral administration is 1 to 200 mg, preferably 5 to 100 mg, more preferably 15 to 60 mg, in terms of edoxaban per dose in one adult, which is administered once to several times a day.

The pharmaceutical composition thus obtained is a novel pharmaceutical composition that can inhibit neointima formation of a blood vessel. Specifically, the present invention can provide a pharmaceutical composition that is useful in the prevention of artery occlusion or reocclusion after percutaneous transluminal angioplasty for acute coronary syndrome, cerebral infarction, peripheral arterial disease, etc. Particularly, the pharmaceutical composition of the present invention is useful in the prevention of reocclusion after endovascular treatment such as percutaneous transluminal angioplasty.

Next, the present invention will be described in detail with reference to Examples. However, the present invention is not intended to be limited by them by any means.

[Examples]

(Test Example) Effects of oral anticoagulant edoxaban on neointima formation of the carotid artery caused by ferric chloride injury in ApoE KO mice

This study was aimed at examining the effects of edoxaban on neointima formation caused by ferric chloride injury of the carotid artery in ApoE KO mice followed by fed with high-cholesterol diets.

Summary of test results

On the day of ferric chloride injury (Day 1), edoxaban at 15 mg/kg was orally administered to the mice 1 hour before ferric chloride injury (edoxaban group treated before injury) and 1 hour after ferric chloride injury (edoxaban group treated after injury). A vehicle was orally administered to a vehicle group 1 hour before ferric chloride injury.

Since after the ferric chloride injury, all of the animals were fed with high-cholesterol diets. On Day 2 to Day 42, the vehicle was orally administered b.i.d. to the vehicle group, while edoxaban at 10 mg/kg was orally administered b.i.d. to the edoxaban group treated before injury and the edoxaban group treated after injury. On the day following Day 42, the whole blood was collected, followed by the histopathological observation of the right carotid artery and image analysis. The time to arrest blood flow for at least 1 minute after initiation of the ferric chloride injury (time to occlusion, TTO) was significantly prolonged in the edoxaban group treated before injury compared to the vehicle group and the edoxaban group treated after injury. The patency rate for 30 minutes after the ferric chloride injury was significantly increased in the edoxaban group treated before injury compared to the vehicle group and the edoxaban group treated after injury.

Cholesterin crystals, foam cells, tissue degeneration, and calcification were observed in the thickened intima and media in all of the groups. Severe stenosis was found in the vehicle group and the edoxaban group treated after injury, whereas the incidence of severe stenosis was decreased and the intima area and the intima/media area ratio (I/M) were significantly decreased in the edoxaban group treated before injury.

As seen from these results, neointima formation caused by ferric chloride injury in the carotid arteries and fed with high-cholesterol diets in ApoE KO mice was inhibited by the administration of edoxaban before the ferric chloride injury and continuous administration thereof during the loading with high-cholesterol diets. When thrombus was formed without the administration of edoxaban before ferric chloride injury, neointima formation was not significantly inhibited even by the continuous administration of edoxaban during the loading with high-cholesterol diets.

(Preparation of dosing solution)

An electronic balance (Mettler Analytical Balance, AG245, Mettler-Toledo International Inc.) was used for weighing a test compound. For doses in terms of a free form, a necessary amount appropriate for each dose was calculated according to the following expression:

Weight (mg) in terms of a free form = Weighed value (mg) in terms of a salt $\times$ Conversion factor for the free form

Preparation method

Edoxaban (15 mg/kg)

6.10 mg (4.5 mg in terms of a free form) of edoxaban tosylate hydrate was weighed, suspended in a vehicle using a mortar, and then adjusted to 3 mL (1.5 mg/mL suspension). The vehicle used was a 0.5 w/v% methylcellulose 400 solution (Wako Pure Chemical Industries, Ltd., Lot No. ECN7001). The dosing solution was used within 5.1 hours after the preparation.

Edoxaban (10 mg/kg)

242.45 mg (180 mg in terms of a free form), 269.41 mg (200 mg in terms of a free form), and 134.70 mg (100 mg in terms of a free form) of edoxaban tosylate hydrate were weighed, each suspended in a vehicle using a mortar, and then adjusted to 180 mL, 200 mL, and 100 mL, respectively (1 mg/mL suspensions). After the preparation, these dosing solutions were stored in a test compound storeroom (cold place, tolerance: 1.5 to 8.4°C, actually measured value: 2.3 to 7.5°C) and used within 14 days after the preparation. The dosing solutions, if allowed to reach room temperature, were used within 4.2 hours.

Ferric chloride (FeCl₃ (Sigma-Aldrich Corp.), 10%)

On the day of use, 92.70 mg and 95.20 mg of FeCl₃ were weighed using the electronic balance and each dissolved in injectable water to have a concentration of 10% according to the weighed weight.

(Testing system)

Animal used

Mice (B6.129P2-Apoe^{tm1Unc}/J, SPF male, 8 weeks old at the time of receipt, Charles River Laboratories Japan, Inc.)

Quarantine and acclimatization

The health condition of each animal at the time of receipt was macroscopically observed, and animals found to have no abnormality were housed in breeding cages. On the day of receipt and the end date of the quarantine period, their body weights were measured using an electronic balance (UW4200S, Shimadzu Corp.). The amount of increase in the body weights of all of the animals fell within mean $\pm$ 3S.D. During the quarantine and acclimatization period, the general conditions of the animals were observed once a day.

Feed

Solid feed MF

Distributor: Oriental Yeast Co., Ltd.

Feeding method: Discretionary intake

High-cholesterol diet (HCC)

Distributor: Oriental Yeast Co., Ltd.

Feeding method: Discretionary intake

The time of start of feeding: Changed from MF after ferric chloride injury.

[Table 1]

Name of raw material	Mixing ratio (%)
Casein	18.50
Methionine	0.30
Sucrose	31.646
Corn Starch	14.9998
Unsalted Butter	24.50
Cholesterol	0.15
Cellulose	5.00
Mineral Mix, AIN-76	3.50
Calcium Carbonate	0.40
Vitamin Mix	1.00
tert-Butylhydroquinone	0.0042

(Test group)

Vehicle group and edoxaban groups treated before ferric chloride injury and after ferric chloride injury (n = 10).

