High dosage topical forms of collagenase
Hochdosierte topische Kollagenaseformen
Formes topiques de collagénase hautement dosées

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Description

[0001] Collagenase ointment has been available in the United States as Collagenase Santyl® Ointment (Advance Biostructures Corp., Lynbrook, NY 11563) for 27 years. It has been used on millions of patients. The concentration of collagenase in Santyl® Ointment has been no greater than 300 ABC units per gram of ointment. Collagenase ointment has also been available in other countries. It is useful for the debridement of burns and of dermal ulcers, particularly bed sores (decubitus ulcers). The debridement of these lesions is necessary to remove dead and dying tissue that is typically a source of microbial infection. In addition, healing does not take place until this necrotic material is removed. Speed of debridement is thus a therapeutic desideratum.

[0002] Collagenase ointment has not been so widely accepted by the burn centers in the treatment of third degree burns as perhaps its efficacy deserves. This lack of acceptance is largely due to the perception that third degree burns in particular require a more rapid debridement than the collagenase ointment can provide. A more rapid debridement of severe burns without the necessity for anesthesia or a surgical operation would constitute a therapeutic advancement.

[0003] A more rapid debridement would also be useful in the treatment of dermal ulcers since it would provide a superior cost/benefit profile.

[0004] Dextrans are polysaccharides produced by certain bacteria (e.g. Leuconostoc mesenteroides) and consist of chains of α-D-glucopyranosyl residues linked predominantly by α-(1→6)-linkages, with a small fraction of α-(1→3)-linkages which give rise to chain branching. Dextrans are available in various molecular-weight fractions. Dextran fractions with weight average molecular weights of 40,000, 70,000 and 75,000 daltons have found therapeutic uses as plasma volume expanders. The 40,000-dalton fraction is also used as a blood flow adjuvant. [ref. Merck Index, Tenth Edition, #29111] In addition, dextran is used in lubricant eye drops and hysteroscopy fluids. [ref. PDR, 45th edition, 1991].

[0005] Dextrans are commercially available as a fine white powder that is approved for pharmacological use. The powder absorbs water readily and hence is useful as a drying agent for wounds, and is completely soluble in sufficient amounts of water.

[0006] Pharmacologically active ingredients such as enzymes, antibiotics, antifungals, anti-inflammatory agents, antipyretics, etc. are usually diluted with an excipient for topical use as creams, ointments, lotions, solutions, etc. Many such excipients decrease the shelf life of drug substances.

[0007] The enzyme collagenase is derived from fermentation by Clostridium histolyticum, and is purified by a chromatographic technique. It possesses the unique ability to digest native and denatured collagen in necrotic tissue. Advantageously, one gram of the admixture contains from 500 to 5,000 ABC units of collagenase; and, as will be explained below, an especially advantageous mixture contains in excess of 2,500 and up to 10,000 ABC units of collagenase per gram of dextran. It is useful for debridement of burns and of decubitus ulcers, generally known as bed sores. The mixture can be shaken or sprayed onto the burn or ulcer, and a homogenous mixture of the dextran and collagenase will thus reach the affected site. The fluids available from the wound will dissolve the dextran and make the active enzyme available where it is needed.

[0008] This invention provides a pharmaceutical composition for topical use comprising an intimate admixture of a non-aqueous excipient and at least 1,500 ABC units of collagenase per gram of excipient. When dextran or another non-aqueous excipient is mixed with the enzyme collagenase at a collagenase concentration much greater than has heretofore been used in practice, and higher than heretofore mentioned in the literature to our knowledge, the resulting compositions, when used topically to treat burns, ulcers and other wounds, provide rapid debridement of dead and dying tissue without causing undesirable side effects.

[0009] The pharmaceutical compositions of this aspect of the invention preferably contain at least 2,500 and up to 10,000 ABC units of collagenase per gram of excipient. For many applications the concentration will exceed 5,000 units/gram of excipient, e.g., 8,000 units/gram of excipient. In general, within these ranges one should use higher concentrations in powdered or liquid compositions than in ointments, because more of the latter can be applied and maintained on the area to be treated. Preferred ranges for ointments are 1,500 to 5,000 and for powders or liquids are 2,500 to 10,000 ABC units collagenase per gram of excipient.

