REDUCTION OF LIPOMA TISSUE

Applicant: Advance Biofactures Corporation, Lynbrook, NY (US)

Inventors: EDWIN H. WEGMAN, Hewlett Bay Park, NJ (US); BURTON BRONSTHER, HEMPTSTEAD, NY (US); ERWIN T. JACOB, STONYBROOK, NY (US)

Appl. No.: 15/384,020

Filed: Dec. 19, 2016

Related U.S. Application Data

Continuation of application No. 15/005,597, filed on Jan. 25, 2016, which is a continuation of application No. 12/724,747, filed on Mar. 16, 2010, now abandoned, which is a continuation of application No. 11/237,543, filed on Sep. 28, 2005, now Pat. No. 7,824,673, which is a continuation of application No. 10/172,601, filed on Jun. 14, 2002, now Pat. No. 6,958,150.

Publication Classification

Int. Cl.
A61K 38/48 (2006.01)
A61K 9/00 (2006.01)
A61K 9/08 (2006.01)
A61K 9/19 (2006.01)
A61K 47/02 (2006.01)

U.S. Cl.
CPC ........ A61K 38/4886 (2013.01); A61K 9/19 (2013.01); A61K 47/02 (2013.01); A61K 9/08 (2013.01); C12Y 304/24003 (2013.01); A61K 38/48 (2013.01); A61K 9/0019 (2013.01)

ABSTRACT

The amount of adipose tissue, including lipomas, at selected locations in the body is reduced by introducing collagenase or collagenase plus another proteinase into the tissue.
REDUCTION OF LIPOMA TISSUE

RELATED APPLICATIONS


BACKGROUND OF THE INVENTION

[0002] Liposuction (otherwise known as suction lipectomy, suction assisted lipectomy dissection, as well as by other names), is a procedure that mechanically removes fat from the subcutaneous tissues. It has been used primarily in cosmetic surgery to extract adipose tissue at specific areas of the male and female human body. Less common uses of liposuction have been removal of lipoma (benign fatty tumor) and much less commonly it has been used for the removal of unusual fatty tumors. It has also been used as a staged procedure for weight loss with questionable success.

[0003] The procedure is carried out by anesthetizing the patient to a varying degree or the area that is to be treated. Small incisions are made at points chosen by the treating physician and a canulla (a long hollow metal tube) having a series of holes along its length is inserted into the subcutaneous adipose tissue. A vacuum of roughly one negative atmosphere is applied and the semi-solid fat is mechanically loosened by the combined forces of the pushing and pulling of the canulla and the vacuum. The loosened fatty tissue is then drawn by vacuum through the canulla and removed from the body.

[0004] Liposuction by mechanical-vacuum means is a very common and desirable procedure performed around the world. However, the mechanical traumatization of the subcutaneous fat caused by this method of removal carries with it significant morbidity and other undesirable post-operative effects including, but not limited to, ecchymosis (black and blue skin), infection, hematoma, prolonged edema, and contour deformity due to uneven removal of fat.

BRIEF SUMMARY OF THE INVENTION

[0005] The invention provides a new method to obtain the reduction of what can be considered by patients and physicians to be excess amounts of unaesthetic and/or redundant subcutaneous adipose tissue. When collagenase with or without other proteinase(s) is introduced into subcutaneous adipose tissue of a living animal body, a dissolution and reduction of the adipose tissue at that location occurs. In a single treatment, reduction of the tissue from its original volume may range from 25% to 75% and higher, up to substantially 100%. This effect is of great benefit as previous methods of fat removal involved mechanical traumatization, incisions, and risks of contour deformities and mechanical injuries to tissues adjacent to the adipose tissue.

[0006] The method is used to rid the patient of unwanted subcutaneous fat cells without necessity of incisions, with-out significant trauma to the subcutaneous tissues, and without significant risk of infection as no metal canulla will be repeatedly introduced into the subcutaneous tissues, thus avoiding the risk of introducing with it bacteria from outside into the wound. Also eliminated is the possibility of mechanical damage to important anatomical structures adjacent to the area of liposuction by inadvertent misplacement canulla thrust. Post-operative ecchymosis may be lessened as well as the post-operative edema due to mechanical disruption.