[Table 2]

Test group	Test substance (<i>p.o.</i>)
Vehicle	Vehicle (on the day of model preparation and 1 hour before injury) + vehicle b.i.d. × 41 days
Administration before ferric chloride injury	Edoxaban 15 mg/kg (on the day of model preparation and 1 hour before injury) + edoxaban 10 mg/kg b.i.d. × 41 days
Administration after ferric chloride injury	Edoxaban 15 mg/kg (on the day of model preparation and 1 hour after injury) + edoxaban 10 mg/kg b.i.d. × 41 days

Grouping

No marked abnormality in general conditions was found before grouping, and all of the animals were grouped as subjects. At the completion of the quarantine period, the body weights of the animals were measured. These animals were grouped into the vehicle group (10 animals), edoxaban group treated before ferric chloride injury (10 animals), and edoxaban group treated after ferric chloride injury (10 animals) according to the stratified random allocation method with the measured body weights as an index using SAS9.3 for Microsoft Windows Workstation 32-bit (SAS Institute Inc.) and its cooperative system EXSUS Ver. 8.0 (CAC EXICARE Corp.).

[Table 3]

Test group	Order of ferric chloride injury					
	1st course			2nd course		
Vehicle	1 st	12 th	13 th		24 th	25 th
Administration before ferric chloride injury	2 nd	11 th	14 th		23 rd	26 th
Administration after ferric chloride injury	3 rd	10 th	15 th		22 nd	27 th
Administration after ferric chloride injury	4 th	9 th		16 th	21 st	28 th

Test group	Order of ferric chloride injury					
	1st course			2nd course		
Administration before ferric chloride injury	5 th	8 th		17 th	20 th	29 th
Vehicle	6 th	7 th		18 th	19 th	30 th

(Testing method)

Administration

Administration route: Oral

Dosing volume: 10 mL/kg

Administration method: Administered using a disposable syringe and an oral probe for mice

The number of doses: Only on Day 1, edoxaban at 15 mg/kg was administered once. On Day 2 to Day 42, edoxaban at 10 mg/kg was administered b.i.d. (first dose: 8:05 to 10:38, second dose: 15:02 to 17:18). The vehicle at 10 mL/kg was administered to the vehicle group.

Preparation of ferric chloride injury model

Vehicle group and edoxaban group treated before ferric chloride injury

Approximately 0.5 hours after the vehicle or test substance (15 mg/kg) administration on the day of ferric chloride injury model preparation, isoflurane (1.5%) anesthesia was started. The right carotid artery of each mouse was decorticated, and a probe for blood flow measurement (MC0.5PSB, Transonic Systems Inc.) was attached to the exposed carotid artery. The blood flow was monitored using an ultrasonic blood flow meter module (TS420, Transonic Systems Inc.). Approximately 1 hour after the vehicle or test substance administration, a filter paper (Qualitative Filter Paper No. 2, Advantec Toyo Kaisha, Ltd.) cut into 1 × 2 mm impregnated with a 10% ferric chloride solution was attached to the carotid artery surface between the probe and the heart. A stopwatch was started after the attachment of the filter paper to manage the measurement time. After 3 minutes, the filter paper was removed, and the time to arrest blood flow for at least 1 minute after initiation of the ferric chloride injury (time to occlusion, TTO, unit: sec) was determined. The arrest of blood flow was identified by the stop of pulsation in an analog meter after decrease of the digital representation of the blood flow

meter to near 0 mL/min. The time read from the stopwatch at the point in time of arrest of blood flow or repatency and described in a chart sheet was used as raw data, and the chart sheet was used as reference data. If no arrest of blood flow longer than 1 minute was observed within 30 minutes after the attachment of the filter paper, TTO for this individual was defined as 30 minutes and the measurement was terminated. The patency rate was determined by subtracting the ratio of the total time of blood flow arrest to the blood flow measurement time of 30 minutes from 100%. After the completion of the blood flow measurement, the incised skin was sutured, and the animals were continuously raised after recovery from anesthesia.

Edoxaban group treated after ferric chloride injury

Before the test substance administration, TTO and patency rate were determined in the same way as in the vehicle group and the edoxaban group treated before ferric chloride injury. After the completion of the blood flow measurement, the incised skin was sutured. 60 minutes after the attachment of the filter paper, the test substance (15 mg/kg) was administered to the animals, which were then continuously raised.

HCC loading

Since after the recovery from anesthesia on the day of model preparation, the animals were fed with HCC.

Blood collection

On the day following Day 42, approximately 0.6 mL of blood was collected from the abdominal vena cava using a disposable syringe and a disposable intravenous injection needle under isoflurane anesthesia and subjected to anticoagulation treatment with EDTA-2Na. Then, the blood was centrifuged ($2000 \times g$, 10 min, 4°C) to obtain plasma. The plasma was dispensed into 3 tubes (80 μL , 120 μL , and the whole remaining portion), then immediately frozen and cryopreserved (ultralow-temperature freezer CLN-35C, set temperature: -80°C , tolerance: -64.5°C or lower, actually measured value: -76.2 to -73.2°C) until delivery.

Histopathological evaluation

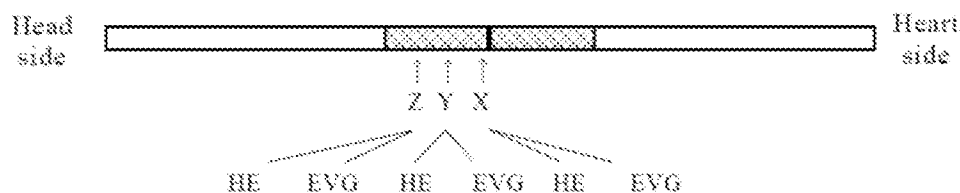
Anatomy

After the completion of the blood collection, the animals were sacrificed by bloodletting through an incision made in the abdominal aorta and vena under perfusion with physiological saline from the left ventricle. Then, the animals were perfusion-fixed in 10% neutral buffered formalin for approximately 5 to 10 minutes. The right carotid

artery including the site injured with ferric chloride was collected from each animal and fixed in 10% neutral buffered formalin.

Histopathological sample preparation

Two slices each, a total of 6 slices, were prepared from a total of 3 positions, i.e., almost the center (X) at the injured site of the carotid artery, and positions 100 μ m (Y) and 200 μ m (Z) from the center toward the head side, and the slices from one position were subjected to two types of staining (hematoxylin-eosin staining and Elastica van Gieson staining).



(Histopathological observation)

As for the 3 hematoxylin-eosin (HE)-stained samples (X, Y, and Z), the tunica intima, the tunica media, and the tunica externa of the wall of the carotid artery, and the lumen of the carotid artery were observed under a microscope to confirm findings such as neointima formation, luminal stenosis, thrombus formation, necrosis of vascular walls, and inflammatory cell infiltration, and the degree of change in each finding. If change was seen in even only one of these events, this change was evaluated as a finding for the individual.

(Image analysis)

As for the 3 Elastica van Gieson (EVG)-stained samples (X, Y, and Z), a vascular lumen area (A), an area surrounded by the internal elastic lamina (cross-sectional area of the blood vessel, B), and an area surrounded by the external elastic lamina (cross-sectional area of the blood vessel, C) were measured using an image analysis apparatus (Win ROOF 2013, Mitani Corp.). The tunica intima was observed in all of the samples, and an intima area (B - A) and a media area (C - B), and an intima/media area ratio (I/M) were determined.

