56498J.18

The invention relates to new preparations for preventing adhesions of organs and/or parts of organs.

After invasive therapeutic intervention or in the course of a disease, adhesions of organs or parts of organs may result in life-theatening situations. The formation of adhesions is also observed after surgical intervention in the thoracic cavities or in the abdominal cavity. In spite of greater efforts to prevent such adhesions, no satisfactory method of treatment has been discovered hitherto.

The role of fibrin formation in the formation of adhesions can be explained as follows: after invasive therapeutic treatment or in inflammatory reactions, plasma proteins as well as fibrinogen and other coagulation proteins are released from the tissue. Fibrinogen is deposited in the form of fibrin. The fibrin network which forms then connects (attaches) adjacent surfaces of organs or other parts of organs. If the fibrin is not dissolved, dense adhesions are formed which may result, for example, in dangerous constrictions of the intestine. Fibrin adhesions which have been freshly formed are gradually surrounded by fibroblasts to form permanent tissue connections.

The degree of possible spontaneous fibrinolysis depends on the release of tissue plasminogen activator (t-PA) from the vascular endothelium, but also from mesothelial cells such as those which occur in the abdominal cavity. After a peritoneal intervention, however, there is a reduction in the fibrinolytic activity in these mesothelial cells. If fibrinolysis is incomplete as a result, the fibrin residues behave like centres into which fibroblasts grow; capillaries are formed which

give rise to fibrin adhesion. They are later replaced by collagen-containing adhesions, the collagen being synthesised by the fibroblasts.

To prevent the formation of post-operative or inflammation-induced adhesions, the systemic administration of ibuprofen has been proposed (US Patent 4.346.108); other proposals concern the parenteral administration of antihistamines, corticosteroids and antibiotics or the intraperitoneal administration of dextran solutions or polyvinylpyrrolidone solutions. The appropriate use of streptokinase, streptodornase and urokinase has also been proposed [cf. Ascherl et al., Medwelt 34, No. 13/83, pages 410-415; Mund- Hoym et al., Geburtsb. u. Frauenheilkunde 44 (1984), pages 463-467; Minju et al., Acta Academiae Medicinae Wuhan 3, (2), pages 77-83].

Human fibrinolysin has also been investigated on its own and in conjunction with other drugs for its ability to suppress post-operative adhesions [cf. Gazzaniga et al., Arch. Surg. Vol. 110, pages 429-432 (1975). Holtz, The Journal of Reproductive Medicine, Vol. 24, No. 4 (1980), pages 141-146, Rivkind et al., Eur. Surg. Res. (Switzerland) 1985, Vol. 17, No. 4, pages 254-258 and Buckman et al., Journal of Surgical Research, Vol. 20, No. 1, pages 1-5] came to the conclusion that the post-operative formation of adhesions in the abdominal cavity is connected with a traumatically or ischaemically induced reduction in the activity of the plasminogen activator.

The direct topical application of tissue plasminogen activator (t-PA) to prevent post-operative intraperitoneal adhesions, which may be concluded from this, was finally described in EP-A-0227400. T-PA which occurs in the body both as a single-stranded and as a double-strained molecule, has a high affinity for fibrin

which forms clots. Natural t-PA is glycosylated and contains fatty acid residues. All these types of t-PA have a specific affinity for fibrin and at the same time activate plasminogen to form plasmin. Plasmin effects the proteolytic breakdown of fibrin. Since fibrin is pathophysiologically responsible for the formation of adhesions, t-PA results in the prevention of adhesion formation and, in some cases, in the removal of adhesions already formed, e.g. in the abdominal cavity after surgical intervention or after inflammatory processes. The t-PA used is isolated from human tissue or obtained using recombinant DNA technology (cf. GB-A-2119804, EP-A-0174835 and 0100982).

According to EP-A-0227400 mentioned above, t-PA is applied topically, for example, in the area of surgical intervention, if possible immediately after surgery has ended or before the wound has started to heal, in order to prevent adhesion of sections of tissue and/or organs or, in the case of inflammatory processes, it is applied to the affected areas and if necessary a t-PA preparation is additionally applied slowly through a catheter which ends at the site of intervention.

Sterile t-PA preparations containing a pharmaceutically acceptable carrier, e.g. a phosphate-buffered saline solution, an isotonic saline solution or purified water, are described. Suitable organic carriers include lipids, e.g. phosphorus lipid micelles or vesicles and also dextran, polymers such as p-dioxanones, lactides and/or glycolides in the form of adsorbable polymers which are microencapsulated or embedded in ointment bases or occur in an aqueous solution of a surfactant substance, e.g. a polyoxy-ethylene/polyoxypropylene block copolymer or a sorbitan fatty acid ester/polyoxyethylene ether. The preferred preparations are those in which t-PA is contained in a carrier with

delayed release of the active substance, which releases the active substance in controlled manner within a period from 1 to 7 days. Examples of carriers with delayed, controlled release of active substance include adsorbent polymers which occur as microcapsules or are present in an ointment base, but more particularly phospholipid vesicles, so-called liposomes.