[0007] While reduction of subcutaneous fat is the principal objective of the invention, the treatment may also be applied to adipose tissue elsewhere in the body.

[0008] The foregoing discussion and that following the heading “Detailed Description” is largely directed to application of the invention for reduction of excess subcutaneous adipose tissue considered to be unaesthetic and/or redundant. The invention is likewise applicable to the reduction and removal of lipomas, whether found at the surface of the skin, within the skin, subcutaneous, or anywhere else in the body.

[0009] Lipomas are tumors of fatty tissues, generally benign. If malignant, they are known as liposarcomas. Benign lipomas contain normal fat that is encapsulated within a fibrous sphere, thus often compressing the fat and causing it to feel more firm than surrounding fat. Many lipomas are asymptomatic and are removed for non-medical reasons. However, a significant number of them cause the patient pain or discomfort and they interfere with normal activity.

[0010] At present, lipomas are usually treated in one of the following ways: 1) wide excision with large dissection, 2) limited incision with limited dissection, or 3) liposuction.

[0011] If a large excision is performed, there is the problem of a very large scar and the accompanying issues of healing such a scar. In addition, removing a large mass from the subcutaneous tissues will leave behind a potential space which can fill with blood resulting in hematoma followed by consolidation of the hematoma and the remnants of a mass of scar tissue. This may be more problematic than the original lipoma.

[0012] Liposuction of a lipoma requires a smaller incision, therefore results in a smaller scar and carries a smaller risk of infection. However, the potential for hematoma formation, followed by scar mass residual, remains an issue of concern.

[0013] The present invention avoids these problems by introducing into lipoma(s) effective amounts of collagenase or collagenase plus another proteinase.

[0014] The invention may be used for the treatment of lipomas and other adipose tissues in humans and in animals, including dogs, cats, birds, and other comfort animals, horses, swine, sheep, and other farm animals, laboratory animals, and wild animals both in their natural state and in zoos.

DETAILED DESCRIPTION

[0015] In the human body, a more or less continuous layer of adipose tissue, composed largely of fat cells, underlies the skin. This subcutaneous fat not only varies in thickness from place to place in the body but also from individual to individual. Usually for cosmetic reasons, it may be desirable to reduce the amount of subcutaneous fat at selected locations.
The present invention accomplishes this by introducing into the tissue effective amounts of collagenase or collagenase plus at least one other proteinase. The said other proteinase may be chosen from any of the four recognized classes of proteinases, viz. the cysteine serine, aspartic and metallo proteinases. Of these, a cysteine proteinase is preferred, and especially clostridin. A serine proteinase such as trypsin or chymotrypsin is also preferred.

The collagenase and other proteinase(s) can be separately introduced, though it is generally more convenient that they both be in a single solution. It is within the skill of the art to select carriers that are pharmaceutically acceptable, including inertness towards the collagenase and other proteinase(s). Examples are normal saline, aqueous dextran solution, aqueous hetastarch solution, preferably suitably buffered. In some instances the physician may prefer a slow release liquid or solid carrier formulation for injection or implantation, in which case the collagenase dosage would usually be somewhat higher than that used in a simple aqueous injection. One can use carrier fibrin glue, comprising fibrin or fibrin precursors, e.g. fibrinogen plus thrombin; see U.S. Pat. No. 5,279,825. Again, selection of carrier and methods of preparing formulations are within the skill of the art. Though water is necessary to activate the enzymes, the aqueous interstitial fluid present in the subcutaneous tissues is sufficient to do this.

The physician will first select what location(s) in the body she/he wishes to treat. In order to limit the amount of enzymes (collagenase and other proteinase) introduced into the body at one time and to permit a preliminary evaluation of results, a limited area—which may be less than the total area—may be chosen for initial treatment. The treatment solution is injected percutaneously into the subcutaneous adipose tissues, preceded if the physician or patient so desires with a local anesthetic.

For maximum effect from a given quantity of the enzyme solution it should be injected in small quantities at a multiplicity of closely spaced points in the area, preferably spaced not more than about two centimeters apart and even much closer.