(Test results)

Test results for TTO and patency rate are shown in Table 4, which summarizes the time to arrest blood flow for at least 1 minute after initiation of ferric chloride injury

(time to occlusion, TTO) and the patency rate in a mouse model of with neointima formation of the carotid artery.

Table 4. Effects of edoxaban on thrombus formation in ApoE KO mice.

Group	Compound	n	TTO (second)	Patency rate (%)
Vehicle ^{a)}	Vehicle ^{a)} (b.i.d.) ×42 days	10	666 ± 33	37.2 ± 1.9
Edoxaban 15 mg/kg before injury	Edoxaban 10 mg/kg (b.i.d.) ×42 days	10	1800 ± 0 (<0.0001) ^{b)} (<0.0001) ^{c)}	100.0 ± 0.0 (<0.0001) ^{b)} (<0.0001) ^{c)}
Edoxaban 15 mg/kg after injury	Edoxaban 10 mg/kg (b.i.d.) ×42 days	10	697 ± 25 (0.4525) ^{b)}	39.6 ± 1.8 (0.3852) ^{b)}

a): 0.5% methylcellulose 400 (10 mL/kg, *p.o.*)
b): *p*-value vs. vehicle group (Student's *t*-test)
c): *p*-value vs. edoxaban treated group after injury (Student's *t*-test)
Each value represents mean ± S.E.

TTO

TTO of each group is shown in Table 4.

TTO was 666 ± 33 seconds for the vehicle group, 1800 ± 0 seconds for the group treated by the oral administration of edoxaban at 15 mg/kg 1 hour before ferric chloride injury, and 697 ± 25 seconds for the group treated by the oral administration of edoxaban at 15 mg/kg 1 hour after ferric chloride injury. See FIGURE 1. TTO was significantly prolonged in the edoxaban group treated before ferric chloride injury compared to the vehicle group ($p < 0.0001$) and the edoxaban group treated after ferric chloride injury ($p < 0.0001$). No significant difference was confirmed between the vehicle group and the edoxaban group treated after ferric chloride injury.

Patency rate

FIGURES 2A-2C show changes in the status of vascular closure and patency caused by ferric chloride injury. FIGURE 3 and Table 4 show the patency rate of each group.

The patency rate was $37.2 \pm 1.9\%$ for the vehicle group, $100.0 \pm 0.0\%$ for the edoxaban 15 mg/kg group treated before ferric chloride injury, and $39.6 \pm 1.8\%$ for the edoxaban 15 mg/kg group treated after ferric chloride injury. The patency rate was significantly increased in the edoxaban group treated before ferric chloride injury compared to the vehicle group ($p < 0.0001$) and the edoxaban group treated after ferric chloride injury ($p < 0.0001$). No significant difference was confirmed between the vehicle group and the edoxaban group treated after ferric chloride injury.

Histopathological observation

FIGURES 4A-4C show images of the HE-stained sections of the carotid artery after administration of the vehicle or edoxaban for 42 days. Table 5 summarizes histopathological findings of sections of the carotid artery after administration of a vehicle or edoxaban to a mouse model (ApoE KO) of neointima formation of the carotid artery for 42 days.

Table 5. Histopathological findings in ApoE KO mice.

Findings	Group					Edoxaban 15 mg/kg before injury					Edoxaban 15 mg/kg after injury				
	Vehicle ^{a)}					Edoxaban 10 mg/kg (b.i.d.) × 42 days					Edoxaban 10 mg/kg (b.i.d.) × 42 days				
	Vehicle ^{a)} (b.i.d.) × 42 days					Edoxaban 10 mg/kg (b.i.d.) × 42 days					Edoxaban 10 mg/kg (b.i.d.) × 42 days				
	10					10					9				
Grade	-	P	+	++	+++	-	P	+	++	+++	-	P	+	++	+++
Stenosis	0	•	1	5	4	9	•	1	0	0	3	•	1	2	3
Thrombus formation	9	1	•	•	•	10	0	•	•	•	9	0	•	•	•
Thickening of tunica intima	0	•	0	1	9	0	•	9	1	0	0	•	3	1	5
Thickening of tunica media	4	•	0	6	0	0	•	0	8	2	0	•	1	4	4
Inflammatory cell infiltration in perivascular tissue	0	•	8	2	0	0	•	10	0	0	0	•	6	3	0

a): 0.5% methylcellulose 400 (10 mL/kg, *p.o.*)

P: present, -: normal, +: mild, ++: moderate, +++: severe

Cholesterol crystals, foam cells, degeneration and ossification were observed in the thickened intima and media.

Severe stenosis was found in 4/10 of the animals in the vehicle group and 3/9 of mild stenosis was found in only 1/10 of the animals in the edoxaban 15 mg/kg group treated before ferric chloride injury and no stenosis was found in the remaining 9/10. Thrombus formation was found in 1/10 of the animals in the vehicle group. Neointima formation was found as a moderate case in 1/10 of the animals and as severe cases in 9/10 of the animals in the vehicle group, as mild cases in 9/10 of the animals and as moderate cases in 1/10 of the animals in the edoxaban 15 mg/kg group treated before ferric chloride injury, and as mild cases in 3/9 of the animals, as moderate cases in 1/9 of the animals, and as severe cases in 5/9 of the animals in the edoxaban 15 mg/kg group treated after ferric chloride injury. Thickening of the tunica media and inflammatory cell infiltration in perivascular tissues were found in all of the groups. Cholesterol crystals, foam cells, tissue degeneration, and calcification were observed in the thickened intima and media.

Image analysis

FIGURES 5A-5C show images of the EVG-stained sections of the carotid artery after administration of the vehicle or edoxaban for 42 days following ferric chloride injury. The image analysis values are summarized in Table 6. Table 6 shows the effects of edoxaban on morphometric parameters of ApoE KO mice after FeCl₃-injury on carotid arteries and summarizes the image analysis values of the sections of the carotid artery after administration of a vehicle or edoxaban to a mouse model (ApoE KO) of neointima formation of the carotid artery for 42 days following ferric chloride injury.

Table 6. Effects of edoxaban on morphometric parameters of ApoE KO mice after FeCl₃-injury on carotid arteries.