WO-A-8 400 111 describes preparations for treating burns, cuts, wounds and grazes of the outer layers of the body (skin). These preparations consist of a heat-sterilisable aqueous gel which contains a pharmaceutically useable glycol and a heat-sterilisable cellulose derivative, e.g. hydroxyethylcellulose, and optionally an antiseptic or antibiotic.

It has now been found that t-PA or rt-PA (r = recombinant), dissolved in a hydroxyethylcellulose gel, will prevent the formation of adhesions almost entirely, even if this hydrogel is applied only once, i.e. shortly after surgical intervention or after an inflammatory irritation on the areas with a tendency to adhesions and accretions.

In order to produce a suitable preparation, a microbiologically pure hydroxyethylcellulose is dissolved in water to form a hydrogel. Salts such as sodium chloride may be added to the water to make it isotonic, as well as buffer substances such as dipotassium hydrogen phosphate and sodium dihydrogen phosphate. The preferably lyophilised t-PA or rt-PA is dissolved in the sterilised hydrogel, and this hydrogel ready for use may also be stored for some days at low temperatures. For longer storage periods, however, it is advantageous to keep the sterilised hydrogel and the lyophilised t-PA or rt-PA in separate containers and prepare the solution only just before it is required by mixing the two

components together.

Tests have shown that even hydroxyethylcellulose hydrogel on its own has an adhesion-preventing effect which is up to 40% compared with cases in which no such preventative treatment is carried out. The presence of t-PA causes an increase in the preventive activity of hydroxyethylcellulose hydrogel.

A hydrogel preparation according to the invention may also readily be instilled, e.g. through a channel in an endoscope, into sites which are at risk, e.g. in body cavities, if the viscosity of the preparation is reduced.

An aqueous hydrogel according to the invention consists of from 1 to 3 g of hydroxyethylcellulose dissolved in 97 to 99 g of water or physiological saline solution and contains 0.1 to 50 mg/ml of t-PA or rt-PA, but preferably 0.3 to 10 mg/ml with or without the addition of basic amino acids, e.g. lysine, arginine.

The water soluble hydroxyethyl ethers of cellulose used are preferably those with an average molecular substitution level of from 1.5 to 3.0 hydroxyethyl groups per unit of anhydroglucose. A preferred hydrogel consists of 2% by weight of hydroxyethyl-cellulose (e.g. Natrosol 250 HX$^{(R)}$), dissolved in water, physiological saline or buffer solution and contains 2 mg/ml of t-PA or rt-PA.

A mixture of 1 mg/ml of rt-PA and 2% by weight of hydroxyethylcellulose in physiological saline solution was investigated by comparison with a mixture of 2% by weight of hydroxyethylcellulose in physiological saline solution and controls (using only physiological saline or buffer solution) in rabbits. Adhesions were produced

a) by applying stitches to the abdominal wall after laparotomy and

b) by irritating the peritoneal tissue in the region of caecum and ileum and the small pelvis with an iodine solution.

When stitches were inserted in the abdominal wall after laparotomy (case a) the test substance was applied and the laparotomy incision was closed up in several layers. The operating surgeon or investigator was not aware of the composition of the test substance. After 1 week, in a large section of skin, the area of the old laparotomy incision was cut and folded open, the length of intestinal tissue adhering to the laparotomy incision was measured and the length which had adhered was expressed as a percentage of the length of the laparotomy incision.

The test substance (with and without t-PA) was applied, in case b), immediately after the intervention and before the abdominal cavity had been closed up. Once again, the operator was not aware of the composition of the test substance.

Seven days after this intervention, the animals were subjected to laparotomy once more under anaesthesis to determine the extent of the adhesions. The area of firm adhesions in the treated sections of intestine in the damaged areas was measured. The results are shown as the percentage adhesion based on the area of damage. In addition, the body weight of the animals was determined on the day of the original laparotomy and the second laparotomy.

The results of these experiments are shown in Table 1. The x axis shows the extent of percentage adhesion or

the percentage change in body weight for

1) the control,

2) Natrosol (HEC), 2% by weight in physiological saline solution, and

3) t-PA in Natrosol (1 mg rt-PA per ml of physiological saline solution containing 2% by weight of HEC).