The physician will estimate the amount of adipose tissue underlying the area to be injected. Dosage usually may range from about 5 or less to about 150 or 500 or more in some cases as much as 3500 or more ABC units of collagenase per gram of adipose tissue treated. Amounts from about 10 to about 100 ABC units per gram are often preferred.

Dosage of other proteinase(s) may range from none to about 350 or more FFC units proteinase activity per gram of adipose tissue.

Collagenase is an enzyme that has the specific ability to digest collagen. It is derived commercially from fermentation by Clostridium histolyticum, and is purified by a chromatographic technique. The potency assay of collagenase is based on the digestion of unclotted 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 solublize ninhydrin reactive material equivalent to 1.09 nanomoles of leucine per minute.

Concentrations of enzymes in the pharmaceutically acceptable carrier are chosen on the principle that sufficient liquid is present to diffuse adequately in the subcutaneous fatty tissue yet no more than adequate to carry the desired amount of actives into the area under treatment. A range from about 50 to 5,000 ABC units collagenase per mL is suitable and considerable latitude within and beyond this range is possible in making the choice for a given situation. Similarly, considerable latitude can be used in choosing the concentration of other proteinase(s), though they will often fall within the scope of 1 to 10,000 FFC units per mL.

Since diffusion into the adipose tissue and the freeing of fat therefrom is seldom complete, it is often desirable to repeat the treatment at least once. This can be done after a few days, say one week.

The invention usually results in significant reduction of adipose tissue within 24 hours. Residue from the adipose tissue in the treated location is at least partly metabolized. If desired, freed fat, cell debris and free cells still present at the location after one or two days may be suctioned off.

Although the invention is intended to be a substitute for liposuction, it may also be used as an adjunct to it. In such ease the treatment is directed to sufficient disruption of the adipose tissue to make it easier to remove by suction. The liposuction stage will follow the application of the invention by one to three days.

Lipomas are normally removed by surgery or liposuction. By use of collagenase or collagenase plus one or more other proteinases as described herein, the lipomatous tumor can be completely removed. The procedures, carriers, dosages and concentrations described above are applicable to the treatment of lipomas. Likewise, as described herein, collagenase essentially free from other proteinases or collagenase plus other proteinases may be used. Fat released from the lipoma is metabolized.

Experimental

A series of experiments was carried out to observe the effect, if any, of injecting different concentrations of collagenase plus proteinase into the fat pads of mature male Zucker rats.

It was generally observed that a dose of between about 250 to about 1,000 ABC units of collagenase per fat pad when injected percutaneously caused moderate to severe tissue disruption in 24 hours. The surgeons who autopsied the rats commented that hemorrhage and trauma were significantly less at all dosage levels than the effect typically observed following mechanical liposuction. It was observed that as the dose was increased, the amount of interstitial hemorrhage tended to increase. Dosages of 2,000 ABC units and higher resulted in considerable local hemorrhage, but at dosages of 500 to 1,000 ABC units hemorrhage was of a generally moderate character. In this regard, it may be mentioned that in typical liposuction the amount of blood is about 25% of the fat removed.

Histopathological analysis of organs of rats treated with the collagenase-plus-proteinase material containing 1,000 and 2,000 ABC units of collagenase reveals normal tissue architecture and cell morphology with no histologic lesions.

It was observed that multi-site percutaneous injection resulted in better fat disruption than single site injection. It was also noted that when the enzyme was slowly infused into the fat pad and the infusion needle moved as the enzyme was being injected, severe interstitial hemorrhage was observed, along with good to moderate fat disruption, when
the animal was sacrificed in 24 hours. However, animals sacrificed at a later date showed no signs of hemorrhage, and there was a moderate disruption of the fat pad.