Group	Compound	n	Intima area (μm^2)	Media area (μm^2)	Thrombus (μm^2)	I/M
Vehicle ^{a)}	Vehicle ^{a)} (b.i.d.) ×42 days	10	198806 ± 18609	63314 ± 10161	7579 ± 7579	4.99 ± 1.34
Edoxaban 15 mg/kg before injury	Edoxaban 10 mg/kg (b.i.d.) ×42 days	10	57357 ± 7979 (<0.0001) ^{b)}	122110 ± 12516 (0.0018) ^{b)}	0 ± 0 (0.3306) ^{b)}	0.47 ± 0.05 (0.0035) ^{b)}
Edoxaban 15 mg/kg after injury	Edoxaban 10 mg/kg (b.i.d.) ×42 days	9	151230 ± 30756 (0.1932) ^{b)} (0.0065) ^{c)}	95262 ± 13809 (0.0760) ^{b)} (0.1668) ^{c)}	0 ± 0 (0.3574) ^{b)} (-) ^{c)}	2.51 ± 1.13 (0.1804) ^{b)} (0.0730) ^{c)}

a): 0.5% methylcellulose 400 (10 mL/kg, p.o.)

b): p-value vs. vehicle group (Student's t-test)

c): p-value vs. edoxaban group treated after injury (Student's t-test, -: all values of two groups are the same.)

Each value represents mean ± S.E

Intima area

The intima area was $198806 \pm 18609 \mu\text{m}^2$ for the vehicle group, $57357 \pm 7979 \mu\text{m}^2$ for the edoxaban 15 mg/kg group treated before ferric chloride injury, and $151230 \pm 30756 \mu\text{m}^2$ for the edoxaban 15 mg/kg group treated after ferric chloride injury. See FIGURE 6. The intima area was significantly decreased in the edoxaban group treated before ferric chloride injury compared to the vehicle group ($p < 0.0001$) and the edoxaban group treated after ferric chloride injury ($p = 0.0065$). No significant difference was confirmed between the vehicle group and the edoxaban group treated after ferric chloride injury.

Media area

The media area was $63314 \pm 10161 \mu\text{m}^2$ for the vehicle group, $122110 \pm 12516 \mu\text{m}^2$ for the edoxaban 15 mg/kg group treated before ferric chloride injury, and $95262 \pm 13809 \mu\text{m}^2$ for the edoxaban 15 mg/kg group treated after ferric chloride injury. See FIGURE 7. The media area was significantly increased in the edoxaban group treated before ferric chloride injury compared to the vehicle group ($p = 0.0018$). No significant difference was confirmed between the vehicle group and the edoxaban group treated after ferric chloride injury and between the edoxaban group treated before ferric chloride injury and the edoxaban group treated after ferric chloride injury.

Intima/media area ratio (I/M)

I/M was 4.99 ± 1.34 for the vehicle group, 0.47 ± 0.05 for the edoxaban 15 mg/kg group treated before ferric chloride injury, and 2.51 ± 1.13 for the edoxaban 15 mg/kg group treated after ferric chloride injury. See FIGURE 8. I/M was significantly decreased in the edoxaban group treated before ferric chloride injury compared to the vehicle group ($p = 0.0035$). No significant difference was confirmed between the vehicle group and the edoxaban group treated after ferric chloride injury and between the edoxaban group treated before ferric chloride injury and the edoxaban group treated after ferric chloride injury.

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A pharmaceutical composition for inhibiting neointima formation of a blood vessel, comprising edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt.

2. A pharmaceutical composition for preventing artery occlusion or reocclusion after percutaneous transluminal angioplasty for acute coronary syndrome, cerebral infarction, or peripheral arterial disease, comprising edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt.

3. A pharmaceutical composition for preventing reocclusion after percutaneous transluminal angioplasty, comprising edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt.

4. A drug-eluting device for preventing reocclusion after percutaneous transluminal angioplasty, comprising edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt.

5. A method for inhibiting neointima formation of a blood vessel, comprising administering an effective amount of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt to a patient.

6. A method for preventing artery occlusion or reocclusion after percutaneous transluminal angioplasty for acute coronary syndrome, cerebral infarction, or peripheral arterial disease, comprising administering an effective amount of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt to a patient.

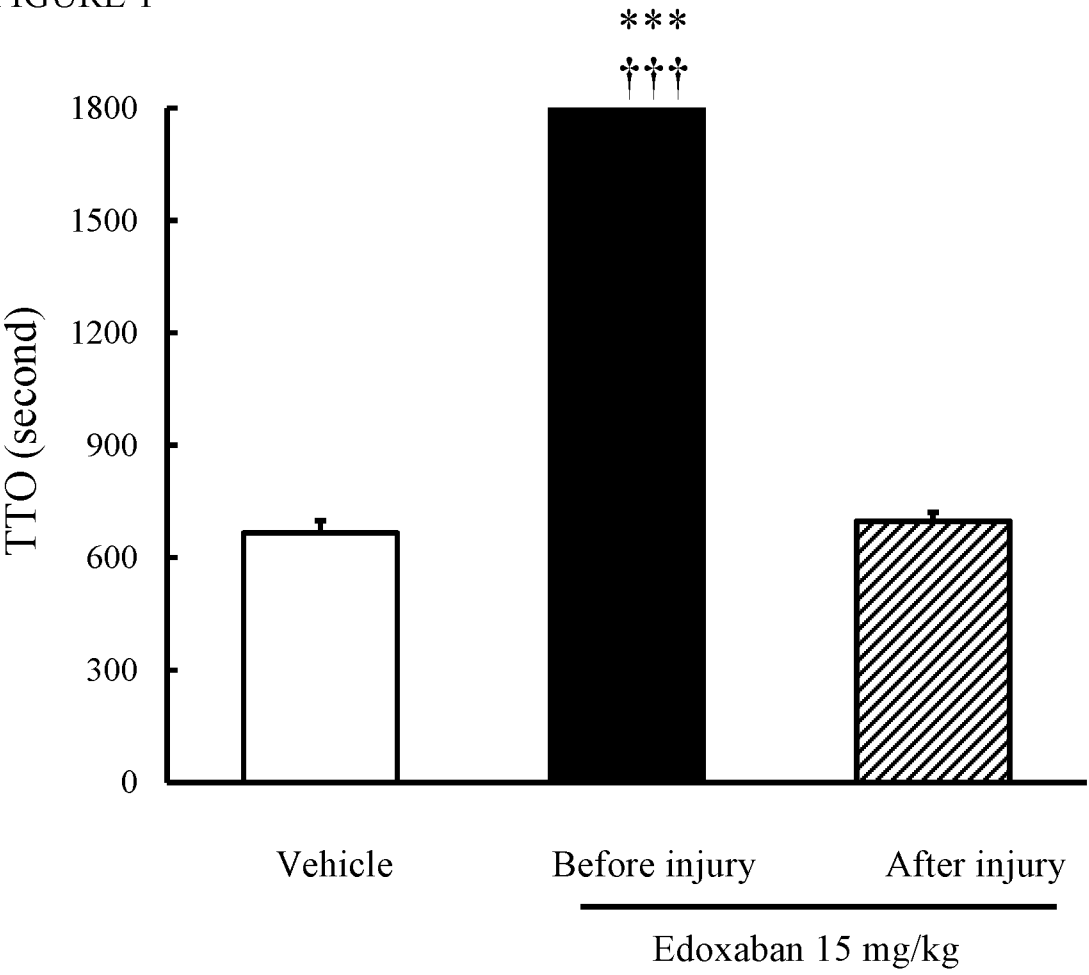
7. A method for preventing reocclusion after percutaneous transluminal angioplasty, comprising administering an effective amount of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt to a patient.