[0010] These pharmaceutical compositions are prepared by intimately admixing a sterilized collagenase powder with a non-aqueous solid or liquid excipient. Excipients that can be used include (but are not limited to) dextran, white petrolatum USP, isopropyl myristate NF, and lactose NF. In addition, an antibiotic or antiseptic powder such as Polysporin® (a mixture of polymyxin B sulfate and bacitracin in powder form), gentamicin, and/or silver sulfadiazine may be added, or may constitute the excipient itself.

[0011] By non-aqueous excipient is meant a liquid or solid material that is inert towards, i.e., does not significantly affect adversely the physiological activity of the collagenase, and that is substantially free from water. Water is an undesired constituent. The water or other aqueous solutions of collagenase taught in the literature, if prepared in advance for use, would generally have a safe shelf life at room temperature of not over two weeks.

[0012] The potency assay of collagenase is based on the digestion of denatured collagen (from bovine tendon) at pH 7.2 and 37°C for 20-24 hours. The number of peptide bonds cleaved are measured by reaction with
ninhydrin. Amino groups released by a trypsin digestion control are subtracted. One net ABC unit of collagenase will solubilize ninhydrin reactive material equivalent to 1.09 nanomoles of leucine per minute.

[0013] Sterilized collagenase powder is available having a minimum assay of 50 ABC units per mg. The assay may range considerably above that from batch to batch, but is taken into account in determining the weight of powder to admix with excipient to give the desired number of collagenase units per gram of excipient.

[0014] Dextran is useful for the delivery of desired amounts of medication to topical wounds, burns, infections, inflammations, lacerations, ulcers. Included in such medications are collagenase and other enzymes, antibiotics, anesthetics, antifungals, anti-inflammatory agents (steroidal and non-steroidal).

[0015] Since the affected area is not touched, the application of the dry powdered mixture of dextran and medication can be less painful than would be the case if the medication were applied as a cream, gel, lotion, ointment, etc. In addition, no cream, gel, lotion, or ointment needs to be removed between dressings; the dextran formulation is soluble, only gentle massage with an appropriate liquid, such as normal saline, is needed to cleanse the area.

[0016] Dry powdered dextran provides the following further advantages for use in admixture with dry powdered pharmaceuticals:

1. They readily dissolve in the fluids available at a wound site.
2. Their dissolution provides an in situ release of the active ingredient.
3. All of the active ingredients are available at the wound site, as opposed to an ointment, wherein some of the active ingredient may remain trapped in the ointment matrix.

[0017] There are also economic advantages to using dextran as described herein since the mixing and filling of dry ingredients is less costly than the mixing of ointments, creams, and lotions and their filling into tubes and/or glass jars.

[0018] Thus dextran of various molecular weights, fine dry powders that have no intrinsic therapeutic activity and that are well tolerated by man and animals, can safely and advantageously be used as carriers for dry pharmaceuticals when used topically.

[0019] Whether application is made by dusting or spraying, a homogeneous dry powdered mixture of the dextran and collagenase will reach the affected site, and the aqueous fluids available from the wound will dissolve the dextran and make the active ingredient available where it is needed.

[0020] Dextran with 1/2 - 20% by weight collagenase has a particular utility as a debriding agent.

[0021] While ranges of weight per cent are given, one skilled in the pharmaceutical arts will make the choice based on activity of the drug and appropriate concentrations for the intended use. More than one concentration of a particular drug may be made available to the physician. In general, for most pharmaceuticals, the concentration will be within the broad range of 0.01 to 30 weight percent pharmaceutical in the mixture.

[0022] Dextran used will ordinarily be in the range of 20,000 to 100,000 daltons molecular weight. The intended use may affect the choice, the higher molecular weights giving a more viscous drug-containing liquid when the powder absorbs exudate from the wound.

[0023] Dextran and most other dry excipients are available commercially as fine dry powders, as is purified collagenase. The mixing of dry powders is within the skill of the art, and various kinds of apparatus can be obtained from commercial suppliers. Taking dextran as an example, it is best to mix and package in a controlled atmosphere of low or zero humidity. For many drugs subject to easy oxidation, an inert atmosphere, e.g. nitrogen or helium, can be used.

[0024] Rather than mixing dry powders, it is possible to dissolve dextran or other soluble excipient and the desired pharmaceutical(s) in a solvent, usually water with or without another water soluble solvent such as a lower alcohol, and either precipitate the solutes as by chilling or adding a non-solvent followed by drying, or spray-dry the solution, or lyophilize the solution, to obtain the dry powdered mixture of pharmaceutical and excipient. Drying of a precipitate followed by grinding, if necessary, should be carried out at near room temperature or lower in a selected atmosphere as described above; likewise spray-drying, which can also advantageously be conducted in vacuo. All such operations are within the skill of the art.

