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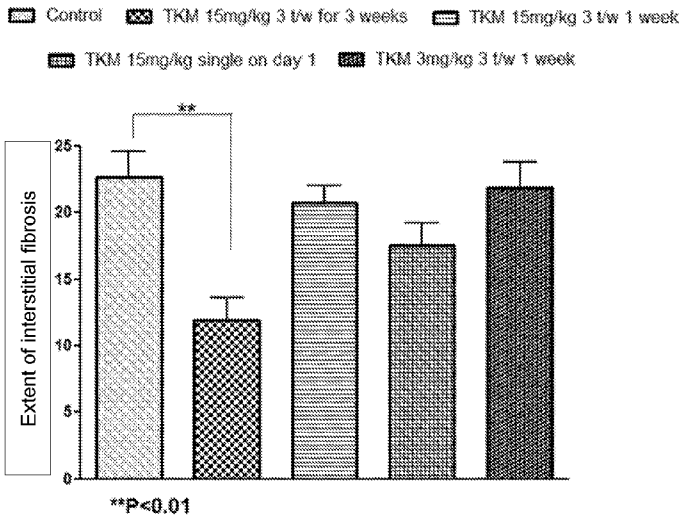

Fig. 1

(57) Abstract: The present embodiments relate to the use of dextran sulfate formulated for systemic administration for treatment, inhibition or prevention of cardiac fibrosis in a subject.

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NEW USE OF DEXTRAN SULFATE

TECHNICAL FIELD

The present embodiments generally relate to fibrosis treatment, and in particular to the use of dextran sulfate for treating, inhibiting or preventing cardiac fibrosis in a subject.

BACKGROUND

Fibrosis is a process involving formation of excess fibrous connective tissue in an organ or tissue in a reparative or reactive process. Fibrosis can be a reactive, benign or pathological state. In response to injury the fibrosis process is sometimes referred to scarring.

Physiologically fibrosis involves depositing connective tissue, which can obliterate the architecture and function of the underlying organ or tissue. Fibrosis is similar to the process of scarring in that both involve stimulated cells laying down connective tissue, including collagen and glycosaminoglycans. Macrophages and damaged tissue release transforming growth factor beta (TGFβ) in response to, for instance, inflammation or tissue damage. This in turn stimulates the proliferation and activation of fibroblasts, which deposit connective tissue.

US 5,605,938 discloses that biocompatible anionic polymers, including dextran sulfate with an average molecular weight of about 40,000 to 2,000,000 Da, can inhibit fibrosis, scar formation and surgical adhesions typically in connection with surgery. The anionic polymers are administered locally at the fibrotic lesions or can be soaked onto an organ or implant in the form of a viscous liquid or gel that preferably also comprises an adhesive protein containing dihydroxyphenylalanine (DOPA) and hydroxyl-containing amino acid residues.

CN 102973593 discloses the use of dextran sulfate in preparing a medicament for treating hepatic fibrosis. The document mentions that dextran sulfate inhibits the activation of astrocytes and promotes macrophages to secrete metalloproteinase.

SUMMARY

It is a general objective to treat, inhibit or reduce cardiac fibrosis in a subject.

This and other objectives are met by embodiments as disclosed herein.

An aspect of the embodiments relates to dextran sulfate, or a pharmaceutically acceptable salt thereof, formulated for systemic administration to a subject for use in treating, inhibiting or preventing cardiac fibrosis in the subject.

Another aspect of the embodiments relates to use of dextran sulfate, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament formulated for systemic administration for treatment, inhibition or prevention of cardiac fibrosis in a subject.

A further aspect of the embodiments relates to a method of treating, inhibiting or preventing cardiac fibrosis in a subject. The method comprises systemically administering dextran sulfate, or a pharmaceutically acceptable salt thereof, to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

The embodiments, together with further objects and advantages thereof, may best be understood by making reference to the following description taken together with the accompanying drawings, in which:

Fig. 1 illustrates estimation of the extent of interstitial fibrosis. Relative area of interstitial fibrosis showed significant suppression of myocardial infarction induced interstitial fibrosis in dextran sulfate group 2M compared to the control group 1M (**P<0.01 according to one-way ANOVA followed by Bonferroni post-hoc comparison).

Figs. 2A-2D are pictures of fibrosis grade in two animals from control group 1M and two animals from dextran sulfate group 2M.

DETAILED DESCRIPTION

The present embodiments generally relate to fibrosis treatment, and in particular to the use of dextran sulfate for treating, inhibiting or preventing cardiac fibrosis in a subject.

The embodiments are based on the discovery that systemically administered dextran sulfate, or a pharmaceutically acceptable salt thereof, is capable of reducing undesired fibrosis formation in a subject, and in particular reducing or inhibiting such fibrosis formation in ischemic tissue, in particular ischemic cardiac tissue.

Fibrotic diseases include a wide spectrum of medical conditions potentially affecting different organs and tissue in a subject's body. These medical conditions are characterized by elevated expression of genes encoding matrix proteins and the resulting fibrosis disrupts the normal architecture of the affected organ or tissue, ultimately leading to its dysfunction or failure.

The embodiments are capable or treating, inhibiting or preventing cardiac fibrosis in terms of treating, inhibiting or preventing adverse effects of fibrosis diseases by reducing the amount of formed fibrosis in the heart or cardiac tissue. Thus, dextran sulfate of the embodiments is capable of treating, inhibiting or preventing deleterious or injurious cardiac fibrosis.

Accordingly, an aspect of the embodiments relates to dextran sulfate, or a pharmaceutically acceptable salt thereof, formulated for systemic administration to a subject for use in treating, inhibiting or preventing cardiac fibrosis in the subject.

It was highly surprising that systemically delivered dextran sulfate could significantly reduce deleterious fibrosis formation in the light of U.S. Patent No. 5,605,938. This patent document discloses that dextran sulfate administered locally could inhibit fibrosis and scar formation in connection with, among others, implantation of various implants. It was speculated therein that the negative charges of the dextran sulfate polymer were involved in inhibiting invasions of various cells at the implantation site. In order for such negative charges to have the cell-invasion-inhibiting effect dextran sulfate needs to be administered locally at the desired site. Accordingly, the implant was advantageously coated with dextran sulfate in U.S. Patent No. 5,605,938.

The experimental data as presented herein shows that dextran sulfate of the embodiments can be systemically administered, i.e. not locally at the target site, and still exert its desired anti-fibrotic effect.

Dextran sulfate, or the pharmaceutically acceptable salt thereof, is formulated for systemic administration to the subject. In an embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, is formulated for parenteral administration as an example of systemic administration to achieve a systemic effect in the subject.

Examples of parenteral administration routes include intravenous (i.v.) administration, intra-arterial administration, intra-muscular administration, intracerebral administration, intracerebroventricular administration, intrathecal administration and subcutaneous (s.c.) administration.

In an embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, is preferably formulated for intravenous (i.v.) or subcutaneous (s.c.) administration to the subject. Accordingly, i.v. and s.c. administration are preferred examples of systemic administration of dextran sulfate, or the pharmaceutically acceptable salt thereof.

Orally delivered dextran sulfate is known to induce colitis and intestinal fibrosis in mice, rats, hamsters and guinea pigs. Accordingly, systemic administration as used herein preferably excludes oral administration of dextran sulfate, or the pharmaceutically acceptable salt thereof. In a particular embodiment, systemic administration of dextran sulfate, or the pharmaceutically acceptable salt thereof, is a systemic administration other than oral administration, preferably other than enteral administration.

In an embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, is formulated as an aqueous injection solution, preferably as an aqueous i.v. or s.c. injection solution. Thus, dextran sulfate, or the pharmaceutically acceptable salt thereof, of the embodiments is preferably formulated as an aqueous injection solution with a selected solvent or excipient. The solvent is advantageously an aqueous solvent and in particular a buffer solution. A non-limiting example of such a buffer solution is a citric acid buffer, such as citric acid monohydrate (CAM) buffer, or a phosphate buffer. For instance, dextran sulfate of the embodiments can be dissolved in saline, such as 0.9 % NaCl saline, and then optionally buffered with 75 mM CAM and adjusting the pH to about 5.9 using sodium hydroxide. Also non-buffered solutions are possible, including aqueous injection solutions, such as saline, i.e. NaCl (aq). Furthermore, other buffer systems than CAM and phosphate buffers could be used if a buffered solution are desired.

Dextran sulfate is preferably a so-called low molecular weight dextran sulfate.

In the following, reference to (average) molecular weight and sulfur content of dextran sulfate applies also to any pharmaceutically acceptable salt of dextran sulfate. Hence, the pharmaceutically acceptable salt of dextran sulfate preferably has the average molecular weight and sulfur content as discussed in the following embodiments.

Dextran sulfate is a sulfated polysaccharide and in particular a sulfated glucan, i.e. polysaccharide made of many glucose molecules. Average molecular weight as defined herein indicates that individual sulfated polysaccharides may have a molecular weight different from this average molecular weight but that the average molecular weight represents the mean molecular weight of the sulfated polysaccharides. This further implies that there will be a natural distribution of molecular weights around this average molecular weight for a dextran sulfate sample.

Average molecular weight (M_w) of dextran sulfate is typically determined using indirect methods such as gel exclusion/penetration chromatography, light scattering or viscosity. Determination of average molecular weight using such indirect methods will depend on a number of factors, including choice of column and eluent, flow rate, calibration procedures, etc.

Average molecular weight (M_w): $\frac{\sum M_i^2 N_i}{\sum M_i N_i}$, typical for methods sensitive to molecular size rather than numerical value, e.g. light scattering and size exclusion chromatography (SEC) methods. If a normal distribution is assumed, then a same weight on each side of M_w, i.e. the total weight of dextran sulfate molecules in the sample having a molecular weight below M_w is equal to the total weight of dextran sulfate molecules in the sample having a molecular weight above M_w.