8. A method for preventing reocclusion after percutaneous transluminal angioplasty, comprising indwelling a drug-eluting device containing an effective amount of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt into a blood vessel of a patient.

9. Edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt for use in inhibiting neointima formation of a blood vessel.

10. Use of edoxaban or a pharmacologically acceptable salt thereof or a hydrate of the compound or the salt for inhibiting neointima formation of a blood vessel.

FIGURE 1



FIGURES 2A-2C

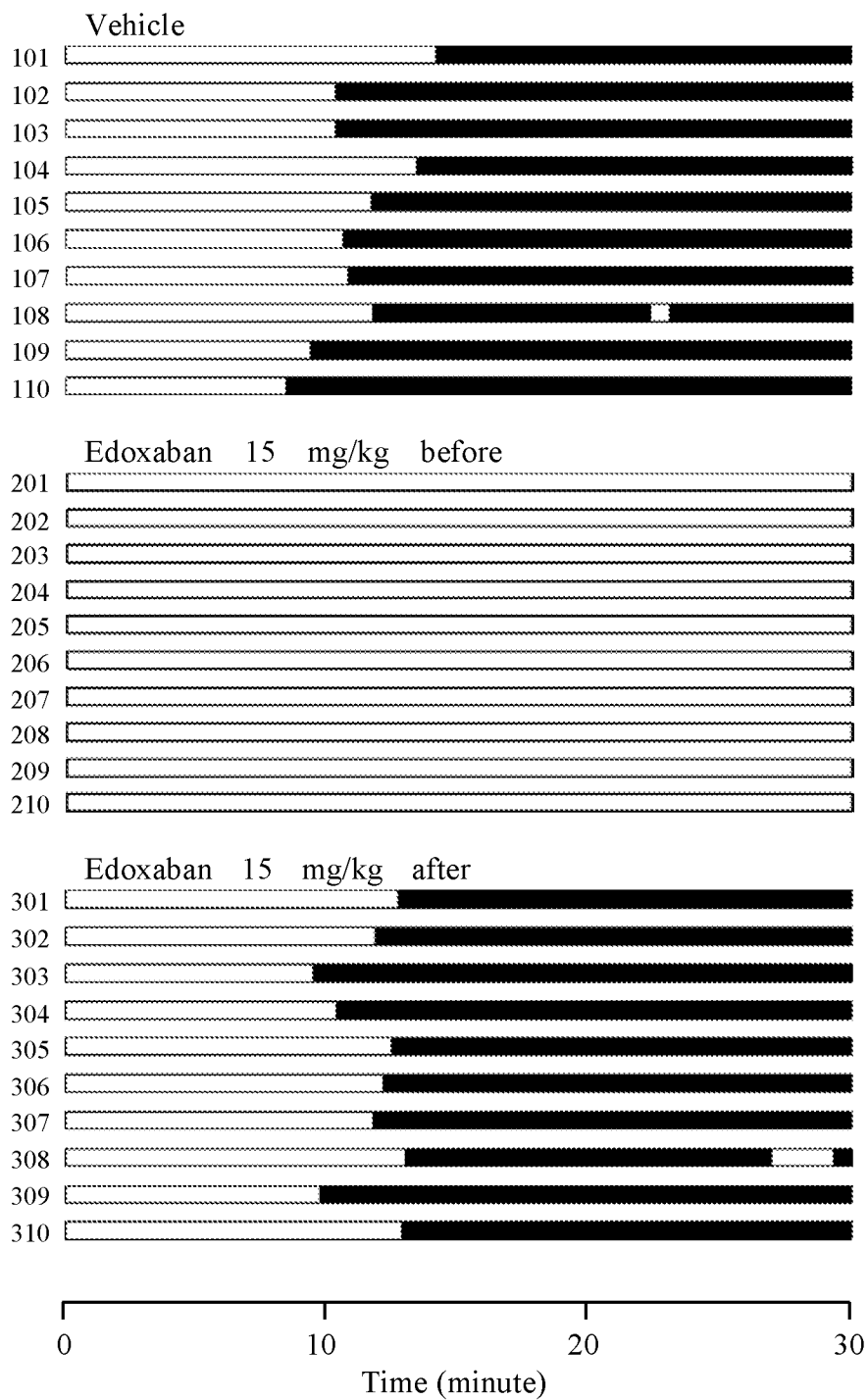
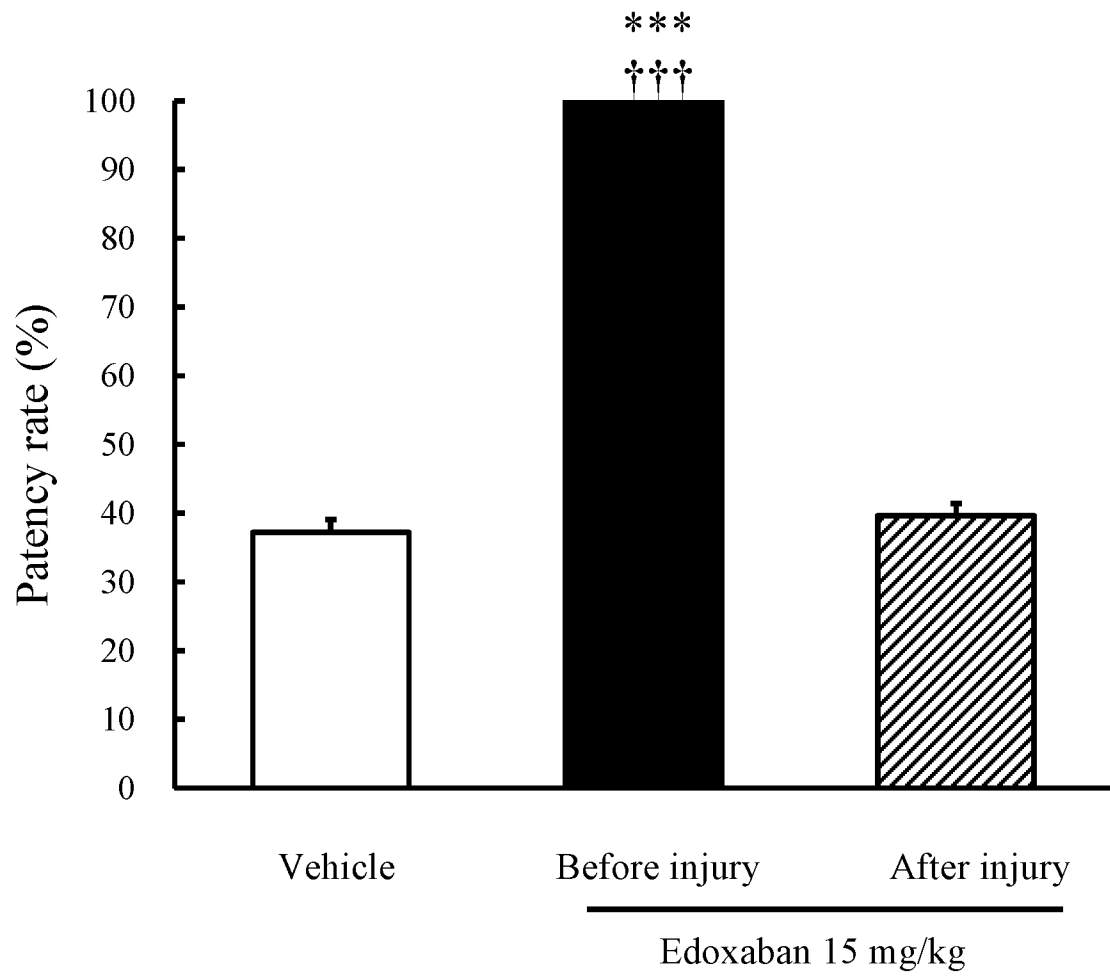
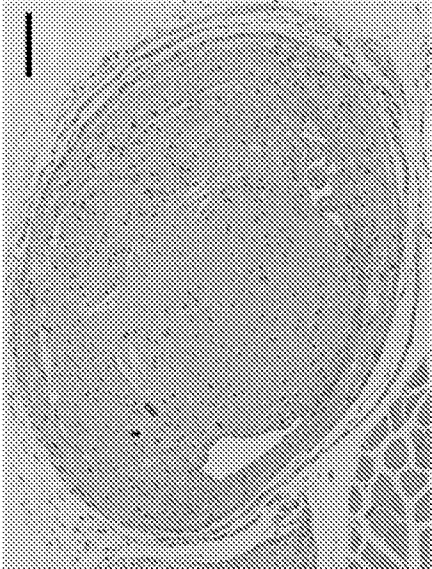