[0025] The particle size of final product is not critical, so long as it dusts or flows easily.

[0026] Since dextran and a number of other powdered excipients absorb moisture easily, and many drug substances are adversely affected by water, our dry powder pharmaceutical compositions should be packaged so as to prevent moisture from entering; therefore, the material from which the package is constructed should be a vapor barrier, and replaceable closures should ensure a tight seal.

[0027] Packages may take on a number of forms, selected and designed for different needs:

1. Shaker containers, whereby the mixture can be dusted over open surface areas.
2. Aerosol containers (atomizers), whereby the mixture can be sprayed onto or into an affected area by gentle gas or air pulses.
3. Single unit envelopes, which may contain, say, from 1/2 to 30 grams of the mixture as a single unit dose. Shaker and/or aerosol containers can be fitted with volume controls so that a predetermined quantity (single unit dose) of the powdered mixture
is released.

[0028] The preparation of ointments by various procedures is within the skill of the art, and various kinds of apparatus can be obtained from commercial suppliers. The high-dosage collagenase ointments of this invention can be packaged in glass jars, squeezable tubes, or in sealed single unit dose envelopes.

[0029] The admixture of finely divided solids with liquids is likewise within the skill of the art, as by using high-speed bladed stirrers or other commercially available apparatus. Liquid compositions of this invention can be packaged in bottles, jars, single unit dose envelopes, or preferably aerosol containers which should be well shaken before use to spray onto or into the area to be treated.

[0030] It may be desirable to include in our pharmaceutical compositions one or more other medicaments. Often an antibiotic or antiseptic is added for general prophylaxis against infection and/or to fight infection already present. Other useful additions are anti-inflammatory agents and local anesthetics or analgesics.

[0031] For the convenience of the physician, nurse, or other user, a pharmaceutical kit may be sold containing a shaker, spray can, tube or other package containing a pharmaceutical composition of this invention together with a separate shaker or spray can or other package containing an antibiotic in any conventional form. Rather than in addition to the antibiotic, one can use in a separate package in the kit any medicament intended to reduce infection or to alleviate pain or to induce general healing.

[0032] With respect to our high-dosage collagenase compositions, in addition to the non-aqueous excipients mentioned above, further examples of those that may be used are powdered cornstarch, talc. A further example of ointment base is lanolin (caution: allergenic to a small percentage of the population). Suitable liquid excipients are mineral oil, glycerol. Any material proposed for use as an excipient must first be tested in the intended formulation to determine that it is indeed substantially inert towards the collagenase over a considerable length of time, i.e., the desired assured shelf life.

DRAWINGS

[0033] Chart 1 shows percentage derbridement as a function of time, as determined in Experiment Number 1 below, using two different concentrations of collagenase in Polysporin(R), one ten times greater than the other.

[0034] Chart 2 shows percentage derbridement as a function of time, as determined in Experiment Number 2 below, using two different concentrations of collagenase in petrolatum, one ten times greater than the other.

[0035] Chart 3 shows percentage derbridement as a function of time, as determined in Experiment Number 3 below, using two different concentrations of collagenase in lactose NF, one five times greater than the other.

[0036] Sterile collagenase powder is available from Advance Biofactures Corporation of Lynbrook, NY 11563.

[0037] White petrolatum USP is commercially available from Witco Chemical.

[0038] Polysporin(R) is commercially available from Burroughs Wellcome.

[0039] Lactose NF is commercially available from a number of sources, e.g., DMV Campina, Inc.

[0040] A number of experiments were carried out to compare the debridging effect of a high-dosage pharmaceutical preparation with a normal dose preparation. In each experiment a number of guinea pigs were anesthetized and were given bilateral third degree burns by being scalded for 20 seconds with a 100-ml beaker containing boiling water. This method produces a well-defined burn and burn eschar of a reproducible size. Some of the burns were treated with the standard amount of collagenase. The other burns were treated with up to ten times the standard amount. All burns were treated with antibiotic. The percentage derbridement was assessed by visual inspection and by serial photographic evidence.

[0041] The following examples illustrate the difference between standard dose preparations and high-dosage preparations.