- **Animal Model**
- **Zucker rat, male.**
- **Autopsy Criteria**
- **Grade 0—No tissue disruption**
- **Grade 1—Mild tissue disruption, mild hemorrhage/necrosis**
- **Grade II—Moderate tissue disruption, mild hemorrhage/necrosis**
- **Grade III—Severe tissue disruption, hemorrhage/necrosis**
- **Grade IV—Complete tissue disruption, hemorrhage/necrosis**
- **Collagenase-Plus-Proteinase Material Used**
- **ABC units collagenase per gram: 960,000**
- **FFC units protease activity per gram: 24,700**
- **Solvent: sterile normal saline**
- **Anesthesia**
- **3 mL Rompun (xylazine) and 7 mL Ketamine HCl**
- **Use 0.1 mL per 100 gm animal weight**
- **Inject IP**
- **(Experiments I and II below, done without anesthesia, established that collagenase and collagenase plus another proteinase, not the anesthesia are the active agents).**
- **Procedure**
- **The rats were anesthetized by intraperitoneal injection. With each rat, one fat pad was injected with normal saline as control and three others were injected with the solution being tested.**

### Experiment A

- **No. of rats: Three Zucker rats**
- **Test solutions: 500 ABC u in 5 mL saline**
- **1,000 ABC u in 6 mL saline**
- **22 gauge, 8" needle used**
- **Injections: Solutions slowly infused into fat pad, moving needle. 12:00 PM day 1**
- **Results: Rat #1 autopsied 10:45 AM day 2**

<table>
<thead>
<tr>
<th>Pad a</th>
<th>Pad b</th>
<th>Pad c</th>
<th>Pad d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat #1</td>
<td>P</td>
<td>P</td>
<td>T</td>
</tr>
<tr>
<td>Rat #2</td>
<td>T</td>
<td>T</td>
<td>P</td>
</tr>
<tr>
<td>Rat #3</td>
<td>P</td>
<td>T</td>
<td>P</td>
</tr>
<tr>
<td>Rat #4</td>
<td>T</td>
<td>P</td>
<td>T</td>
</tr>
<tr>
<td>Rat #5</td>
<td>P</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>Rat #6</td>
<td>T</td>
<td>P</td>
<td>T</td>
</tr>
</tbody>
</table>

### Objective

- To determine the effect of highly purified collagenase on the subcutaneous fat pads of rats.

### Materials and Methods

- **Collagenase:** Nucleolysin®, which is a collagenase purified by a chromatographic technique and essentially free from other proteinases, available from Advance Biofactures Corp., Lynbrook, N.Y. 11563.
- **Diluent:** The lyophilized Nucleolysin® was reconstituted in a diluent consisting of water for injection USP, sodium chloride and calcium chloride. Each mL of reconstitution Diluent contained 9.0 mg NaCl USP and 0.297 mg CaCl₂ USP.
- **Six female Zucker rats.**
- **Four subcutaneous fat pads were designated:**
  - a=right anterior, b=left anterior, c=right posterior, d=left posterior.
- To avoid the possibility that administration of pain killers might interfere with the results, and in view of the short term nature of the experiment, no anesthesia was used.
- Each of the four subcutaneous fat pads of each rat was injected with either 250 ABC units collagenase in the form of Nucleolysin® dissolved in 0.2 mL Diluent (T), or with 0.2 mL Diluent only (P) according to the following schedule:

- **Thinning of fat grade II and III, 40% adipolysis.**
- **Site 3: 1,000 u, Mild adipose tissue degradation, no hemorrhage, grade I.**
- **Site 4: 1,000 u, Full thickness digestion of ½ of fat pad. Free fat floating over tissue, no hemorrhage, no necrosis, grade III.**
Twenty-four hours after injection all six rats were sacrificed at the same time in a CO₂ chamber.

Analysis and Interpretation of Results

Table 1 gives the weights of the rats before injection and before sacrifice. All of the animals appeared normal during the course of the experiment; there were no outward signs of pain.

After the rats were sacrificed, they were all incised to reveal the fat pads. A scale of 0 to 9 was used to describe the amount of disruption of the fat pads. An assigned value of 0 meant that no disruption was observed; an assignment of 9 was given to the fat pad(s) showing the most disruption (relative to the other fat pads). The assignment of a value to each fat pad was achieved through a consensus reached between two investigators neither of whom was aware of which injection was received by each fat pad. The results are given in Table 2.

Note that all of the fat pads that received an injection of Diluent only were rated 0, whereas the pads that received an injection of Nucleolysin® received ratings of 2 to 9. These results are highly significant, and the Mann-Whitney test indicates there is a significant difference between a diluent injection and a Nucleolysin® injection at the 99% degree of confidence.