In an embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, preferably has an average molecular weight equal to or below 40 000 Da, more preferably equal to or below 20 000 Da and in particular equal to or below 10 000 Da.

Dextran sulfate of a molecular weight exceeding 10 000 Da generally has a lower effect vs. toxicity profile as compared to dextran sulfate having a lower average molecular weight. This means that the maximum dose of dextran sulfate that can be safely administered to a subject is lower for larger dextran sulfate molecules (>10 000 Da) as compared to dextran sulfate molecules having an average molecular weight within the preferred range. As a consequence, such larger dextran sulfate molecules are less appropriate in clinical uses when the dextran sulfate is to be systemically administered to subjects *in vivo*.

In an embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, has an average molecular weight within a range of 2 000 and 10 000 Da. In another embodiment, the average molecular weight is within a range of 2 500 and 10 000 Da. In a particular preferred embodiment, the average molecular weight is within a range of 3 000 to 10 000 Da.

In an optional, but preferred embodiment, less than 40 % of the dextran sulfate molecules have a molecular weight below 3 000 Da, preferably less than 35 %, such as less than 30 % or less than 25 % of the dextran sulfate molecules have a molecular weight below 3 000 Da. In addition, or alternatively, less than 20 % of the dextran sulfate molecules have a molecular weight above 10 000 Da, preferably less than 15 %, such as less than 10 % or less than 5 % of the dextran sulfate molecules have a molecular weight above 10 000 Da. Thus, in a particular embodiment, the dextran sulfate has a substantially narrow molecular weight distribution around the average molecular weight.

In a particular embodiment, the average molecular weight of dextran sulfate, or the pharmaceutically acceptable salt thereof, is within a range of 3 500 and 9 500 Da, such as within a range of 3 500 and 8 000 Da.

In another particular embodiment, the average molecular weight of dextran sulfate, or the pharmaceutically acceptable salt thereof, is within a range of 4 500 and 7 500 Da.

In a further particular embodiment, the average molecular weight of dextran sulfate, or the pharmaceutically acceptable salt thereof, is within a range of 4 500 and 5 500 Da.

Thus, in a currently preferred embodiment the average molecular weight of dextran sulfate, or the pharmaceutically acceptable salt thereof, is preferably approximately 5 000 Da or at least substantially close to 5 000 Da, such as 5 000 ± 500 Da, for instance 5 000 ± 400 Da, preferably 5 000 ± 300 Da or 5 000 ± 200 Da, such as 5 000 ± 100 Da. Hence, in an embodiment, the average molecular weight of dextran sulfate, or the pharmaceutically acceptable salt thereof, is 4.5 kDa, 4.6 kDa, 4.7 kDa, 4.8 kDa, 4.9 kDa, 5.0 kDa, 5.1 kDa, 5.2 kDa, 5.3 kDa, 5.4 kDa or 5.5 kDa.

In a particular embodiment, the average molecular weight of dextran sulfate, or the pharmaceutically salt thereof as presented above is average M_w, and preferably determined by gel exclusion/penetration chromatography, size exclusion chromatography, light scattering or viscosity-based methods.

In a particular embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, consists, on average, of about or slightly above 5 glucose units and has an average sulfate number per glucose unit of at least 2.0, such as of at least 2.5.

Dextran sulfate is a polyanionic derivate of dextran and contains sulfur. The average sulfur content for dextran sulfate of the embodiments is preferably 15 to 20 % and more preferably approximately 17 %, generally corresponding to about or at least two sulfate groups per glucosyl residue. In a particular embodiment, the sulfur content of dextran sulfate is preferably equal to or at least close to the maximum possible degree of sulfur content of the corresponding dextran molecules.

In a particular embodiment, dextran sulfate of the embodiments has a number average molecular weight (M_n) as measured by nuclear magnetic resonance (NMR) spectroscopy within an interval of 1850 and 2000 Da.

In another particular embodiment, dextran sulfate of the embodiments has on average 5.1 glucose units and an average sulfate number per glucose unit of 2.6 to 2.7, typically resulting in a number average molecular weight (M_n) as measured by nuclear magnetic resonance (NMR) spectroscopy within an interval of 1850 and 2000 Da.

Number average molecular weight (M_n): $\frac{\sum M_i N_i}{\sum N_i}$, typically derived by end group assays, e.g. NMR spectroscopy or chromatography. If a normal distribution is assumed, then a same number of dextran sulfate molecules can be found on each side of M_n, i.e. the number of dextran sulfate molecules in the sample having a molecular weight below M_n is equal to the number of dextran sulfate molecules in the sample having a molecular weight above M_n.

A dextran sulfate, or pharmaceutically salt thereof, that can be used according to the embodiments is described in WO 2016/076780.

The dextran sulfate according to the embodiments can be provided as a pharmaceutically acceptable salt of dextran sulfate. Such pharmaceutically acceptable salts include e.g. a sodium or potassium salt of dextran sulfate.

Suitable dose ranges for the dextran sulfate, or the pharmaceutically acceptable salt, of the embodiments may vary according to the size and weight of the subject, the condition for which the subject is treated, and other considerations. In particular for human subjects, a possible dosage range could be from 1 µg/kg to 150 mg/kg of body weight, preferably from 10 µg/kg to 100 mg/kg of body weight.

In preferred embodiments, dextran sulfate, or the pharmaceutically acceptable salt thereof, is formulated to be systemically administered at a dosage in a range from 0.05 to 50 mg/kg of body weight of the subject, preferably from 0.05 or 0.1 to 40 mg/kg of body weight of the subject, and more preferably from 0.05 or 0.1 to 30 mg/kg, or 0.1 to 25 mg/kg or from 0.1 to 15 mg/kg or 0.1 to 10 mg/kg body weight of the subject.

Systemic administration of dextran sulfate, or the pharmaceutically acceptable salt thereof, of the embodiments is preferably initiated as soon as possible after occurrence of an event or condition that may otherwise cause fibrosis and in particular deleterious fibrosis in the subject. For instance, fibrosis often occur following an injury or other condition causing ischemia or a cardiovascular disease in the subject. In such a case, systemic administration of dextran sulfate, or the pharmaceutically acceptable salt thereof, is preferably performed as soon as possible following detection or diagnosis of the injury or other condition causing ischemia or a cardiovascular disease.

Systemic administration of dextran sulfate, or the pharmaceutically acceptable salt thereof, does not necessarily have to be limited to treatment of a present medical condition but could alternatively, or in addition, be used for prophylaxis. In other words, dextran sulfate of the embodiments could be systemically administered to a subject that will undergo a medical procedure, such as surgery, that may cause or induce cardiac fibrosis.

Dextran sulfate, or the pharmaceutically acceptable salt thereof, of the embodiments can be systemically administered at a single administration occasion, such as in the form of a single bolus injection. This bolus dose can be injected quite quickly to the patient but is advantageously infused over time so that the dextran sulfate solution is infused over a few minutes of time to the patient, such as during 5 to 10 minutes or more.

Alternatively, dextran sulfate, or the pharmaceutically acceptable salt thereof, of the embodiments can be systemically administered at multiple, i.e. at least two, occasions during a treatment period. Thus, dextran sulfate of the embodiments could be systemically administered once or at multiple times per day, once or at multiple times per week, once or at multiple times per month as illustrative examples.

In a particular embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, is formulated for systemic administration at multiple times, such as 2-5 times, preferably 3 times, a week for multiple consecutive weeks, such as at least 2-5 consecutive, preferably at least 3 consecutive weeks.

In an embodiment, systemic administration of dextran sulfate, or the pharmaceutically acceptable salt thereof, could be initiated as soon as possible following an event or condition causing deleterious fibrosis in the subject, such as soon as possible following an ischemic event or heart infarct as mentioned above. Alternatively, the systemic administration could be initiated at a time period following the event or condition causing deleterious fibrosis. A reason for such a delay in systemic administration is that the fibrosis process generally takes a period of time following a fibrosis causing event or condition. For instance, systemic administration of dextran sulfate, or the pharmaceutically acceptable salt thereof, could be initiated within the first week or from one week following an event or condition causing deleterious fibrosis in the subject.

In an embodiment, the subject is a mammalian subject, preferably a primate, and more preferably human subject. Although the embodiments are in particular directed towards treating, inhibiting or preventing cardiac fibrosis in human subjects, the embodiments may also, or alternatively, be used in veterinary applications. Non-limiting example of animal subjects include primate, cat, dog, pig, horse, mouse, rat.

In an embodiment, the subject is suffering from a disease, condition or disorder causing cardiac fibrosis and in particular detrimental, deleterious or injuries fibrosis. Such detrimental, deleterious or injurious fibrosis causes disruption of the normal architecture of the affected organ or tissue, ultimately leading to its dysfunction and failure. This means that detrimental, deleterious or injurious fibrosis is a pathological state or pathological fibrosis of excess deposition of fibrous tissue that will have a negative and detrimental effect on the organ or tissue where the fibrosis takes place.

Accordingly, in an embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, is for use in treating, inhibiting or preventing pathological fibrosis causing excess deposition of fibrous tissue in the heart or cardiac tissue of the subject causing dysfunction of the organ or tissue.

In a particular embodiment, the disease, condition or disorder causing fibrosis is selected from a group consisting of endomyocardial fibrosis, fibrosis following myocardial infarction or atrial fibrosis.

Dextran sulfate, or the pharmaceutically acceptable salt thereof, is thereby used to treat, inhibit or prevent the fibrosis component of any of the above mentioned diseases, conditions or disorders. Accordingly, dextran sulfate, or the pharmaceutically acceptable salt thereof, does not necessarily treat, inhibit or prevent the disease, condition or disorder per se but reduces the fibrosis process and thereby the amount of fibrotic tissue resulting from the disease, condition or disorder.