Experiment Number 1: Eight guinea pigs were given bilateral third degree burns. Seven of the burns were controls and were treated daily by sprinkling approximately 1 g of Polysporin(R) which contained 800 ABC units of collagenase powder. The lesion was then covered with a 3x3-in sterile gauze pad containing a thin layer of sterile petrolatum. This procedure was repeated for 4 days. The test sides were treated in an identical manner, except that each gram of Polysporin(R) contained 8,000 ABC units of collagenase powder. (The presence of a Polysporin(R)-resistant Proteus mirabilis infection necessitated the use of gentamicin powder, which was sprinkled on the wound after treatment with collagenase/Polysporin(R) but before covering with the gauze pad. Sides 61L, 61R, 62L, 62R, 63L, 63R, 64L, and 64R were treated with gentamicin on the second, third, and fourth days subsequent to burning.)

[0042] The results of this experiment are presented in Table 1 and Chart 1. Note that the average percentage derbridement with 8,000 ABC units is significantly better (at the 99% degree of confidence, based on the Wilcoxon test) than the derbridement seen with 800 ABC units for all four days.

Experiment Number 2: Eight guinea pigs were given bilateral third degree burns. Half of the burns were controls and were treated daily by applying a sterile gauze pad containing about 3 g of an ointment of white petrolatum USP containing 270 ABC units of collagenase per gram of petrolatum. This procedure was repeated for 4 days. The test sides were treated in an identical man-
ner, except that the petrolatum used contained 2,700 ABC units of collagenase powder per gram of petrolatum. Gentamicin powder was sprinkled onto the burns of animals 80, 81, 82, and 83 before the collagenase/petrolatum ointment was applied. Similarly, silver sulfadiazine powder was used on animals 86, 87, 88, and 89.

The results of this experiment are presented in Table 2 and Chart 2. Note again that the high-dosage treatment debrided significantly faster than the standard-dose treatment.

**Experiment Number 3:** Seven guinea pigs were given bilateral third degree burns. Half of the burns were controls and were treated daily by sprinkling on the wound silver sulfadiazine followed by approximately 1 g of lactose NF that contained 800 ABC units of collagenase powder. The burn was then covered with a 3x3-in sterile gauze pad containing a thin layer of sterile petrolatum. This procedure was repeated for 4 days. The test sides were treated in an identical manner, except that each gram of lactose NF contained 4,000 ABC units of collagenase powder.

The results of this experiment are presented in Table 3 and Chart 3. Note again that the high-dosage treatment debrided significantly faster than the standard dose treatment.

**EXAMPLES WITH DEXTRAN**

Comparisons were made of the rate of debridement of burns when treated with dextran/collagenase combinations and with collagenase-containing ointment (Santyl<sup>(R)</sup> Ointment; contains 250 ABC units of collagenase per gram of white petrolatum USP; manufactured by Advance Biofactures Corp. of Lynbrook, NY 11563).

Four burn experiments, comprising a total of eighteen guinea pigs, were carried out to compare the debriding effect of dextran/collagenase combinations to that of Santyl<sup>(R)</sup> Ointment. An antibiotic was used in all cases. Thirteen sides were each treated with 3 grams of Santyl<sup>(R)</sup> Ointment. Seven sides were treated with a dextran/collagenase mixture. Sterile gauze pads with a thin layer of sterile petrolatum to avoid sticking were used on all surfaces containing the dextran/collagenase application. 0.5 gm of the dextran/collagenase combination is one application on a burn surface.

**Experiment Number I:** Neosporin powder was used as the antibiotic in conjunction with Santyl<sup>(R)</sup> Ointment. The powder was first sprinkled on the surface of the wound. Gentamicin cream was used as the antibiotic in conjunction with dextran containing 750 ABC units of collagenase powder per gram of dextran. The dextran/collagenase combination showed faster debridement in the first 48 hours of the experiment. By the fourth day, all sides showed equal percentage debridement. The edge of the burn area was more completely debrided when using the dextran/collagenase powder combination.

**Experiment Number II:** Santyl<sup>(R)</sup> Ointment containing 0.1% Gentamicin sulfate powder was compared to dextran/collagenase/Gentamicin sulfate powder at 750 ABC units/g and 375 ABC units/g concentrations of collagenase powder in the dextran. The powder contained 0.1% Gentamicin sulfate. The dextran/collagenase/Gentamicin powder with 750 ABC units/g concentration of collagenase showed faster debridement than the other two test preparations in the first 48 hours. By the fourth day, all sides showed equal percentage debridement. The edge of the burn area was more completely debrided when using the dextran/collagenase combinations.