<table>
<thead>
<tr>
<th>Rat</th>
<th>weight before injection, g</th>
<th>weight before sacrifice, g</th>
<th>weight difference, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>383.5</td>
<td>383.3</td>
<td>-0.2</td>
</tr>
<tr>
<td>#2</td>
<td>273.8</td>
<td>276.1</td>
<td>+2.3</td>
</tr>
<tr>
<td>#3</td>
<td>301.9</td>
<td>307.8</td>
<td>+6.9</td>
</tr>
<tr>
<td>#4</td>
<td>406.0</td>
<td>410.1</td>
<td>+4.1</td>
</tr>
<tr>
<td>#5</td>
<td>380.1</td>
<td>385.8</td>
<td>+5.7</td>
</tr>
<tr>
<td>#6</td>
<td>355.6</td>
<td>357.9</td>
<td>+2.3</td>
</tr>
<tr>
<td>avg</td>
<td>351.2</td>
<td>352.1</td>
<td>+1.9</td>
</tr>
</tbody>
</table>

Table 2

Summary of Disruption of Fat Pads

<table>
<thead>
<tr>
<th>Rat</th>
<th>Pad a</th>
<th>Pad b</th>
<th>Pad c</th>
<th>Pad d</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>#2</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>#3</td>
<td>P</td>
<td>T</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>#4</td>
<td>T</td>
<td>5</td>
<td>T</td>
<td>5</td>
</tr>
<tr>
<td>#5</td>
<td>P</td>
<td>0</td>
<td>T</td>
<td>8</td>
</tr>
<tr>
<td>#6</td>
<td>T</td>
<td>2</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

Experiment I

Objective

To determine whether highly purified collagenase, and collagenase containing other proteinase, have different effects on the subcutaneous fat pads of rats.

Materials and Methods

(1) Purified collagenase: Nucleolysin®, as described in Experiment H.

(2) Collagenase containing other proteinase: Collagenase having an activity of 202 ABC units collagenase per mg and 436 FFC units other proteinase per mg.

(3) Diluent: Lyophilized (1) and (2) were reconstituted in a diluent consisting of water for injection USP, sodium chloride and calcium chloride. Each mL of reconstitution Diluent contained 9.0 mg NaCl USP and 0.294 mg CaCl₂ USP.

(4) Six female Zucker rats.

(5) The four subcutaneous fat pads of each rat were designated as in Experiment H.

(6) No anesthesia was used, for the reasons stated in Experiment H.

(7) Each of the four subcutaneous fat pads of each rat was injected with either 94 ABC units collagenase in the form of Nucleolysin® dissolved in 0.2 mL of Diluent (N) or with 93 ABC units collagenase plus 201 FFC units other proteinase dissolved in 0.2 mL of Diluent (C) according to the following schedule.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Pad a</th>
<th>Pad b</th>
<th>Pad c</th>
<th>Pad d</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>C</td>
<td>C</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>#2</td>
<td>N</td>
<td>N</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>#3</td>
<td>C</td>
<td>N</td>
<td>C</td>
<td>N</td>
</tr>
<tr>
<td>#4</td>
<td>N</td>
<td>C</td>
<td>N</td>
<td>C</td>
</tr>
<tr>
<td>#5</td>
<td>C</td>
<td>N</td>
<td>N</td>
<td>C</td>
</tr>
<tr>
<td>#6</td>
<td>N</td>
<td>C</td>
<td>C</td>
<td>N</td>
</tr>
</tbody>
</table>

Twenty-two-and-one-half hours after injection all six rats were sacrificed at the same time in a CO₂ chamber.

Analysis and Interpretation of Results

Table 1 gives the weights of the rats before injection and before sacrifice. All of the animals appeared normal during the course of the experiment; there were no outward signs of pain.

After the rats were sacrificed, they were all incised to reveal the fat pads. The same two investigators who evaluated the fat pads in Experiment H applied the same scale that was used in that study to the evaluation of the fat pads in this study. The results are given in Table 2.