A comparison of the results in Table 1 shows that hydroxyethylcellulose hydrogel on its own brought about, on average, a 40 to 45% reduction (based on the controls) in the adhesions induced by the conditions, in the caecum and ileum, but in conjunction with rt-PA there was an almost total (95%) reduction in the caecum and a reduction of approximately 85% in the ileum. As for the adhesion of the laparotomy incision (abdominal wall) with the underlying tissue, hydrogel on its own results in a substantial reduction; however, the presence of rt-PA prevents adhesion completely.

The use of the hydrogel preparations according to the invention containing t-PA and rt-PA significantly minimises the loss of body weight during the first stage of healing.

The Examples which follow describe the production of preparations according to the invention.

Example 1

rt-PA set containing 0.1 mg rt-PA/ml

1) In a suitable container, 2 g of microbiologically pure hydroxyethylcellulose are sprinkled into 98 g of sterile-filtered physiological saline solution

and dissolved therein with stirring at ambient temperature. The gel formed is transferred, under laminar air flow, into 50 ml vials with insertable caps, then sealed with rubber stoppers and flanged, then sterilised for 30 minutes at 121°C in pressurised steam. The sealed gel can be stored for two years at ambient temperature.

2) rt-PA is lyophilised in quantities of 10, 20 and 30 mg under sterile conditions in vials provided with insertable caps, then sealed with rubber stoppers and flanged. The sealed vial can be stored for up to two years at ambient temperature (up to 25°C).

3) Solution ready for use:
5 mg of lyophilised rt-PA are introduced, under laminar air flow conditions, into 50 g of sterile 2% by weight hydroxyethylcellulose gel and dissolved therein with stirring using a sterile glass rod.

This gel ready for use may either be applied to the stitches in the wound immediately or may be stored for a few days at 4°C in a refrigerator.

Example 2

rt-PA gel containing 1.0 mg of rt-PA/ml

Before use, 50 mg of lyophilised rt-PA are introduced into 50 g of sterile, 2% by weight hydroxyethylcellulose gel which has been made isotonic and is dissolved therein by stirring with a sterile glass rod.

The gel ready for use may be stored for a few days in the refrigerator.

Example 3

rt-PA gel containing 10.0 mg of rt-PA/ml

Before use, 500 mg of lyophilised rt-PA are introduced into 50 g of sterile, 2% by weight hydroxyethylcellulose gel which has been made isotonic.

The finished gel may be stored for a few days in the refrigerator.

Claims

for the contracting states: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Preparations for preventing adhesions of organs and parts of organs after invasive therapeutic intervention or in the course of a disease, containing an aqueous hydrogel based on hydroxyethylcellulose and t-PA or rt-PA.

2. Preparations according to claim 1, characterised in that isotonic additives and optionally buffer substances are also added to the hydroxyethylcellulose hydrogel containing the t-PA or rt-PA.

3. Preparations according to claim 1 and 2, characterised in that they contain as hydrogel 1 to 3% by weight of hydroxyethylcellulose in aqueous solution and 0.1 to 50 mg/ml of t-PA or rt-PA.

4. Preparation according to claim 3, characterised in that it contains 0.3 to 10 mg/ml of t-PA or rt-PA.

5. Preparations according to claims 1, 2, 3 and 4, characterised in that they contain as hydroxy-ethylethers of cellulose those having an average molecular substitution level of from 1.5 to 3.0 hydroxyethyl groups per unit of anhydroglucose.

6. Preparation according to claims 1 to 5, characterised in that it contains 2% by weight of hydroxyethylcellulose and 1 to 2 mg/ml of rt-PA in a buffer solution.

7. Preparations according to claims 1 to 6, characterised in that the sterilised hydrogel and the lyophilised t-PA or rt-PA are stored in separate

containers which enable the finished solution to be made up just before use by mixing these two components.

8. Use of hydroxyethylcellulose and t-PA or rt-PA in aqueous solution for the production of preparations for preventing adhesions of organs or parts of organs after invasive interventions or in the course of a disease according to claims 1 to 7.

* C L M S *

Claims for the contracting states ES, GR

1. Process for producing preparations for preventing adhesions of organs and parts of organs after invasive therapeutic intervention or in the course of a disease, characterised in that a sterilised hydroxyethylcellulose is dissolved in water to form a hydrogel and t-PA or rt-PA is dissolved in this hydrogel.

2. Process according to claim 1, characterised in that salts are added to the water to render it isotonic and optionally buffer substances are also added.

3. Process according to claims 1 and 2, characterised in that 1 to 3 g of hydroxyethylcellulose are dissolved in 97 to 99 g of water or physiological saline solution and 0.05 to 50 mg/ml of t-PA or rt-PA are dissolved in the solution.

4. Process according to claim 3, characterised in that a hydroxyethylcellulose with an average molecular substitution level of from 1.5 to 3 hydroxyethyl groups per unit of anhydroglucose is used to prepare the hydrogel.