In an embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, formulated for systemic administration is used to treat, inhibit or prevent cardiac fibrosis in a subject suffering from cardiac fibrosis.

The pathological accumulation of extracellular matrix (fibrous connective tissue) is a key contributor to cardiac heart failure (CHF) in both diabetic and non-diabetic patients, resulting in progressive stiffening of the ventricular walls and loss of contractility of the heart. Heart failure is a global health problem, appearing most commonly in patients with previous myocardial infarction (MI). Cardiac remodeling, due to fibrosis, seen in both the infarcted and non-infarcted myocardium is recognized to be a major determinant of the development of impaired ventricular function, leading to a poor prognosis.

Accordingly, fibrosis may occur in heart (cardiac fibrosis) in the form of, for instance, endomyocardial fibrosis, fibrosis following myocardial infarction or atrial fibrosis. Fibrosis often occurs following ischemia at a site in the heart muscle, which may have severe and negative consequences in terms of heart wall stiffening, loss of contractility and cardiac remodeling.

In an embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, is for use in treating, inhibiting or preventing cardiac fibrosis in a subject having suffered from myocardial infarction or another ischemic condition in the heart, e.g. myocardial ischemia.

In a particular embodiment, dextran sulfate, or the pharmaceutically acceptable salt thereof, is for use in treating, inhibiting or preventing interstitial fibrosis in an infarct area of a heart of the subject.

Experimental data as presented herein show that dextran sulfate has an anti-fibrosis effect in a myocardial infarction model. Dextran sulfate resulted in significantly less cardiac fibrosis and in particular significantly less interstitial fibrosis in the infarct area of the test subjects.

Fibrosis may also occur in connection with transplantation of the heart or cardiac tissue in a subject. Dextran sulfate, or the pharmaceutically acceptable salt thereof, may accordingly be systemically administered to a subject that will be subject to or has recently been subject to heart or cardiac tissue transplantation. Non-limiting examples of such transplanted cardiac tissue includes heart valves, etc.

Dextran sulfate, or the pharmaceutically acceptable salt thereof, may also be systemically administered to a subject in connection with implantation of a medical device, such as pacemaker, stent, prosthesis, in or in connection with the heart of the subject. Thus, pathological cardiac fibrosis may also occur following implantation of medical devices.

Another aspect of the embodiments relates to use of dextran sulfate, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament formulated for systemic administration for treatment, inhibition or prevention of cardiac fibrosis in a subject.

A further aspect of the embodiments relates to a method of treating, inhibiting or preventing cardiac fibrosis in a subject. The method comprises systemically administering dextran sulfate, or a pharmaceutically acceptable salt thereof, to the subject.

EXPERIMENTS

Evaluation of anti-fibrosis effect of dextran sulfate in a myocardial infarction model

The present study assessed the effect of dextran sulfate in inhibiting or reducing fibrosis in a rat model of myocardial infarction (MI).

Materials and methods

The myocardial infarction model in rat involved ligations of the left coronary artery permanently with an intramural stitch. The surgery caused obstruction of the blood flow and subsequently to severe ischemic damage and cardiac walls infarct.

In total 120 female SD rats having an average body weight of 178 g at study initiation (Day 0) were obtained from Harlan Laboratories, Israel. Animals were fed *ad libitum* a commercial rodent diet (Teklad Certified Global 18 % Protein Diet). Animals had free access to autoclaved and acidified drinking water (pH between 2.5 and 3.5) obtained from the municipality supply. Animals were housed under standard laboratory conditions, air conditioned and filtered (HEPA F6/6) with adequate fresh air supply (minimum

15 air changes/hour). Animals were kept in a climate controlled environment with a temperatures range of 20–24°C and RH range of 30–70 % with 12 hours light and 12 hours dark cycle.

Dextran sulfate with an average molecular weight within a range of 5-7 kDa was dissolved in 0.9 % NaCl (saline) (Teva Pharmaceutical Industries Ltd) to be injected subcutaneous at doses of 15 mg/kg or 3 mg/kg.

On the day of surgery the animals were anesthetized with a combination of 90 mg/kg ketamine and 10 mg/kg xylazine, and animals were intubated and mechanically ventilated. In order to induce MI, under anesthesia, the rat chest was opened by left thoracotomy, the pericardium was removed and the proximal left coronary artery was permanently occluded with an intramural stitch (*Circulation* 2008, 117: 1388-1396). Two hours post-surgery, each animal in all treated groups were injected s.c. with dextran sulfate or saline vehicle according to Table 1.

Table 1 – Group allocation

Group	Treatment	Volume	S.C. Administration
1M (n=23)	Vehicle control	0.5 ml/kg	3 times a week, starting on day 1 for 3 weeks
2M (n=23)	Dextran sulfate 15 mg/kg		3 times a week, starting on day 1 for 3 weeks
3M (n=26)	Dextran sulfate 15 mg/kg		3 times a week, starting on day 1 for 1 week
4M (n=21)	Dextran sulfate 15 mg/kg		single dose on day 1
5M (n=27)	Dextran sulfate 3 mg/kg		3 times a week, starting on day 1 for 1 week

On day 36 after MI induction, the rats were sacrificed by CO_2 inhalation and the hearts were harvested and fixed in buffered formalin solution. Routine paraffin embedding was performed using standard histological procedures.

Masson's Trichrome Staining was used for fibrosis evaluation. The hearts were sectioned transversely into five sections that were imbedded in paraffin. Five paraffin sections at 5 µm were performed on a Lika microtome. All sections were stained according to standard Masson's trichrome protocol. The collagen fibers were stained blue, the nuclei were stained black and the background was stained red. The sections were visualized in a computer-imaging system and infarct size was marked and calculated using the ImageJ program. For each animal, five serial sections including one containing the ligature were analyzed and the mean value of all sections for each heart was treated as one value for statistical analysis.

Statistical analysis was performed by two ways ANOVA for repeated measures, followed by Bonferroni post-hoc test.

Results of cardiac fibrosis analysis

Interstitial fibrosis in the marginal area of the infarct was estimated manually in heart sections stained with Masson's Trichrome Staining. The area of interstitial fibrosis was calculated as the percent of the total area of the left ventricle. For each rat five cross-sections were analyzed. The extent of interstitial fibrosis in the margin zone of the infarct is shown in Fig. 1.

The extent of interstitial fibrosis was significantly lower in the dextran sulfate treated group 2M (11.9 $\pm$ 0.9 %) as compared to the control vehicle treated group 1M (22.7 $\pm$ 1.9 %). The extent of interstitial fibrosis was also lower in the other dextran sulfate treated groups 3M-5M, although the difference was not significant.

The effect of dextran sulfate seems to be primarily during the time fibrosis is developing following MI. Accordingly, treatment group 2M comprising treatment with dextran sulfate during 3 weeks significantly decreased fibrosis while the effect in the other groups (3M-5M) with shorter treatment was less pronounced. This seems to be in agreement with the pathogenesis of fibrosis in the rat model of myocardial infarction (*American Journal of Pathology* 1995, 147(2): 325-338). This article discloses that the fibrosis process in infarcted cardiac tissue is regulated differently than fibrosis in dermal wounds and in non-infarcted areas of the heart.

Figs. 2A-2D are pictures of fibrosis grade in two animals from group 1M (Figs. 2A and 2B) and two animals from group 2M (Fig. 2C and 2D). Fibrotic area stained blue are indicated by arrows in the figures.

The results thereby revealed that dextran sulfate treatment significantly decreased fibrosis at five weeks post-infarction compared to the vehicle control treated group. Dextran sulfate is thereby capable of decreasing the fibrogenesis following myocardial infarction.

The embodiments described above are to be understood as a few illustrative examples of the present invention. It will be understood by those skilled in the art that various modifications, combinations and changes may be made to the embodiments without departing from the scope of the present invention. In

particular, different part solutions in the different embodiments can be combined in other configurations, where technically possible. The scope of the present invention is, however, defined by the appended claims.

CLAIMS

1. Dextran sulfate, or a pharmaceutically acceptable salt thereof, formulated for systemic administration to a subject for use in treating, inhibiting or preventing cardiac fibrosis in said subject.

2. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to claim 1, formulated for systemic administration to said subject for use in treating, inhibiting or preventing cardiac fibrosis in said subject having suffered from myocardial infarction or myocardial ischemia.

3. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to claim 1 or 2, wherein said dextran sulfate, or said pharmaceutically acceptable salt thereof, is formulated for intravenous or subcutaneous administration to said subject.

4. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to any of the claims 1 to 3, wherein said dextran sulfate, or said pharmaceutically acceptable salt thereof, has an average molecular weight equal to or below 10 000 Da.

5. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to claim to 4, wherein said average molecular weight is within a range of 2 000 and 10 000 Da, preferably within a range of 3 000 and 10 000 Da, more preferably within a range of 3 500 and 9 500 Da, such as within a range of 4 500 and 7 500 Da, and preferably within a range of 4 500 and 5 500 Da.

6. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to claim 4, wherein said dextran sulfate, or said pharmaceutically acceptable salt thereof, has a number average molecular weight (M_n) as measured by nuclear magnetic resonance (NMR) spectroscopy within an interval of 1850 and 2000 Da.

7. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to claim 6, wherein said dextran sulfate, or said pharmaceutically acceptable salt thereof, has on average 5.1 glucose units and an average sulfate number per glucose unit of 2.6 to 2.7.

8. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to any of the claims 1 to 7, wherein said dextran sulfate, or said pharmaceutically acceptable salt thereof, has an average sulfur content in a range from 15 to 20 %.

9. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to claim 8, wherein said average sulfur content is about 17 %,

10. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to any of the claims 1 to 9, wherein said dextran sulfate, or said pharmaceutically acceptable salt thereof, is formulated as an aqueous injection solution.

11. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to any of the claims 1 to 10, wherein said dextran sulfate, or said pharmaceutically acceptable salt thereof, is formulated for systemic administration at multiple times, such as 3 times, a week for multiple consecutive weeks, such as 3 consecutive weeks.

12. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to any of the claims 1 to 11, wherein said dextran sulfate, or said pharmaceutically acceptable salt thereof, is formulated to be administered at a dosage in a range from 0.05 to 50 mg/kg of body weight of said subject, preferably from 0.05 to 30 mg/kg of body weight of said subject, and more preferably from 0.1 to 15 mg/kg or 0.1 to 10 mg/kg body weight of said subject.

13. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to any of the claims 1 to 12, wherein said subject is suffering from a disease or disorder selected from a group consisting of endomyocardial fibrosis, fibrosis following myocardial infarction or atrial fibrosis.

14. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to any of the claims 1 to 13, wherein said dextran sulfate, or said pharmaceutically acceptable salt thereof, is for use in treating, inhibiting or preventing interstitial fibrosis in an infarct area of a heart of said subject.

15. Dextran sulfate, or said pharmaceutically acceptable salt thereof, for use according to any of the claims 1 to 14, wherein said pharmaceutically acceptable salt thereof is a sodium salt of dextran sulfate.

16. Use of dextran sulfate, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament formulated for systemic administration for treatment, inhibition or prevention of cardiac fibrosis in a subject.

17. A method of treating, inhibiting or preventing cardiac fibrosis in a subject, said method comprising systemically administering dextran sulfate, or a pharmaceutically acceptable derivative thereof, to said subject.

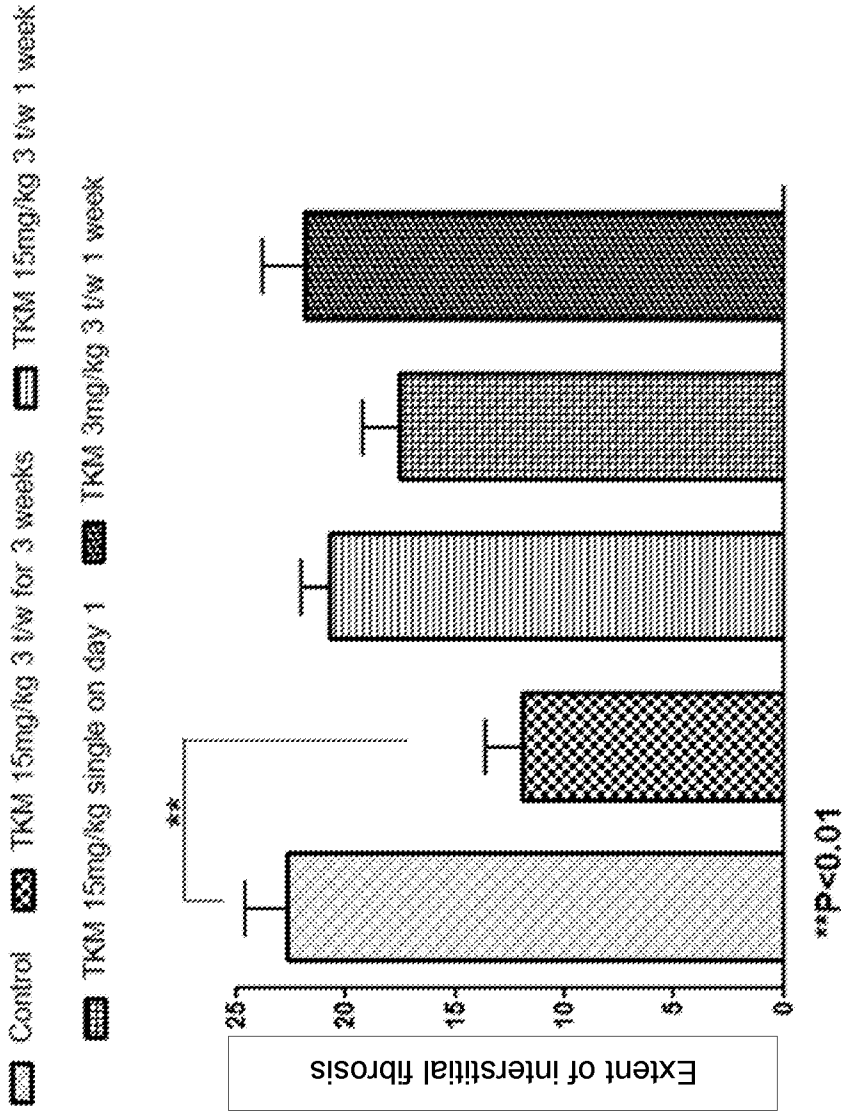
Control
TKM 15mg/kg 3 t/w for 3 weeks
TKM 15mg/kg 3 t/w 1 week
TKM 15mg/kg single on day 1
TKM 3mg/kg 3 t/w 1 week
Extent of interstitial fibrosis
25
20
15
10
5
0
**
**p<0.01

Fig. 1

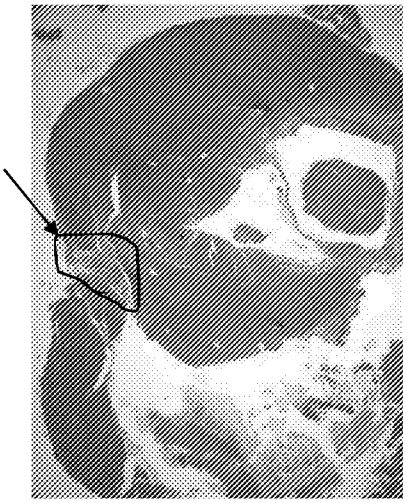

Fig. 2C

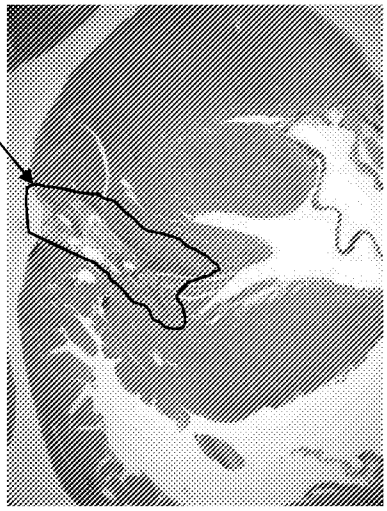

Fig. 2D

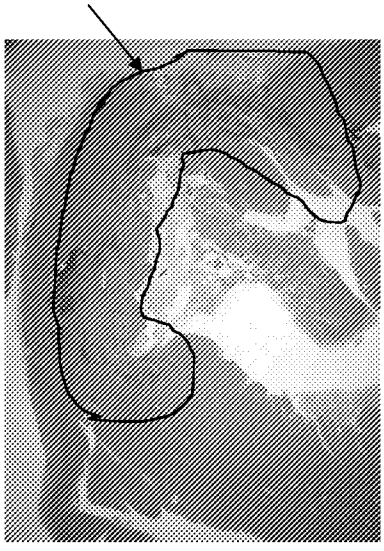

Fig. 2A

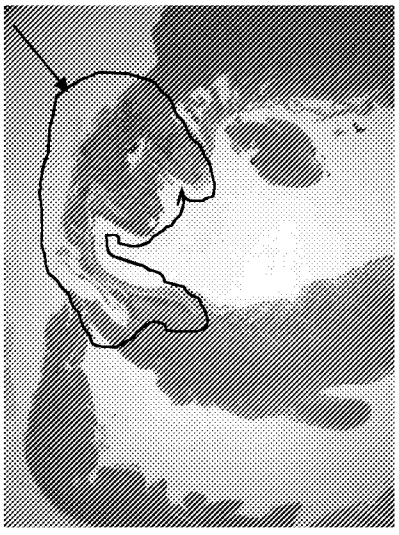

Fig. 2B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2016/050720

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet

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)

IPC: A61K, A61P, C08B, C08L

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

SE, DK, FI, NO classes as above

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

EPO-Internal, PAJ, WPI data, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, INSPEC, MEDLINE, PUBCHEM, IBM-TDB, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BANZ, Y. et al, "Locally targeted cytoprotection with dextran sulfate attenuates experimental porcine myocardial ischaemia/reperfusion injury" Eur Heart J, 2005, vol. 26, p. 2334-2343; whole document; page 2335, column 1, paragraph [0003]; page 2335, column 1, paragraph [0005]; page 2339, column 2, paragraph [0001]; page 2341, column 1, paragraph [0001] --	1-17
A	KRENNING, G. et al, "The Origin of Fibroblasts and Mechanism of Cardiac Fibrosis" J Cell Physio, 2010, vol. 225, p. 631-637; page 631, column 1, paragraph [0002] --	1-17

☒ 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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"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	Date of mailing of the international search report
23-09-2016	23-09-2016

Name and mailing address of the ISA/SE	Authorized officer
Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Elias Pershagen Telephone No. + 46 8 782 28 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2016/050720

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MERCER, P. F. et al "Coagulation and coagulation signalling in fibrosis"Biochim Biophys Acta, 2013, p. 1018-1027; whole document; page 1022, paragraph [0044]	1-17
	--	
A	Dextran sulfate product specification [online] Sigma Aldrich [retrieved on 19-09-2016] Retrieved from the Internet: <URL: http://www.sigmaaldrich.com/Graphics/COfAInfo/SigmaSAPQM/SPEC/D7/D7037/D7037-BULK_____SIAL_____.pdf>; whole document	1-17
	--	
A	Dextran sulfate product information [online] Sigma Aldrich [retrieved on 19-09-2016] Retrieved from the Internet: <URL: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/d8906pis.pdf>; whole document	1-17
	--	
A	WO 2008134430 A1 (NOVELMED THERAPEUTICS INC ET AL), 6 November 2008 (2008-11-06); whole document	1-17
	--	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2016/050720