The Mann-Whitney statistical test indicates there was a significantly greater disruption in the fat pads that received purified collagenase than in those that received collagenase, plus other proteinase (99.5% degree of confidence). (In comparing the present results with the results obtained in Experiment H, there was no significant difference between the fat pads that received 250 ABC units of purified collagenase and the present fat pads that received 94 ABC units (95% degree of confidence.)

The weight of the incised fat pads are given in Table 3, along with its weight as a percentage of the rat’s total body weight before sacrifice. A two-way analysis of variance (ANOVA) revealed no significant difference (α=0.05) in the weight of the fat pads (as a percentage of total body weight) as a function of fat pad location and injected sample; however, one-way ANOVA revealed a significant difference between the weights of anterior vs. posterior fat pads (although there was no significant difference between the two treatments or between right vs. left fat pads). There was also no significant correlation between the weight of the fat pad and its “disruption value” shown in Table 2.
TABLE 1

Weights of the Zucker Rats

<table>
<thead>
<tr>
<th>Rat</th>
<th>weight before injection, g</th>
<th>weight before sacrifice, g</th>
<th>weight difference, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>432.9</td>
<td>436.1</td>
<td>+3.2</td>
</tr>
<tr>
<td>#2</td>
<td>491.2</td>
<td>494.3</td>
<td>+3.1</td>
</tr>
<tr>
<td>#3</td>
<td>372.2</td>
<td>372.0</td>
<td>-0.2</td>
</tr>
<tr>
<td>#4</td>
<td>416.1</td>
<td>419.1</td>
<td>+3.0</td>
</tr>
<tr>
<td>#6</td>
<td>336.1</td>
<td>336.5</td>
<td>+0.4</td>
</tr>
<tr>
<td>#6</td>
<td>396.5</td>
<td>398.3</td>
<td>+1.8</td>
</tr>
<tr>
<td>avg</td>
<td>407.5</td>
<td>409.4</td>
<td>+1.9</td>
</tr>
</tbody>
</table>

TABLE 2

Summary of Disruption of Fat Pads

<table>
<thead>
<tr>
<th>Pad a</th>
<th>Pad b</th>
<th>Pad c</th>
<th>Pad d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Inj.</td>
<td>Result</td>
<td>Inj.</td>
</tr>
<tr>
<td>#1</td>
<td>C</td>
<td>2</td>
<td>C</td>
</tr>
<tr>
<td>#2</td>
<td>N</td>
<td>6</td>
<td>N</td>
</tr>
<tr>
<td>#3</td>
<td>C</td>
<td>5</td>
<td>C</td>
</tr>
<tr>
<td>#4</td>
<td>N</td>
<td>0+</td>
<td>C</td>
</tr>
<tr>
<td>#5</td>
<td>C</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>#6</td>
<td>N</td>
<td>7</td>
<td>C</td>
</tr>
</tbody>
</table>

TABLE 3

Weights of Fat Pads

<table>
<thead>
<tr>
<th>Pad a</th>
<th>Pad b</th>
<th>Pad c</th>
<th>Pad d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>g</td>
<td>%</td>
<td>g</td>
</tr>
<tr>
<td>#1</td>
<td>5.5</td>
<td>1.3</td>
<td>9.3</td>
</tr>
<tr>
<td>#2</td>
<td>10.4</td>
<td>2.1</td>
<td>8.8</td>
</tr>
<tr>
<td>#3</td>
<td>8.0</td>
<td>2.2</td>
<td>9.1</td>
</tr>
<tr>
<td>#4</td>
<td>7.8</td>
<td>1.9</td>
<td>10.0</td>
</tr>
<tr>
<td>#5</td>
<td>7.8</td>
<td>2.3</td>
<td>6.9</td>
</tr>
<tr>
<td>#6</td>
<td>8.0</td>
<td>2.0</td>
<td>12.2</td>
</tr>
<tr>
<td>avg</td>
<td>7.9</td>
<td>2.0</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Collagenase Toxicity Study in Rats

Objective

To observe the effect of daily injections of different concentrations of collagenase/protease solutions into the fat pads of Wistar rats.

