(12) UK Patent Application (19) GB (11) 2 102 811 A

(21) Application No **8219500**
(22) Date of filing **6 Jul 1982**
(30) Priority data
(31) **3337/81**
(32) **28 Jul 1981**
(33) **Austria (AT)**
(43) Application published
9 Feb 1983
(51) **INT CL**^{3}
C07G 7/00
A61K 37/02
(52) Domestic classification
C3H K1 K2
A5B 170 180 215 216 21Y
320 32Y 38Y 39X J
U1S 1311 1320 C3H
(56) Documents cited
None
(58) Field of search
C3H
A5B
(71) Applicants
Immuno AG fur
Chemischmedizinische
Produkte,
(At
72
12
At
(72) In
H
T
Y
(74) /
I

(54) A tissue adhesive and a method of producing the same

(57) A tissue adhesive based on human or animal proteins contains factor XIII, fibrinogen and an antibiotic. In order to achieve a high straining capacity of the adherences and a retarded antimicrobial efficacy, the ratio of factor XIII to fibrinogen, expressed in units of factor XIII per gram of fibrinogen, amounts to at least 500. The antibiotic is chosen among amino-glucosides, beta-lactams, polypeptides or tetracyclines. In a method of producing the tissue adhesive the concentration ratio of factor XIII to fibrinogen is adjusted in a fibrinogen-containing blood plasma fraction by the addition of factor XIII. The antibiotic is added either before deepfreezing or lyophilization of the resulting preparation, or after thawing or reconstitution of the same.

ERRATUM

SPECIFICATION NO. 2102811 A

Front page, Heading (71) Applicants *for* Immuno AG fur *read* Immuno Aktiengesellschaft für

THE PATENT OFFICE
27 May 1983

Bas 251492.

GB 2 102 811 A

(12) UK Patent Application (19) GB (11) 2 102 811 A

(21) Application No **8219500**
(22) Date of filing **6 Jul 1982**
(30) Priority data
(31) **3337/81**
(32) **28 Jul 1981**
(33) **Austria (AT)**
(43) Application published
9 Feb 1983
(51) **INT CL**^{3}
C07G 7/00
A61K 37/02
(52) Domestic classification
C3H K1 K2
A5B 170 180 215 216 21Y
320 32Y 38Y 39X J
U1S 1311 1320 C3H
(56) Documents cited
None
(58) Field of search
C3H
A5B
(71) Applicants
Immuno AG fur Chemischmedizinische Produkte,
(Austria),
72 Industriestrasse,
1220 Vienna,
Austria.
(72) Inventors
Heinz Redl,
Thomas Seelich,
Yendra Linnau.
(74) Agents
Page, White and Farrer,
27 Chancery Lane,
London WC2A 1NT.

(54) **A tissue adhesive and a method of producing the same**

(57) A tissue adhesive based on human or animal proteins contains factor XIII, fibrinogen and an antibiotic. In order to achieve a high straining capacity of the adherences and a retarded antimicrobial efficacy, the ratio of factor XIII to fibrinogen, expressed in units of factor XIII per gram of fibrinogen, amounts to at least 500. The antibiotic is chosen among amino-glucosides, beta-lactams, polypeptides or tetracyclines. In a method of producing the tissue adhesive the concentration ratio of factor XIII to fibrinogen is adjusted in a fibrinogen-containing blood plasma fraction by the addition of factor XIII. The antibiotic is added either before deepfreezing or lyophilization of the resulting preparation, or after thawing or reconstitution of the same.

GB 2 102 811 A

SPECIFICATION

Improvements in or relating to a tissue adhesive and a method of producing the same

The invention relates to a tissue adhesive based on human or animal proteins, containing factor XIII, fibrinogen and an antibiotic, as well as to a method of producing the same.

From British Patent application No. 8003775 and British patent application No. 8003776 methods are already known for producing a tissue adhesive containing fibrinogen and factor XIII, in which certain concentration ratios of factor XIII to fibrinogen and, if desired, albumin are adjusted, and the preparations are deepfrozen or lyophilized. These preparations basically exhibited satisfactory properties, i.e. a high straining capacity of the adherences and a good absorbability; however, it is desirable to improve these preparations with a view to an antimicrobial efficacy.

To be sure, it has already been proposed in U.S. patent No. 2,533,004 as well as by Fellinger et al. in the journal "Der Tuberkulosearzt" (6/11,1952) to add antibiotics to fibrinogen solutions and to use them as wound adhesives, yet these solutions to be prepared directly at the beside do not give the fibrin clots formed therefrom a sufficient durability and straining capacity.