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 17
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 17 relates to a method for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods, see PCT rule 41bis.1(b) and PCT rule 67.1.(iv). Nevertheless, an examination has been conducted for this claim. The examination has been made in respect of the technical content of the claim.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

International application No.
PCT/SE2016/050720

Continuation of: second sheet

International Patent Classification (IPC)

A61K 31/721 (2006.01)
A61P 9/10 (2006.01)
C08B 37/02 (2006.01)
C08L 5/02 (2006.01)

WO	2008134430	A1	06/11/2008	CA	2685208	A1	06/11/2008
				US	9023831	B2	05/05/2015
				US	20100087393	A1	08/04/2010

(19)中华人民共和国国家知识产权局

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(85)PCT国际申请进入国家阶段日
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PCT/SE2016/050720 2016.07.15

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(51)Int.Cl.
A61K 31/721(2006.01)
A61P 9/10(2006.01)
C08B 37/02(2006.01)
C08L 5/02(2006.01)

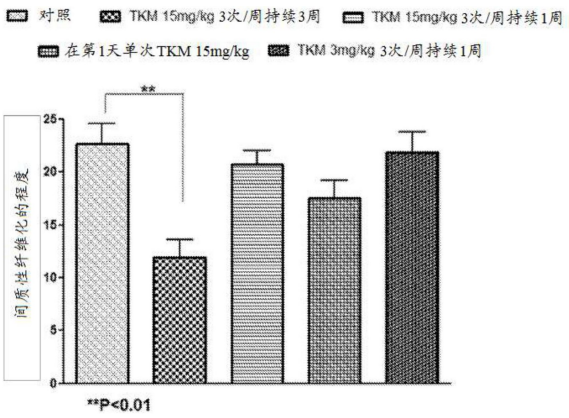
权利要求书2页 说明书8页 附图3页

(54)发明名称

硫酸葡聚糖的新用途

(57)摘要

本发明实施方案涉及硫酸葡聚糖的用途,所述硫酸葡聚糖被配制用于全身施用以用于治疗、抑制或预防受试者的心脏纤维化。



CN 107921055 A

1.一种硫酸葡聚糖或其药学上可接受的盐,其被配制用于向受试者全身施用以用于治疗、抑制或预防所述受试者的心脏纤维化。

2.根据权利要求1所述的硫酸葡聚糖或所述其药学上可接受的盐,其被配制用于向所述受试者全身施用以用于治疗、抑制或预防患有心肌梗塞或心肌缺血的所述受试者的心脏纤维化。

3.根据权利要求1或2所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述硫酸葡聚糖或所述其药学上可接受的盐被配制用于静脉内或皮下施用于所述受试者。

4.根据权利要求1至3中任一项所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述硫酸葡聚糖或所述其药学上可接受的盐的平均分子量等于或低于10 000Da。

5.根据权利要求4所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述平均分子量在2 000与10 000Da的范围内,优选地在3 000与10 000Da的范围内,更优选地在3 500与9 500Da的范围内,如在4 500与7 500Da的范围内,并且优选地在4 500与5 500Da的范围内。

6.根据权利要求4所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述硫酸葡聚糖或所述其药学上可接受的盐的数均分子量(M_n)如通过核磁共振(NMR)光谱法所测量在1850与2000Da的区间内。

7.根据权利要求6所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述硫酸葡聚糖或所述其药学上可接受的盐具有平均5.1个葡萄糖单元并且每个葡萄糖单元的平均硫酸根数目是2.6至2.7。

8.根据权利要求1至7中任一项所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述硫酸葡聚糖或所述其药学上可接受的盐的平均硫含量在15%至20%的范围内。

9.根据权利要求8所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述平均硫含量是约17%。

10.根据权利要求1至9中任一项所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述硫酸葡聚糖或所述其药学上可接受的盐被配制为注射水溶液。

11.根据权利要求1至10中任一项所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述硫酸葡聚糖或所述其药学上可接受的盐被配制用于全身施用,一周多次,如3次,连续多周,如连续3周。

12.根据权利要求1至11中任一项所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述硫酸葡聚糖或所述其药学上可接受的盐被配制为以0.05至50mg/kg所述受试者的体重、优选地0.05至30mg/kg所述受试者的体重并且更优选地0.1至15mg/kg或0.1至10mg/kg所述受试者的体重范围内的剂量施用。

13.根据权利要求1至12中任一项所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述受试者患有选自由以下各项组成的组的疾病或病症:心内膜心肌纤维化、心肌梗塞后纤维化或心房纤维化。

14.根据权利要求1至13中任一项所述的硫酸葡聚糖或所述其药学上可接受的盐,其中所述硫酸葡聚糖或所述其药学上可接受的盐用于治疗、抑制或预防所述受试者的心脏梗塞区域的间质性纤维化。

15.根据权利要求1至14中任一项所述的硫酸葡聚糖或所述其药学上可接受的盐,其中

所述其药学上可接受的盐是硫酸葡聚糖的钠盐。

16.一种硫酸葡聚糖或其药学上可接受的盐的用途,其用于制造被配制用于全身施用以治疗、抑制或预防受试者心脏纤维化的药物。

17.一种治疗、抑制或预防受试者的心脏纤维化的方法,所述方法包含向所述受试者全身施用硫酸葡聚糖或其药学上可接受的衍生物。

硫酸葡聚糖的新用途

技术领域

[0001] 本发明实施方案通常涉及纤维化治疗，并且具体涉及硫酸葡聚糖用于治疗、抑制或预防受试者的心脏纤维化的用途。

[0002] 发明背景

[0003] 纤维化是在修复或反应过程中在器官或组织中形成过量纤维结缔组织的过程。纤维化可以是反应性的、良性的或病态的。为了应对损伤，纤维化过程有时被称为瘢痕形成。

[0004] 生理性纤维化涉及沉积结缔组织，其可以消除下面的器官或组织的结构和功能。纤维化与瘢痕形成过程类似，都涉及受刺激的细胞产生包括胶原蛋白和糖胺聚糖的结缔组织。巨噬细胞和受损组织响应于例如炎症或组织损伤而释放转化生长因子β(TGFβ)。这反过来又刺激成纤维细胞的增殖和活化，成纤维细胞沉积结缔组织。

[0005] US 5,605,938公开了生物相容性阴离子聚合物(包括平均分子量约40,000至2,000,000Da的硫酸葡聚糖)可以抑制纤维化、瘢痕形成和通常与手术相关的手术粘连。阴离子聚合物在纤维化损伤处局部施用，或者可以粘性液体或凝胶形式浸泡到器官或植入物上，所述粘性液体或凝胶优选还包含含有二羟苯丙氨酸(DOPA)和含羟基氨基酸残基的粘附蛋白。

[0006] CN 102973593公开了硫酸葡聚糖在制备用于治疗肝纤维化的药物中的用途。所述文件提到硫酸葡聚糖抑制星形胶质细胞的激活并促使巨噬细胞分泌金属蛋白酶。

发明概要

[0007] 总体目标是治疗、抑制或减少受试者的心脏纤维化。

[0008] 这个和其它目标由在此公开的实施方案来满足。

[0009] 实施方案的一个方面涉及配制用于全身施用于受试者的硫酸葡聚糖或其药学上可接受的盐，用于治疗、抑制或预防受试者的心脏纤维化。

[0010] 实施方案的另一方面涉及硫酸葡聚糖或其药学上可接受的盐在制造配制用于全身施用以治疗、抑制或预防受试者心脏纤维化的药物中的用途。

[0011] 实施方案的另一方面涉及治疗、抑制或预防受试者心脏纤维化的方法。所述方法包含向受试者全身施用硫酸葡聚糖或其药学上可接受的盐。

[0012] 图式简单说明

[0013] 结合附图参考以下描述，可以最好地理解实施方案及其进一步的目的和优点，其中：

[0014] 图1示出了间质性纤维化程度的估计。间质性纤维化的相对面积显示与对照组1M相比，硫酸葡聚糖组2M中的心肌梗塞诱导的间质性纤维化的显著抑制(根据单因素方差分析，随后的邦弗朗尼(Bonferroni)事后比较，**P<0.01)。

[0015] 图2A-2D是来自对照组1M的两只动物和来自硫酸葡聚糖组2M的两只动物的纤维化等级的图片。

[0016] 实施方式

[0017] 本发明实施方案大体上涉及纤维化治疗，并且具体涉及硫酸葡聚糖用于治疗、抑制或预防受试者的心脏纤维化的用途。

[0018] 实施方案基于以下发现：全身施用的硫酸葡聚糖或其药学上可接受的盐能够减少受试者中不希望的纤维化形成，特别是减少或抑制局部缺血组织，特别是局部缺血性心脏组织中的这种纤维化形成。

[0019] 纤维化疾病包括可能影响受试者体内不同器官和组织的多种医学病况。这些医学病况的特征在于编码基质蛋白的基因表达升高，并且由此产生的纤维化破坏了受影响的器官或组织的正常结构，最终导致其功能障碍或衰竭。

[0020] 实施方案在治疗、抑制或预防纤维化疾病的不利作用方面能够通过减少心脏或心脏组织中形成的纤维化的量来治疗、抑制或预防心脏纤维化。因此，实施方案的硫酸葡聚糖能够治疗、抑制或预防有害或致伤的心脏纤维化。