FIGURE 3

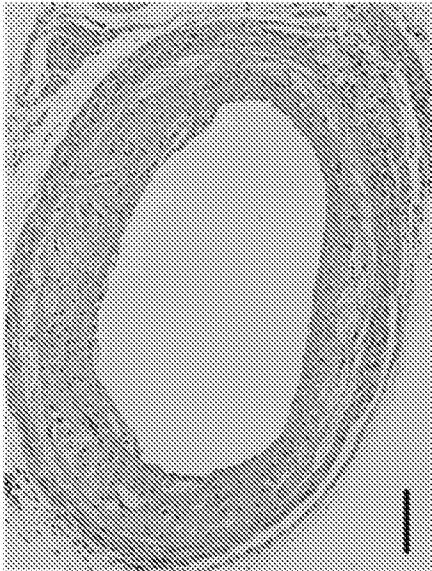


FIGURES 4A-4C

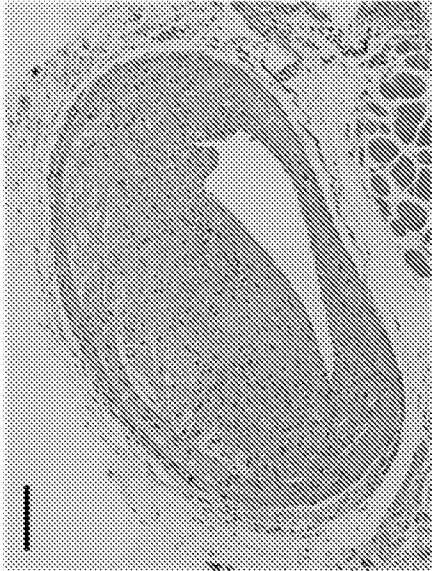
Vehicle



Before injury

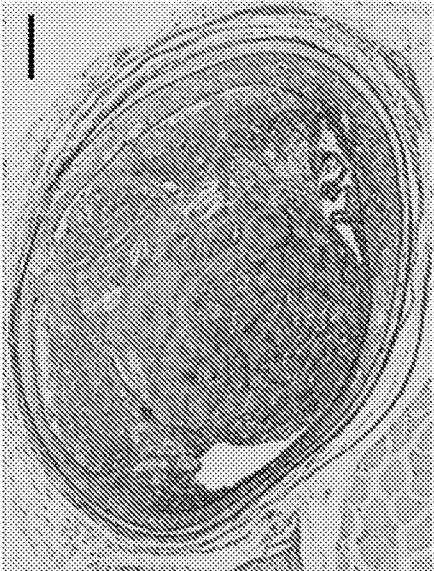


After injury