Furthermore, it is known from the work by Bösch et al., Archiv für orthopädische und Unfall-Chirurgie, Vol. 90 (1977), pages 63 to 75, to apply a fibrin adhesive system for filling bone defects in connection with bone transplants, with the fibrin forming at the chosen site immediately at the bone cavity by the addition of thrombin to a fibrinogen solution. As required, commonly available combination preparations of pulverized neomycin were added.

Finally, it was proposed according to the PCT application No. 80/00083 to prepare a fibrinogen antibiotic gel, wherein a mixture of cryoprecipitate with tobramycin and gentamicin as antibiotics to be prepared at the bedside is used.

According to experiments carried out by Applicant it was found that the described and known tissue adhesives that contain fibrinogen, factor XIII and antibiotic do not possess the desired combination of properties, i.e. a high straining capacity of the adherences and an antimicrobial efficacy, but that an adverse interaction between the antibiotics and factor XIII takes place, which results in a strong decrease of the cross-linking ability of fibrinogen and a negative effect on the coagulability. Consequently, the adhesive exhibits a poorer rigidity and adhering capacity on the wound and tissue surfaces.

A further disadvantage of the known preparations resides in that the release of the antibiotic to the tissue takes place too quickly so that the retardation of the antibiotic does not suffice to be effective over a longer period of time and to achieve a high active substance release.

The invention aims at avoiding these disadvantages and difficulties and has as its object to provide a tissue adhesive of human or animal origin that meets the above-mentioned combination of properties and guarantees an improved efficacy of the antibiotic.

The set object is achieved with a tissue adhesive of the initially defined kind in that the ratio of factor XIII to fibrinogen, expressed in units of factor XIII per gram of fibrinogen, amounts to at least 500 and that an antibiotic selected from the group consisting of aminoglucosides, betalactams, polypeptides and tetracyclines is contained therein.

Advantageously, factor XIII is contained in a deepfrozen tissue adhesive in an amount of at least 40 units/ml.

According to another embodiment at least 33 % of fibrinogen is contained in a lyophilized tissue adhesive, factor XIII being present in an amount of at least 170 units/g of lyophilisate.

Suitably, a plasmin inhibitor or plasminogen-activator inhibitor selected from the group consisting of aprotinin, α_2-antiplasmin, α_2-macroglobulin, α_1-antitrypsin, ε-aminocaproic acid and tranexamic acid is additionally contained.

Advantageously, the tissue adhesive is a two-component preparation, factor XIII, fibrinogen and the plasmin inhibitor or plasminogen-activator inhibitor being contained in the first component and the antibiotic, thrombin and bivalent calcium being contained in the second component.

Preferably, the antibiotic is contained in the form of a hardly soluble derivative. A variant of this embodiment consists in that, in addition to the hardly soluble derivative, also an easily soluble one is used, if desired distributed in the two components of the tissue adhesive. This embodiment has the advantage that the easily soluble derivative is released quickly, thus ensuring a high initial efficacy, whereas the hardly soluble derivative causes a lasting efficacy.

Moreover, the invention comprises a method of producing the tissue adhesive with a modification consisting in that a concentration ratio of factor XIII to fibrinogen, expressed in units of factor XIII/g of fibrinogen, of at least 500 is adjusted in a fibrinogen-containing blood plasma fraction by the addition of factor XIII, whereupon the antibiotic is added and the preparation is deepfrozen or lyophilized.

According to another modification a concentration ratio of factor XIII to fibrinogen, expressed in units of factor XIII/g of fibrinogen, of at least 500 is adjusted in a fibrinogen-containing blood plasma fraction by the addition of factor XIII, whereupon the preparation is deepfrozen or lyophilized and, after thawing or reconstitution, is combined with an antibiotic-containing solution. With this embodiment the antibiotic may be added after thawing or to the reconstituted solution. However, with these embodiments it has to be taken care that the concentration of factor XIII does not fall below a minimum concentration; it is to be above 40

units/ml.

According to preferred embodiment in which the fibrinogen-containing blood plasma fraction is washed with a buffer solution, the washing procedure is carried out until a factor XIII concentration of 200 units of factor XIII/g of fibrinogen is reached, whereupon factor XIII is supplied in an amount of at least 300 units/g of fibrinogen in the form of a concentrate of lyophilisate.

The tissue adhesive according to the invention and the method of producing the same will be explained in more detail by way of the following examples.