[0021] 因此，实施方案的一个方面涉及配制用于向受试者全身施用的硫酸葡聚糖或其药学上可接受的盐，用于治疗、抑制或预防受试者的心脏纤维化。

[0022] 根据美国专利第5,605,938号，全身递送的硫酸葡聚糖可以显著减少有害的纤维化形成，这是非常令人惊讶的。这一专利文献公开了局部施用硫酸葡聚糖可以抑制尤其与各种植入物的植入有关的纤维化和瘢痕形成。其中据推测，硫酸葡聚糖聚合物的负电荷涉及抑制植入部位处的各种细胞的侵入。为了使这种负电荷具有细胞侵入抑制效果，硫酸葡聚糖需要在所需部位局部施用。因此，在美国专利5,605,938中有利地用硫酸葡聚糖包覆植入物。

[0023] 这里给出的实验数据表明，实施方案的硫酸葡聚糖可以全身施用，即不在目标部位局部施用，并且仍然发挥其期望的抗纤维化作用。

[0024] 配制硫酸葡聚糖或其药学上可接受的盐用于向受试者全身施用。在一个实施方案中，将硫酸葡聚糖或其药学上可接受的盐配制成用于肠胃外施用作为全身施用的实例，以在受试者中实现全身作用。

[0025] 肠胃外施用途径的实例包括静脉内(i.v.)施用、动脉内施用、肌内施用、脑内施用、脑室内施用、鞘内施用和皮下(s.c.)施用。

[0026] 在一个实施方案中，硫酸葡聚糖或其药学上可接受的盐优选配制成静脉(i.v.)或皮下(s.c.)施用于受试者。因此，i.v.和s.c.施用是硫酸葡聚糖或其药学上可接受的盐的全身施用的优选实例。

[0027] 已知口服硫酸葡聚糖可诱导小鼠、大鼠、仓鼠和豚鼠的结肠炎和肠纤维化。因此，如本文所用的全身施用优选不包括硫酸葡聚糖或其药学上可接受的盐的口服。在一个具体实施方案中，硫酸葡聚糖或其药学上可接受的盐的全身施用是除口服以外的全身施用，优选不是肠内施用。

[0028] 在一个实施方案中，硫酸葡聚糖或其药学上可接受的盐被配制成注射水溶液，优选配制成i.v.或s.c.注射水溶液。因此，实施方案的硫酸葡聚糖或其药学上可接受的盐优选配制成具有选择的溶剂或赋形剂的注射水溶液。溶剂有利地是含水溶剂，特别是缓冲溶液。这种缓冲溶液的非限制性实例是柠檬酸缓冲液，如柠檬酸单水合物(CAM)缓冲液或磷酸盐缓冲液。例如，可以将实施方案的硫酸葡聚糖溶解在生理盐水(如0.9％NaCl生理盐水)中，然后任选用75mM CAM缓冲并使用氢氧化钠将pH调节至约5.9。非缓冲溶液也是可能的，

包括注射水溶液，如生理盐水，即NaCl(aq)。此外，如果需要缓冲溶液，那么可以使用除CAM和磷酸盐缓冲液之外的其它缓冲系统。

[0029] 硫酸葡聚糖优选是所谓的低分子量硫酸葡聚糖。

[0030] 在下文中，提及硫酸葡聚糖的(平均)分子量和硫含量也适用于硫酸葡聚糖的任何药学上可接受的盐。因此，硫酸葡聚糖的药学上可接受的盐优选具有如以下实施方案中所讨论的平均分子量和硫含量。

[0031] 硫酸葡聚糖是一种硫酸化多糖，尤其是一种硫酸化葡聚糖，即由许多葡萄糖分子组成的多糖。如本文所定义的平均分子量指示单独的硫酸化多糖可能具有不同于这一平均分子量的分子量，但是所述平均分子量表示硫酸化多糖的平均分子量。这进一步意味着硫酸葡聚糖样品将在这个平均分子量附近具有分子量的自然分布。

[0032] 硫酸葡聚糖的平均分子量(M_w)通常使用间接方法如凝胶排阻/穿透色谱法、光散射或粘度来测定。使用这种间接方法测定平均分子量将取决于许多因素，包括柱和洗脱液的选择、流速、校准程序等。

[0033] 平均分子量(M_w)：$\frac{\sum M_i^2 N_i}{\sum M_i N_i}$，典型用于对分子大小而非数值敏感的方法，例如光散射和尺寸排阻色谱(SEC)方法。如果假定正态分布，那么在M_w的每一侧的重量相同，即样品中分子量低于M_w的硫酸葡聚糖分子的总重量等于样品中分子量高于M_w的硫酸葡聚糖分子的总重量。

[0034] 在一个实施方案中，硫酸葡聚糖或其药学上可接受的盐优选具有等于或低于40 000Da，更优选等于或低于20 000Da，尤其等于或低于10 000Da的平均分子量。

[0035] 分子量超过10 000Da的硫酸葡聚糖与具有较低平均分子量的硫酸葡聚糖相比通常具有较低的效果比毒性特征。这意味着与具有优选范围内的平均分子量的硫酸葡聚糖分子相比，对于较大的硫酸葡聚糖分子(>10 000Da)，可以安全施用于受试者的硫酸葡聚糖的最大剂量较低。因此，当将硫酸葡聚糖全身施用于受试者体内时，这种较大的硫酸葡聚糖分子在临床应用中不太合适。

[0036] 在一个实施方案中，硫酸葡聚糖或其药学上可接受的盐的平均分子量在2 000至10 000Da的范围内。在另一个实施方案中，平均分子量在2 500至10 000Da的范围内。在一个特别优选的实施方案中，平均分子量在3 000至10 000Da的范围内。

[0037] 在一个任选但优选的实施方案中，小于40％的硫酸葡聚糖分子的分子量低于3000Da，优选小于35％，如小于30％或小于25％的硫酸葡聚糖分子的分子量低于3 000Da。另外或替代地，小于20％的硫酸葡聚糖分子的分子量超过10 000Da，优选小于15％，如小于10％或小于5％的硫酸葡聚糖分子的分子量超过10 000Da。因此，在一个具体实施方案中，硫酸葡聚糖在平均分子量附近具有相当窄的分子量分布。

[0038] 在一个具体实施方案中，硫酸葡聚糖或其药学上可接受的盐的平均分子量在3500至9 500Da的范围内，如在3 500至8 000Da的范围内。

[0039] 在另一个具体实施方案中，硫酸葡聚糖或其药学上可接受的盐的平均分子量在4500至7 500Da的范围内。

[0040] 在另一个具体实施方案中，硫酸葡聚糖或其药学上可接受的盐的平均分子量在4500至5 500Da的范围内。

[0041] 因此，在目前优选的实施方案中，硫酸葡聚糖或其药学上可接受的盐的平均分子量优选为约5 000Da或至少基本上接近5 000Da，如5 000±500Da，例如5 000±400Da，优选5 000±300Da或5 000±200Da，如5 000±100Da。因此，在一个实施方案中，硫酸葡聚糖或其药学上可接受的盐的平均分子量是4.5kDa、4.6kDa、4.7kDa、4.8kDa、4.9kDa、5.0kDa、5.1kDa、5.2kDa、5.3kDa、5.4kDa或5.5kDa。

[0042] 在一个具体实施方案中，如上所示的硫酸葡聚糖或其药学上的盐的平均分子量是平均M_w，并且优选通过凝胶排阻/穿透色谱法、尺寸排阻色谱法、光散射或基于粘度的方法测定。

[0043] 在一个具体实施方案中，硫酸葡聚糖或其药学上可接受的盐平均包含约5个或略高于5个葡萄糖单元，并且每个葡萄糖单元的平均硫酸根数目为至少2.0，如至少2.5。

[0044] 硫酸葡聚糖是葡聚糖的聚阴离子衍生物并含有硫。实施方案中硫酸葡聚糖的平均硫含量优选为15至20％，更优选约17％，通常对应于每个葡糖基残基约或至少两个硫酸根基团。在一个具体实施方案中，硫酸葡聚糖的硫含量优选等于或至少接近相应葡聚糖分子的最大可能的硫含量。

[0045] 在一个具体实施方案中，实施方案的硫酸葡聚糖通过核磁共振(NMR)光谱法测量的数均分子量(M_n)在1850和2000Da的区间内。

[0046] 在另一个具体实施方案中，实施方案的硫酸葡聚糖平均具有5.1个葡萄糖单元，并且每个葡萄糖单元的平均硫酸根数为2.6至2.7，通常使得通过核磁共振(NMR)光谱法测量的数均分子量(M_n)在1850至2000Da的区间内。

[0047] 数均分子量(M_n)：$\frac{\sum M_i N_i}{\sum N_i}$，通常通过端基测定法例如NMR光谱法或色谱法导出。如果假定正态分布，那么可以在M_n的每一侧发现相同数量的硫酸葡聚糖分子，即样品中分子量低于M_n的硫酸葡聚糖分子的数量等于样品中分子量高于M_n的硫酸葡聚糖分子的数量。

[0048] WO 2016/076780中描述了可以根据实施方案使用的硫酸葡聚糖或其药学上的盐。

[0049] 根据实施方案的硫酸葡聚糖可以作为硫酸葡聚糖的药学上可接受的盐提供。这样的药学上可接受的盐包括例如硫酸葡聚糖的钠盐或钾盐。

[0050] 用于实施方案的硫酸葡聚糖或其药学上可接受的盐的合适的剂量范围可以根据受试者的大小和体重、受试者的治疗条件和其它考虑因素而变化。特别对于人类受试者，可能的剂量范围可以是1μg/kg至150mg/kg体重，优选10μg/kg至100mg/kg体重。

[0051] 在优选实施方案中，将硫酸葡聚糖或其药学上可接受的盐配制成以0.05至50mg/kg受试者体重，优选0.05或0.1至40mg/kg受试者体重，更优选0.05或0.1至30mg/kg或0.1至25mg/kg或0.1至15mg/kg或0.1至10mg/kg受试者体重范围内的剂量全身施用。