Materials

1. 6 adult Wistar rats — 3 male, 3 female, fed ad libitum
2. Collagenase/protease material: 990,000 ABC units collagenase and 24,700 FFC units protease activity.
3. Solutions of 1,000 ABC units in 0.4 mL saline and 2,000 ABC units in 0.4 mL saline.
4. Acepromazine, 2 mg/cc

Procedure

The rats are tranquilized by injecting 1.0 cc/Kg of a solution of Acepromazine subcutaneously. The animals appear tranquilized in about five minutes. The rats are treated as follows: Using a 1,000 units in 0.4 mL saline solution, 0.1 mL is injected percutaneously into each of the anterior thigh and hind leg fat pads. This is done for one male and one female rat. This procedure is repeated with a 2,000 units in 0.4 mL solution using another male and female rat. Two control animals are injected using 0.1 mL of saline into each of the areas. This procedure is done daily for a period of fourteen days. The rats are weighed daily. At the end of the test period the animals are sacrificed and autopsied. Sections of the spleen, liver, lungs, pancreas and kidney are sent for histopathology.

Necropsy Observation

1. Controls: Fat pads intact. All organs normal.
2. 1,000 units: Slight digestion of fat pads, some oil.
3. No hemorrhage. All organs normal.
4. 2,000 units: Slight to moderate digestion of fat pads.
5. Yellowing of abdominal wall at site of injection.
6. All organs normal.

Conclusion

Four human patients suffering from lipoma of the skin were treated with collagenase.

The collagenase was purified by chromatographic techniques so as to be substantially free from other proteins. It was supplied in lyophilized vials containing 2,200 ABC units of collagenase and sterile diluent.

Histopathology Report

Both control and test tissue sections exhibit normal tissue architecture and cell morphology. No histologic lesions.

Experiment J

Four human patients suffering from lipoma of the skin were treated with collagenase.

This 57-year-old female patient was diagnosed with a 3 cm by 4 cm x 0.5 cm lipoma located on her left anterior thigh. This patient has an unremarkable past medical history and was not taking any concomitant medications. Upon injection of the collagenase, she experienced adverse events of swelling, bruising and tenderness with the lipoma volume remaining static on the 1st and 3rd days post-injection. On the 7th day post-injection, the lipoma volume had decreased by 20% followed by an additional 20% decrease by the 14th day post-injection. By one month post-injection, the lipoma volume had decreased by 100% with no increase/change in volume apparent at the 6th month post-injection visit.
Patient 2

[0123] This 34-year-old female patient was diagnosed with a 1 cm x 1 cm x 0.5 cm lipoma located on her right lower arm. This patient has an unremarkable past medical history and was not taking any concomitant medications. Upon injection of the collagenase, she experienced adverse events of swelling, bruising and tenderness with an increase in the lipoma volume to 1.3 cm x 1.3 cm x 0.6 cm on the 1st and 3rd days post-injection. On the 7th day post-injection, the lipoma volume had decreased and was essentially static (as compared to baseline) and remained so at the 14th day post-injection. By one month post-injection, the lipoma volume had decreased by 50%, and by three months post-injection, the lipoma volume had decreased by 100% with no increase/change in volume apparent at the 6th month post-injection visit.

Patient 3

[0124] This 40-year-old female patient was diagnosed with a 5 cm x 5 cm x 1.5 cm lipoma located on her left upper arm. This patient has a past medical history significant for hypercholesterolemia, asthma, herniated disc, laryngitis and allergies to cats; however, she was not taking any concomitant medications. Upon injection of the collagenase, she experienced adverse events of swelling, bruising and tenderness with an increase in the lipoma volume to 5.5 cm x 5.5 cm x 1.5 cm on the 1st and 3rd days post-injection. On the 7th day post-injection, the lipoma volume had decreased and was essentially static (as compared to baseline) with a slight decrease (10%) at the 14th day post-injection. By one month post-injection, the lipoma volume had decreased by 100% with no increase/change in volume apparent at the 6th month post-injection visit.