Example 1:

Cryoprecipitate (100 g) was gained from frozen fresh human plasma by heating to 2° C, separated by centrifugation and washed twice in a buffer solution containing Na_3 citrate, NaCl, glycine, glucose, aprotinin and heparin at a pH of 6.5, and the separated precipitate was dissolved in a glycine-containing buffer solution (255 ml) at a pH of 7.9. It was found that a ratio of factor XIII to fibrinogen of 226 units of factor XIII/g of fibrinogen was contained in this solution. To adjust the ratio desired according to the invention, of more than 500 U/g of fibrinogen, a pulverized factor XIII preparation with 9,000 units was added to the solution, the concentration ratio of the solution thus having been increased to 826 units of factor XIII/g of fibrinogen. This solution was sterile-filtered; then 1.7g of gentamicin were added under sterile conditions, the mixture was filled into final containers (2.5 ml), deepfrozen and lyophilized.

Example 2:

The preparation of the tissue adhesive basis from cryoprecipitate was effected in the same manner as in Example 1, with the difference that the precipitation was liquefied by heating to 37° C after a single washing, and 13,600 units of pulverized facter XIII were added. A ratio of factor XIII to fibrinogen of 967 factor XIII units/g of fibrinogen was obtained.

5.67 g 7-[(thienyl)-(2)-acetamido]-cephalosporanic acid were added to the solution as an antibiotic. The suspension thus obtained was filled into final containers (1 ml) and deepfrozen. The filled-in preparation has a content of factor XIII amounting to 87 U/ml.

The application of the tissue adhesives prepared according to Examples 1 and 2 advantageously is realized by mixing the thawed or reconstituted mixture with thrombin and calcium chloride and applying it onto the tissue to be connected. It is also possible to apply the two components separately onto the tissue to be connected or filled.

Example 3:

The method according to Example 1 was repeated except for adding the antibiotic. The washed precipitate, after dissolving in a buffer solution, was sterile-filtered, filled into final containers (2,5 ml), deepfrozen and lyophilized, the first component of the tissue adhesive according to the invention thus having been made storable. The second component was prepared prior to application from a solution of thrombin and calcium chloride by adding 7-[(thienyl)-(2)-acetamido]-cephalosporanic acid (30 mg/ml).

Example 4:

The procedure according to Example 2 was repeated, wherein gentamicin (1.89 g) was added after dissolving the cryoprecipitate, the solution was filled into final containers (1 ml) and deepfrozen. Thus, the first component of the adhesive according to the invention is present in a storable form. The second component containing 30 mg of 7-[(thienyl)-2(2)-acetamido]-cephalosporanic acid per ml of a calcium chloride thrombin solution was prepared prior to application.

Instead of the aprotinin added in accordance with Examples 1 to 4, one or more of the following compounds may be used as plasmin inhibitor or plasminogen-activator inhibitor: α_2-antiplasmin, α_2-macroglobulin, α_1-antitrypsin, ε-aminocaproic acid and tranexamic acid.

The tissue adhesives prepared according to the invention are generally applicable for the seamless connection of human of animal tissue or organ parts, to dress wounds and stop bleedings, their antimicrobial efficacy being substantially improved.

The improved adhesive properties with an equally improved antimicrobial efficacy of the tissue adhesives according to the invention can be taken from the following comparative examples summarized in a table; the degree of crosslinking of tissue adhesives according to the invention with an increased factor XIII/fibrinogen ratio was compared to the crosslinking degree of known tissue adhesives without an increased factor XIII/fibrinogen ratio, using different antibiotics for each case. The α-crosslinking degree has been determined according to the sodiumlaurylsulfate (SDS) polyacrylamide gel electrophoresis method, which is carried out in a manner that, after having mixed the tissue adhesive with an equal volume of a solution containing 40 µMol $CaCl_2$ and 15 NIH units U.S. National Institute of Health units) of thrombin per ml, the mixture is incubated at 37° C. The α-crosslinking degree is determined after stopping the reaction and reductive cleavage of the disulfide bridges contained in the proteins, by the addition of a mixture of urea, sodiumdodecylsulfate and β-mercaptoethanol by means of gel electrophoresis.

In a further part of the table the clot rigidity of a tissue adhesive according to the invention was compared to a known one in a thrombelastograph, with gentamicin having been added as antibiotic.

Finally, the table includes comparative values of the tearing resistance of a tissue adhesive according to

the invention and of a known one, with gentamicin being used as an antibiotic.