[0052] 实施方案的硫酸葡聚糖或其药学上可接受的盐的全身施用优选在可能以其它方式引起受试者的纤维化和特别是有害纤维化的事件或病况发生之后尽可能快地开始。例如，纤维化常常在造成受试者局部缺血或心血管疾病的损伤或其它病况之后发生。在这种情况下，优选在检测或诊断造成局部缺血或心血管疾病的损伤或其它病况之后，尽快进行硫酸葡聚糖或其药学上可接受的盐的全身施用。

[0053] 硫酸葡聚糖或其药学上可接受的盐的全身施用不一定限于治疗目前的医学病况，而且可以替代地或另外用于预防。换句话说，实施方案中的硫酸葡聚糖可以全身性地施用

于将经历可能引起或诱发心脏纤维化的医疗程序(如手术)的受试者。

[0054] 实施方案的硫酸葡聚糖或其药学上可接受的盐可以在单次施用时全身施用,如以单次快速浓注的形式施用。这一推注剂量可以相当快速地注射给患者,但是随着时间推移适宜输注,使得硫酸葡聚糖溶液在几分钟的时间内输注给患者,例如5至10分钟或更长时间。

[0055] 或者,实施方案的硫酸葡聚糖或其药学上可接受的盐可以在治疗期间以多个(即至少两个)时刻全身施用。因此,实施方案的硫酸葡聚糖可以每天一次或多次,每周一次或多次,每月一次或多次全身施用,作为说明性实例。

[0056] 在一个具体实施方案中,硫酸葡聚糖或其药学上可接受的盐被配制用于一周多次(如2-5次,优选3次),持续连续多周(如连续至少2-5,优选连续至少3周)的全身施用。

[0057] 在一个实施方案中,可以在引起受试者的有害纤维化的事件或病况之后尽可能快地,如在上述缺血事件或心脏梗塞之后尽可能快地开始硫酸葡聚糖或其药学上可接受的盐的全身施用。或者,全身施用可以在引起有害纤维化的事件或病况之后的时间段开始。这样延迟全身施用的原因是纤维化过程通常在引起纤维化的事件或病况后需要一段时间。例如,硫酸葡聚糖或其药学上可接受的盐的全身施用可以在引起受试者的有害纤维化的事件或病况后的第一周或一周内开始。

[0058] 在一个实施方案中,受试者是哺乳动物受试者,优选灵长类动物,更优选人类受试者。尽管实施方案特别涉及治疗、抑制或预防人类受试者的心脏纤维化,但是实施方案也可以或替代地用于兽医应用。动物受试者的非限制性实例包括灵长类动物、猫、狗、猪、马、小鼠、大鼠。

[0059] 在一个实施方案中,受试者患有引起心脏纤维化的疾病、病况或病症,特别是不利的、有害的或损伤性纤维化。这种不利的、有害的或损伤性纤维化引起受影响的器官或组织的正常结构的破坏,最终导致其功能障碍和衰竭。这意味着不利的、有害的或损伤性纤维化是纤维组织过量沉积的病理状态或病理性纤维化,其将对发生纤维化的器官或组织具有负面和有害作用。

[0060] 因此,在一个实施方案中,硫酸葡聚糖或其药学上可接受的盐用于治疗、抑制或预防导致器官或组织中的功能障碍的受试者的心脏或心脏组织中纤维组织过量沉积的病理性纤维化。

[0061] 在一个具体实施方案中,引起纤维化的疾病、病况或病症选自由以下各项组成的组:心内膜心肌纤维化、心肌梗塞后纤维化或心房纤维化。

[0062] 因此,硫酸葡聚糖或其药学上可接受的盐用于治疗、抑制或预防任何上述疾病、病况或病症的纤维化成分。因此,硫酸葡聚糖或其药学上可接受的盐本身并不一定治疗、抑制或预防疾病、病况或病症,而是减少纤维化过程并由此减少由疾病、病况或病症导致的纤维化组织的量。

[0063] 在一个实施方案中,施用配制用于全身施用的硫酸葡聚糖或其药学上可接受的盐治疗、抑制或预防患有心脏纤维化的受试者的心脏纤维化。

[0064] 细胞外基质(纤维结缔组织)的病理累积是糖尿病患者和非糖尿病患者心脏衰竭(CHF)的关键因素,导致心室壁逐渐变硬并丧失心脏收缩性。心脏衰竭是全球性的健康问题,最常见于早先有心肌梗塞(MI)的患者。由于纤维化,在梗塞心肌和非梗塞心肌中所见的

心脏重塑被认为是出现心室功能受损的主要决定因素，从而导致预后不良。

[0065] 因此，纤维化可以例如心内膜心肌纤维化、心肌梗塞后纤维化或心房纤维化的形式出现在心脏(心脏纤维化)中。纤维化通常在心肌缺血部位发生，这在心脏壁硬化、收缩性丧失和心脏重塑方面可能具有严重和负面的后果。

[0066] 在一个实施方案中，硫酸葡聚糖或其药学上可接受的盐用于治疗、抑制或预防患有心肌梗塞或心脏中另一种局部缺血症状(例如心肌缺血)的受试者的心脏纤维化。

[0067] 在一个具体实施方案中，硫酸葡聚糖或其药学上可接受的盐用于治疗、抑制或预防受试者心脏梗塞区域的间质性纤维化。

[0068] 如本文所示的实验数据显示硫酸葡聚糖在心肌梗塞模型中具有抗纤维化作用。硫酸葡聚糖使得心脏纤维化显著减少，并且特别是在测试受试者的梗塞区域中显著减少间质纤维化。

[0069] 还可能发生与受试者心脏或心脏组织移植有关的纤维化。因此，硫酸葡聚糖或其药学上可接受的盐可以全身施用于将要经受或最近已经受心脏或心脏组织移植的受试者。这种移植的心脏组织的非限制性实例包括心脏瓣膜等。

[0070] 硫酸葡聚糖或其药学上可接受的盐还可以全身施用于与受试者的心脏中或与受试者的心脏相关联地植入如起搏器、支架、假体的医疗装置相关的受试者。因此，植入医疗装置后也可能发生病理性心脏纤维化。

[0071] 实施方案的另一个方面涉及硫酸葡聚糖或其药学上可接受的盐的用途，其用于制造配制用于全身施用以治疗、抑制或预防受试者心脏纤维化的药物。

[0072] 实施方案的另一方面涉及治疗、抑制或预防受试者心脏纤维化的方法。所述方法包含向受试者全身施用硫酸葡聚糖或其药学上可接受的盐。

[0073] 实验

[0074] 心肌梗塞模型中硫酸葡聚糖的抗纤维化作用的评价

[0075] 本研究评估了硫酸葡聚糖在心肌梗塞(MI)的大鼠模型中抑制或减少纤维化的作用。

[0076] 材料和方法

[0077] 大鼠心肌梗塞模型包括左冠状动脉通过壁内缝合永久结扎。手术造成血流阻塞，继而导致严重的缺血性损伤和心壁梗塞。

[0078] 在研究开始时(第0天)，从以色列Harlan实验室获得总共120只平均体重为178g的雌性SD大鼠。动物随意喂食市售啮齿动物饲料(Teklad Certified Global 18％蛋白质饲料)。动物可以自由饮用从市政供应获得的高压灭菌和酸化饮用水(pH值在2.5和3.5之间)。将动物饲养在标准的实验室条件下，具有足够新鲜空气供应(最少15次换气/小时)的空气调节和过滤(HEPA F6/6)。将动物保持在气候受控的环境中，温度范围为20-24℃，RH范围为30-70％，12小时光照和12小时黑暗循环。

[0079] 将平均分子量在5-7kDa范围内的硫酸葡聚糖溶解在0.9％NaCl(生理盐水)(梯瓦制药工业有限公司(Teva Pharmaceutical Industries Ltd))中，以15mg/kg或3mg/kg的剂量皮下注射。

[0080] 在手术当天，将动物用90mg/kg氯胺酮和10mg/kg甲苯噻嗪的组合麻醉，并对动物进行插管和机械通气。为了诱导MI，在麻醉下，通过左侧开胸手术打开大鼠胸部，取出心包，

并通过壁内缝合永久性阻塞左冠状动脉近端(《循环(Circulation)》2008,117:1388-1396)。手术后两小时,所有处理组中的每只动物根据表1皮下注射硫酸葡聚糖或生理盐水媒剂。

[0081] 表1-组分配

[0082]

组	处理	体积	皮下施用
1M (n=23)	媒剂对照	0.5 ml/kg	一周 3 次，从第 1 天开始持续 3 周
2M (n=23)	硫酸葡聚糖 15 mg/kg		一周 3 次，从第 1 天开始持续 3 周
3M (n=26)	硫酸葡聚糖 15 mg/kg		一周 3 次，从第 1 天开始持续 1 周
4M (n=21)	硫酸葡聚糖 15 mg/kg		在第 1 天的单次剂量
5M (n=27)	硫酸葡聚糖 3 mg/kg		一周 3 次，从第 1 天开始持续 1 周

[0083] 在MI诱导后的第36天,通过CO_2吸入处死大鼠并收集心脏再将其固定在缓冲的福尔马林溶液中。使用标准组织学程序进行常规石蜡包埋。

[0084] 使用马森三色染色(Masson's Trichrome)进行纤维化评估。将心脏横向切成5块石蜡包埋的切片。在Lika切片机上进行5μm的5个石蜡切片。所有切片根据标准马森三色方案染色。胶原纤维染成蓝色,细胞核染成黑色,背景染成红色。在计算机成像系统中观察切片并使用ImageJ程序标记和计算梗塞面积。对于每只动物,分析包括一个含有结扎的五个连续切片,并将每个心脏的所有切片的平均值作为一个值进行统计学分析。