**Experiment Number III:** This was the same as Experiment Number II, except that an additional two animal sides were treated with a dextran/collagenase/Gentamicin combination containing 1500 ABC units/g concentration of collagenase. The results showed that the dextran/collagenase combination using all three different concentrations of collagenase exhibited faster debridement in the first 48 hours, with the 1500 ABC units/g concentration being the fastest compared to Santyl<sup>(R)</sup> Ointment.

**Experiment Number IV:** The debridement effect of Santyl<sup>(R)</sup> Ointment was compared to that of a dextran/collagenase combination with 1500 ABC units of collagenase powder per gram of dextran. The antibiotic used was silver sulfadiazine powder sprinkled onto the wound surface before the application of the treatment. At the end of the experiment (4 days), the dextran/collagenase treated side had a greater percentage of debridement than the Santyl<sup>(R)</sup> Ointment treated side.

**Claims**

1. A pharmaceutical composition for topical use comprising an intimate admixture of a non-aqueous excipient and at least 1,500 ABC units of collagenase per gram of excipient.

2. A composition according to claim 1 in the form of an ointment.

3. A composition according to claim 2 wherein the excipient is petrolatum.

4. A composition according to either of claims 2 or 3 containing from 1,500 to 5,000 ABC units of collagenase per gram of excipient.

5. A composition according to claim 1 wherein the excipient is a dry powder.

6. A composition according to claim 5 wherein the excipient is lactose.

7. A composition according to claim 5 wherein the
8. A composition according to claim 5 wherein the excipient is dextran.

9. A composition according to any of claims 5-8 containing from 2,500 to 10,000 ABC units of collagenase per gram of excipient.

10. The use of an intimate admixture of a non-aqueous excipient and at least 1500 ABC units of collagenase per gram of excipient in the preparation of a medicament for topical application to wounds, burns, scalds and dermal ulcers.

Patentansprüche


2. Zusammensetzung nach Anspruch 1 in der Form einer Salbe.

3. Zusammensetzung nach Anspruch 1, worin der Träger Vaseline ist.

4. Zusammensetzung nach einem der Ansprüche 2 oder 3 mit einem Gehalt von 1.500 bis 5.000 ABC-Einheiten Collagenase je Gramm des Trägers.

5. Zusammensetzung nach Anspruch 1, worin der Träger ein trockenes Pulver ist.


Revendications

1. Une composition pharmaceutique pour usage local comportant un mélange intime d’un excipient non aqueux et au moins 1.500 unités ABC de collagénase par gramme d’excipient.

2. Une composition selon la revendication 1, sous la forme d’une pommade.

3. Une composition selon la revendication 2, dans laquelle l’excipient est le pétrolatum.

4. Une composition selon l’une quelconque des revendications 2 ou 3, contenant de 1.500 à 5.000 unités ABC de collagénase par gramme d’excipient.

5. Une composition selon la revendication 1, dans laquelle l’excipient est une poudre sèche.

6. Une composition selon la revendication 5, dans laquelle l’excipient est le lactose.

7. Une composition selon la revendication 5, dans laquelle l’excipient est un mélange de sulfate de polymixine B et de bacitracine sous forme de poudre.

8. Une composition selon la revendication 5, dans laquelle l’excipient est le dextrane.

9. Une composition selon l’une quelconque des revendications 5 à 8, contenant de 2.500 à 10.000 unités ABC de collagénase par gramme d’excipient.

10. L’utilisation d’un mélange intime d’un excipient non aqueux et d’au moins 1.500 unités ABC de collagénase par gramme d’excipient dans la préparation d’un médicament pour application locale à des plaies, à des brûlures, à des ébouillantements et à des ulcères cutanés.
Chart 1: Collagenase/Polysporin

Debridement as a function of time

% Debridement

Days after scalding

- Control
- Test
Table 2: Percentage Debridement in Experiment Number 2 (Collagenase/Petrolatum)

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8.5*  

*significant at the 95% degree of confidence (two-tailed test)
Chart 1: Collagenase/Polysporin

Debridement as a Function of Time

% Debridement

Days after scalding

- control
- test
**Table 1: Percentage Debridement in Experiment Number 1**
(Collagenase/Polysporin®)

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\[ U^* = 0.5 \]

*All U values are significant at the 99% degree of confidence (two-tailed test).*
Table 3: Percentage Debridement in Experiment Number 3  
(Collagenase/Lactose)

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</table>

\[ U \]
8* 4* 11

*significant at the 95% degree of confidence (two-tailed test)
Chart 3: Collagenase/Lactose

Debridement as a Function of Time

% Debridement

Days after scalding

control

Test