FIGURES 5A-5C

Vehicle



Before injury



After injury



FIGURE 6

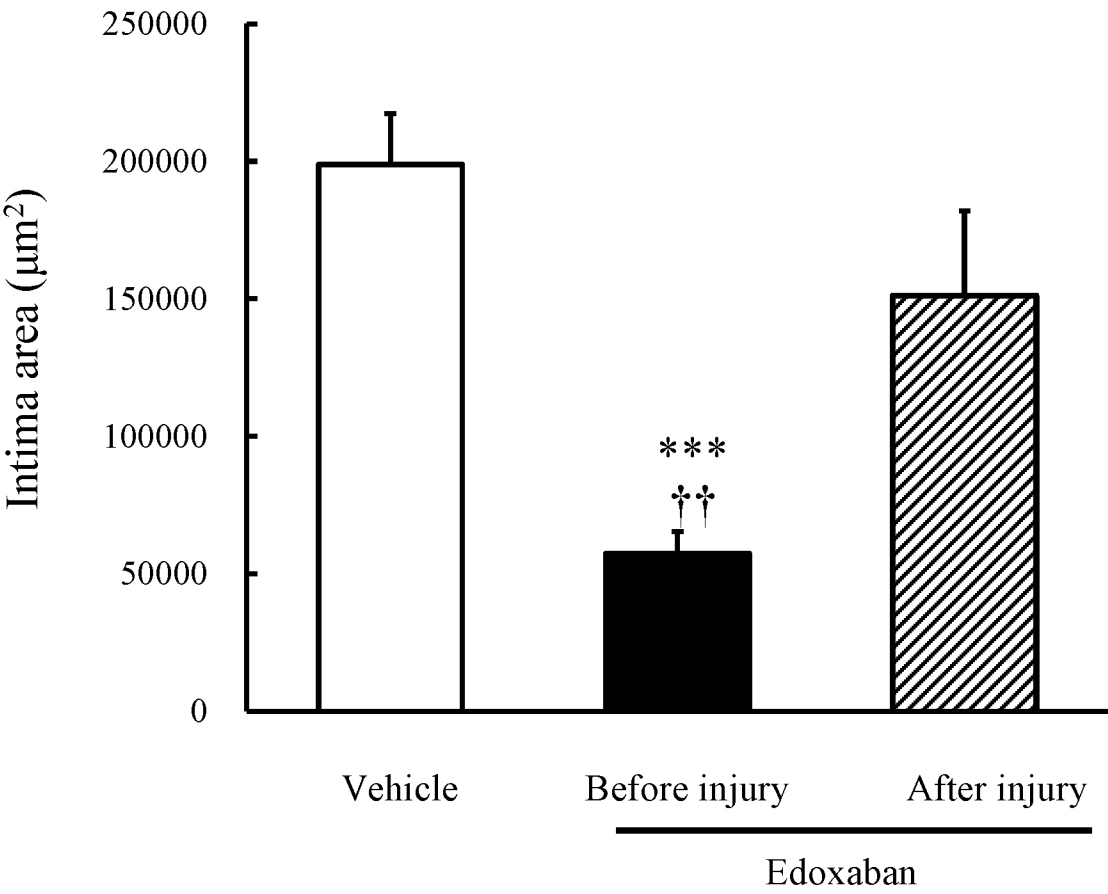


FIGURE 7

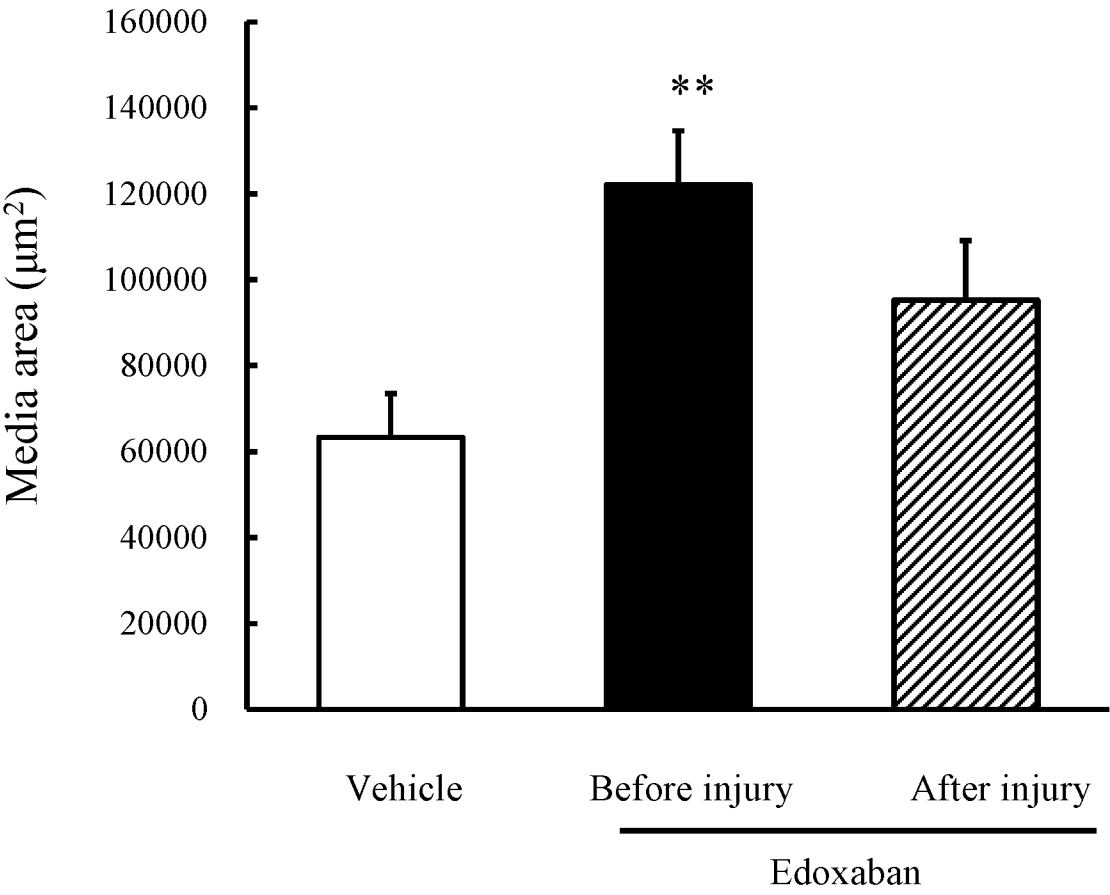
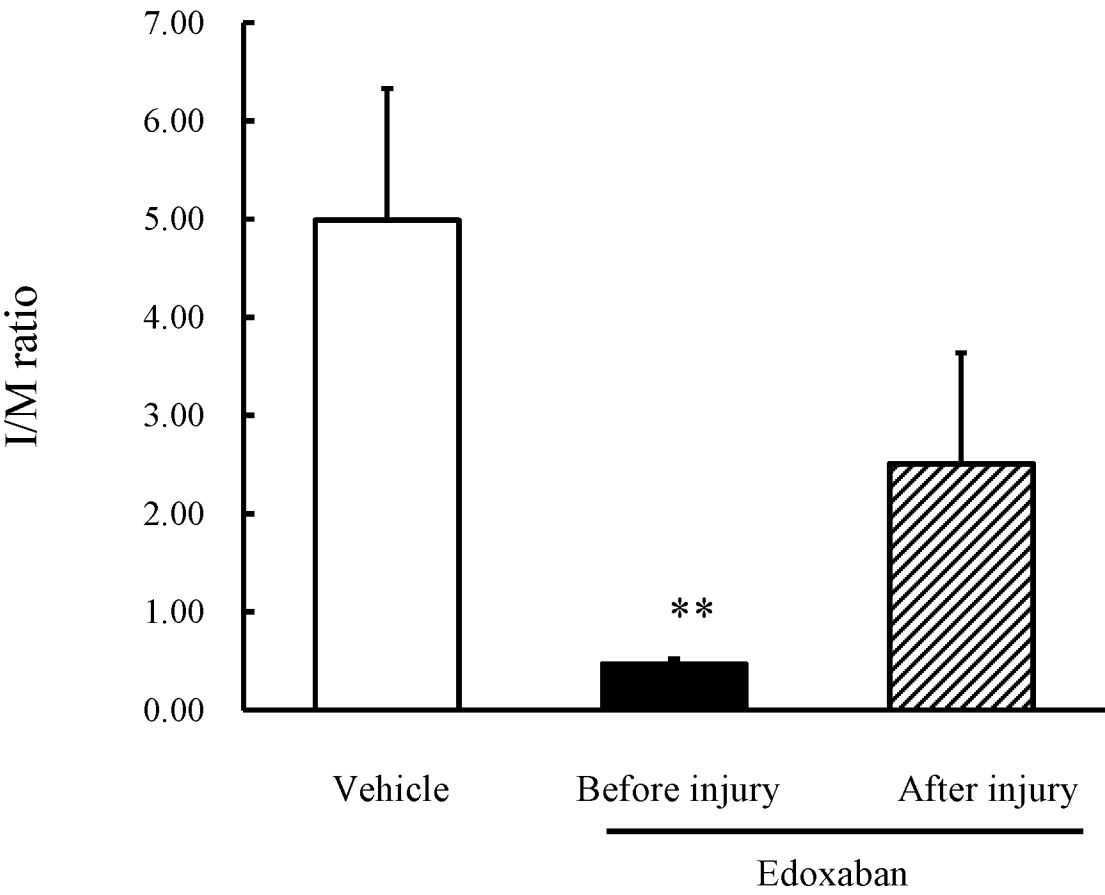