Patient 4

[0125] This 66-year-old male patient was diagnosed with a 1 cm x 1 cm x 0.5 cm lipoma located on his right distal forearm. This patient has a past medical history significant for obesity, multiple skin nodules (neurofibromatosis) as well as an increased PSA level (biopsy results were negative); and he was taking multivitamins and aspirin as concomitant medications. Upon injection of the collagenase, he experienced adverse events of swelling and tenderness with an increase in the lipoma volume to 1.2 cm x 1.2 cm x 0.5 cm on the 1st day post-injection. On the 3rd and 7th days post-injection, the lipoma volume was static. Whereas on the 14th day post-injection, the lipoma volume had decreased slightly by 20%. No further reduction in lipoma volume was noted with this patient. The Principal Investigator feels that this was due to the nature of his lipoma—a fibrous/multi-lobulated one—that due to the presence of extensive scar tissue is resistant to the effects of the collagenase.

[0126] The principal investigator noted that patients 1, 2 and 3 were very pleased with the results of their participation in the study. Be also noted the fact that patients that present with fibrous/multi-lobulated lipomas, as in the case of patient 4, are not good candidates for the study due to the extensive scar tissue associated with their lipoma that is resistant to the effects of the collagenase.

1-12. (canceled)


14. The method of claim 13 wherein the treatment results in a reduction of lipoma tissue volume of about 25% to about 75% after a single administration of the composition.

15. The method of claim 13 wherein the treatment results in a reduction of lipoma tissue volume of about 100% after a single administration of the composition.

16. The method of claim 13 wherein the lipoma is found at a location selected from the group consisting of the surface of the skin, within the skin, subcutaneous, or anywhere else in the subject.

17. The method of claim 13 wherein the lipoma is a liposarcoma.

18. The method of claim 13 wherein the composition is administered into the lipoma is an amount of about 500 or more ABC units of collagenase per gram of treated tissue.

19. The method of claim 13 wherein the composition is administered into the lipoma is an amount of about 3500 or more ABC units of collagenase per gram of treated tissue.

20. The method of claim 13 wherein the collagenase is purified by chromatographic techniques.

21. The method of claim 13 wherein the collagenase is lyophilized and supplied in vials containing about 2,200 ABC units of collagenase and a sterile diluent.

22. A method of treating lipoma in a subject in need thereof, comprising the steps of:
   a. providing a vial of lyophilized collagenase comprising about 2,200 ABC units of collagenase;
   b. reconstituting the lyophilized collagenase with a diluent comprising about 0.9% sodium chloride and about 2 mM calcium chloride so as to provide a concentration of about 2,000 ABC units per mL; and
   c. injecting an effective amount of the reconstituted collagenase into the lipoma.

23. The method of claim 22 wherein the effective amount of reconstituted collagenase is about 1,000 ABC units collagenase per centimeter of the diameter of the lipoma.

24. The method of claim 13 wherein the lipoma excludes fibrous or multi-lobulated types of lipoma.

25. The method of claim 13 further comprising the step of adding a pharmaceutically acceptable carrier to the composition.

26. The method of claim 25 wherein the pharmaceutically acceptable carrier is inert.

27. The method of claim 25 wherein the pharmaceutically acceptable carrier is selected from the group consisting of normal saline, aqueous dextran solution, and aqueous hestarch solution.

28. The method of claim 25 wherein the pharmaceutically acceptable carrier is buffered.

29. A method for treating lipoma in a subject in need thereof comprising the step of administering a collagenase and at least one other proteinase.

30. The method of claim 29 wherein the proteinase is selected from the group consisting of the class of cysteine proteinases, the class of serine proteinases, the class of aspartic proteinases, and the class of metallo proteinases.

31. The method of claim 29 wherein the collagenase and the at least one other proteinase are administered separately.

32. The method of claim 29 wherein the collagenase and the at least one other proteinase are administered in a single solution.

33. The method of claim 29, further comprising a pharmaceutically acceptable carrier.
34. The method of claim 33, wherein the pharmaceutically acceptable carrier is inert.

35. The method of claim 34, wherein the pharmaceutically acceptable carrier is selected from the group consisting of normal saline, aqueous dextran solution, and aqueous hestasarch solution.

36. The method of claim 34, wherein the pharmaceutically acceptable carrier is buffered.

37. The method of claim 29, wherein the lipoma volume is reduced in an amount of about 25% to about 100% after treatment.

38. The method of claim 29 wherein the collagenase is purified by chromatographic techniques.

* * * * *