Fibrin α-crosslinking (at 37°C after 60 min.)

Addition of antibiotic	Tissue adhesive of invention with increased factor XIII content > 500 U/g fibrinogen	Tissue adhesive without increased factor XIII content
Gentamicin	70 %	30 %
Neomycin	41 %	21 %
Fosfomycin	47 %	24 %
Axlocillin	66 %	42 %
Doxycyclin	65 %	26 %
Cefoxitin	54 %	44 %
Clot rigidity in thrombelastograph (37° C - 60 min.) ε = elasticity module		
Gentamicin	1150	426
Tear resistance in g/cm^2 (37° C - 30 min.)		
Gentamicin	1283	999

Finally, a comparative example was carried out with respect to the antibiotics release of a tissue adhesive prepared according to Example 4, 85 % of gentamicin having been released from a clot produced by this tissue adhesive already after 72 hours in an in vitro test. After 96 hours a release of gentamicin was not recognized any more, while 7-[(thienyl)-(2)-acetamido]-cephalosporanic acid was still detectable even after 8 days.

CLAIMS

1. A deepfrozen or lyophilized tissue adhesive based on human or animal proteins which comprises factor XIII, fibrinogen and an antibiotic, wherein the ratio of factor XIII to fibrinogen, expressed in units of factor XIII per gram of fibrinogen, amounts to at least 500, and said antibiotic is selected from the group consisting of aminoglucosides, betalactams, polypeptides and tetracyclines.

2. A deepfrozen tissue adhesive as set forth in claim 1, wherein factor XIII is contained in an amount of at least 40 units/ml.

3. A lyophilized tissue adhesive as set forth in claim 1, wherein at least 33 % by weight of fibrinogen is contained, factor XIII being present in an amount of at least 170 units/gram of lyophilisate.

4. A tissue adhesive as set forth in claim 1, further comprising a plasmin inhibitor or plasminogen-activator inhibitor selected from the group consisting of aprotinin, α_2-antiplasmin, α_2-macroglobulin, α_1-antitrypsin, ε-aminocaproic acid and tranexamic acid.

5. A tissue adhesive as set forth in claim 1, comprising a first component and a second component, said first component containing factor XIII and fibrinogen and said second component containing said antibiotic, thrombin and bivalent calcium.

6. A tissue adhesive as set forth in claim 4, comprising a first component and a second component, said first component containing factor XIII, fibrinogen and said plasmin inhibitor or plasminogen-activator inhibitor and said second component containing said antibiotic, thrombin and bivalent calcium.

7. A tissue adhesive as set forth in claim 1, wherein said antibiotic is present in the form of a hardly soluble derivative.

8. A method of producing a tissue adhesive based on human or animal proteins and comprising factor XIII, fibrinogen, a plasmin inhibitor or plasminogen-activator inhibitor, and an antibiotic selected from the group consisting of aminoglucosides, betalactams, polypeptides and tetracyclines, which method comprises the steps of adjusting in a fibrinogen-containing blood plasma fraction a concentration ratio of factor XIII to fibrinogen, expressed in units of factor XIII/gram of fibrinogen, of at least 500 by adding factor XIII, adding said antibiotic, and deepfreezing or lyophilizing the resulting preparation.

9. A method of producing a tissue adhesive based on human or animal proteins and comprising factor XIII, fibrinogen, a plasmin inhibitor or plasminogen-activator inhibitor, and an antibiotic selected from the group consisting of aminoglucosides, betalactams, polypeptides and tetracyclines, which method comprises the steps of

adjusting in a fibrinogen-containing blood plasma fraction a concentration ratio of factor XIII to fibrinogen, expressed in units of factor XIII/gram of fibrinogen, of at least 500 by adding factor XIII,

deepfreezing or lyophilizing the resulting preparation,

thawing or reconstituting the preparation, and

combining the preparation with a solution containing said antibiotic.

10. A method as set forth in claim 8 or 9, further comprising washing said fibrinogen-containing blood plasma fraction with a buffer solution in a washing procedure, and wherein said washing procedure is carried out until a factor XIII concentration of 200 units of factor XIII/gram of fibrinogen is reached, whereupon factor XIII is added in an amount of at least 300 units/gram of fibrinogen in the form of a concentrate or lyophilisate.

11. A tissue adhesive substantially as hereinbefore described with reference to the accompanying examples.

12. A method substantially as hereinbefore described with reference to the accompanying examples.

Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1983.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.