FIGURE 8



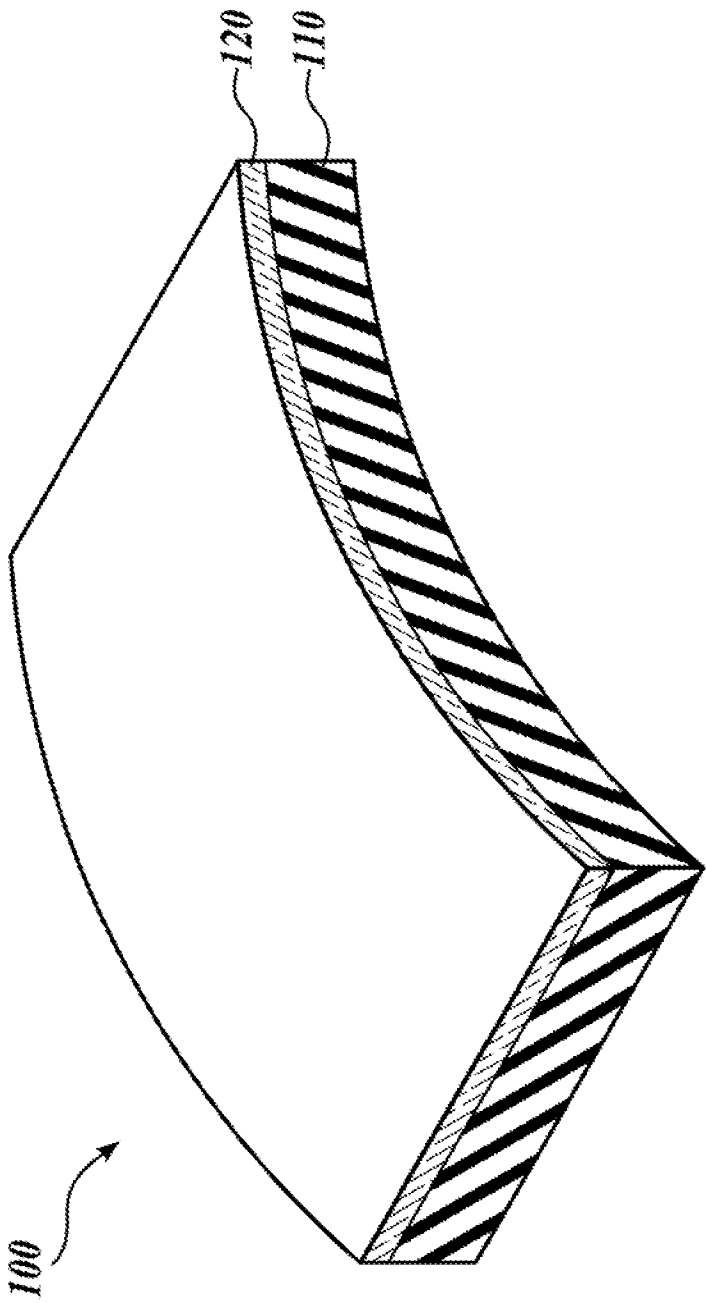


FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/001409

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/444 A61P9/14
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T. Hara et al.: "Abstract 13438: Rivaroxaban, a Direct Factor Xa Inhibitor, Attenuates Neointima Formation after Mechanical Vascular Injury Circulation", 25 November 2014 (2014-11-25), XP055446347, Retrieved from the Internet: URL:http://circ.ahajournals.org/content/13 0/Suppl_2/A13438 [retrieved on 2018-01-31] the whole document ----- -/--	1-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

5 February 2018

Date of mailing of the international search report

14/02/2018

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Büttner, Ulf

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/001409

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BLANDINE MAUREL ET AL: "In stent restenosis and thrombosis assessment after EP224283 injection in a rat model", ATHEROSCLEROSIS, vol. 229, no. 2, 1 August 2013 (2013-08-01), pages 462-468, XP055446349, AMSTERDAM, NL ISSN: 0021-9150, DOI: 10.1016/j.atherosclerosis.2013.06.009 table 1	1-10
X	----- BRIGITTE KAISER ET AL: "A Synthetic Inhibitor of Factor Xa, DX-9065a, Reduces Proliferation of Vascular Smooth Muscle Cells in Vivo in Rats", THROMBOSIS RESEARCH, vol. 98, no. 2, 1 April 2000 (2000-04-01), pages 175-185, XP055446365, US ISSN: 0049-3848, DOI: 10.1016/S0049-3848(99)00213-3 figure 3	1-10
X	----- WO 2016/110269 A1 (TSENG WEI-KUNG [CN]; HSU CHIA-CHUN [CN]; WU WAN-LIN [CN]) 14 July 2016 (2016-07-14) claims	4
X	----- Anonymous: "Edoxaban with aspirin may be a "paradigm shifting" treatment for peripheral artery disease patients treated with endovascular techniques - Vascular News", 16 February 2016 (2016-02-16), XP055446402, Retrieved from the Internet: URL:https://vascularnews.com/edoxaban-with-aspirin-may-be-a-paradigm-shifting-treatment-for-peripheral-artery-disease-patients-treated-with-endovascular-techniques/ [retrieved on 2018-01-31] the whole document -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2017/001409

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016110269 A1	14-07-2016	TW 201625238 A	16-07-2016
		US 2018000750 A1	04-01-2018
		WO 2016110269 A1	14-07-2016