[0085] 通过两因素方差分析对重复测量进行统计学分析,随后进行邦弗朗尼事后检验。

[0086] 心脏纤维化分析的结果

[0087] 在通过马森三色染色染色的心脏切片中手动估算梗塞边缘区域的间质性纤维化。以左心室总面积的百分比形式计算间质性纤维化面积。对于每只大鼠,分析五个横截面。梗塞边缘区的间质性纤维化程度如图1所示。

[0088] 与对照媒剂处理组1M(22.7±1.9%)相比,硫酸葡聚糖处理组2M的间质性纤维化程度显著降低(11.9±0.9%)。其它硫酸葡聚糖处理组3M-5M的间质性纤维化程度也降低,但差异不显著。

[0089] 硫酸葡聚糖的作用似乎主要是在MI后出现纤维化的时期。因此,包含在3周期间用硫酸葡聚糖处理的处理组2M显著减少了纤维化,而在其它组(3M-5M)中较短处理的效果不太明显。这似乎与心肌梗塞大鼠模型中纤维化的发病机理一致(《美国病理学杂志(American Journal of Pathology)》1995,147(2):325-338)。这篇文章公开了梗塞心脏组织中的纤维化过程与皮肤伤口中的纤维化和心脏的非梗塞区域受到不同地调节。

[0090] 图2A-2D是来自1M组的两只动物(图2A和2B)和来自2M组的两只动物(图2C和2D)的纤维化等级的图片。染成蓝色的纤维化区域在图中用箭头指示。

[0091] 结果借此表明，与媒剂对照处理组相比，硫酸葡聚糖处理在梗塞后五周显著减少了纤维化。因此，硫酸葡聚糖能够减少心肌梗塞后的纤维形成。

[0092] 上述实施方案应被理解为本发明的一些说明性实例。本领域技术人员应当理解，在不脱离本发明的范围的情况下，可以对实施方案进行各种修改、组合和改变。具体而言，在技术上可能的情况下，不同实施方案中的不同部分解决方案可以其它配置进行组合。然而，本发明的范围由所附权利要求限定。

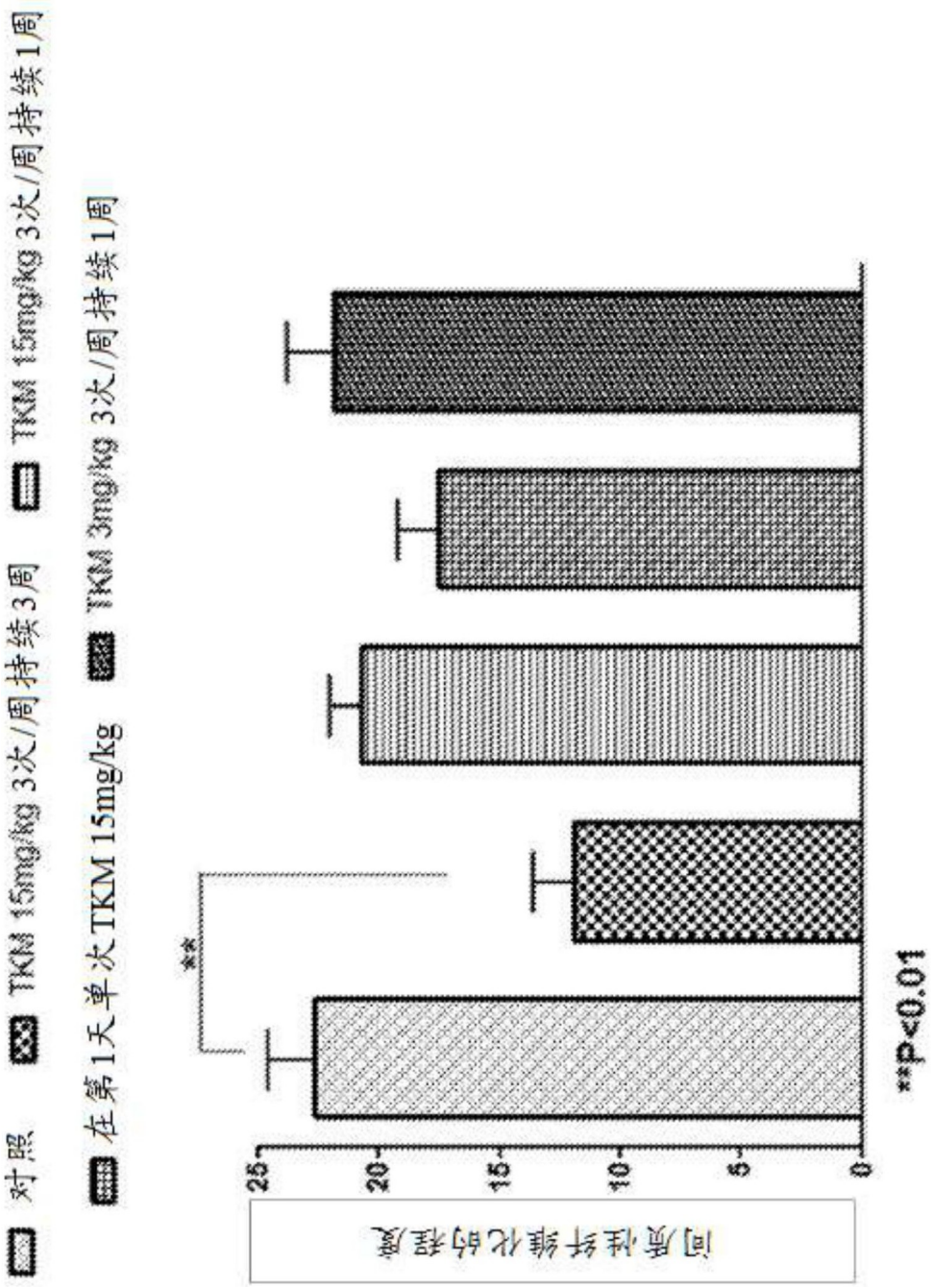

对照
TKM 15mg/kg 3次/周持续3周
TKM 15mg/kg 3次/周持续1周
在第1天单次TKM 15mg/kg
TKM 3mg/kg 3次/周持续1周
间质性纤维化的程度
25
20
15
10
5
0
**
**P<0.01

图1

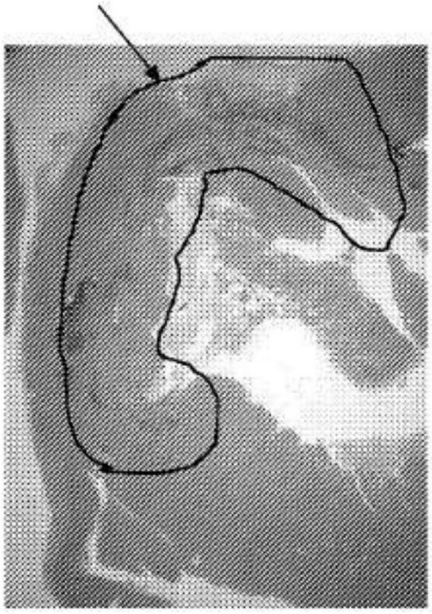


图2A

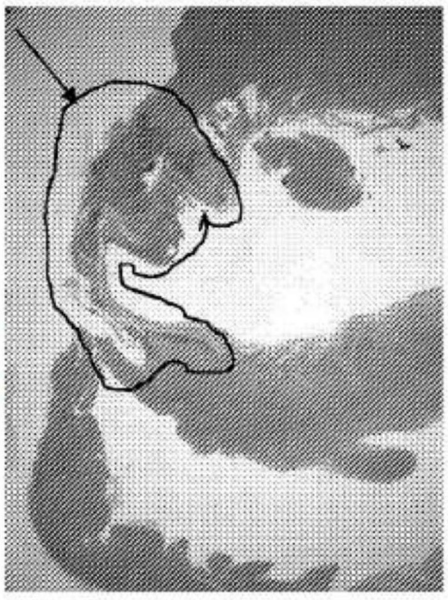


图2B

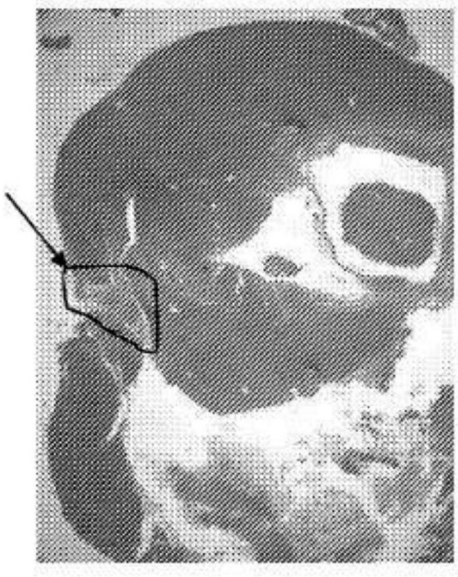


图2C

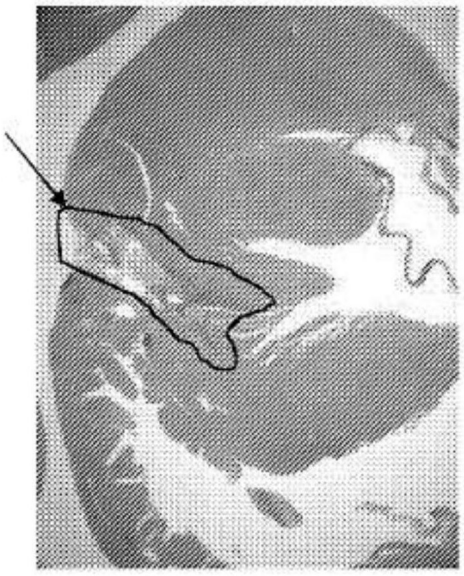


图2D