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## (54) COMPOSITIONS CONTAINING N-(2-AMINO-3,5 -DIBROMOBENZYL)-N-METHYL-CYCLOHEXYLAMINE AND/OR ITS SALTS


(71) We, DR. KARL THOMAE GESELLSCHAFT MIT BESCHRANKTER HAFTUNG, a Body Corporate organised under the laws of the Federal Republic of Germany of Biberach an der Riss, Federal Republic of Germany, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-


This invention relates to pharmaceutical compositions for use in the treatment of diabetic nephropathy.

Diabetic nephropathy is a frequent complication of diabetes mellitus. It is generally believed that thickening of the glomerular basement membranes is responsible for kidney damage.

Glycoproteins perform a decisive role in the structure of basement membranes, the carbohydrate portion of such glycoproteins being linked to the apoprotein principally through asparagine and hydroxylysine residues. It has been found that, in diabetics, the amount of hydroxyproline, hydroxylysine and saccharide part of the glycoproteins in the basement membranes of the kidney is increased as compared with normal healthy subjects (see Diabetes *25*, 909-913 [1976]).

The present invention is based on the surprising discovery that the metabolism of glycoproteins by diabetics may be substantially normalised, i.e. the proportion of saccharide and glycopeptide excreted strongly reduced to levels equivalent to those of a normal healthy subject by the administration of N-(2-amino-3,5 -dibromo-benzyl) -N-methyl -cyclohexylamine or a physiologically compatible acid addition salt thereof. Desirably the N-(2-amino-3,5-dibromo -benzyl)-N-methyl-cyclohexylamine or physiologically compatible acid addition salt thereof is administered in dosages of from 15 to 30 mg each, 3 to 4 times per day.

This effect is in contradistiction to the effect of these compounds on healthy subjects where the amount of saccharide and glycopeptide excreted (molecular fraction: M 500 - 10,000) increases.

N-(2-amino-3,5-dibromo-benzyl) -N-methyl-cyclohexylamine and its acid addition salts, as well as processes for their preparation, are described in British Patent Specification No. 968,254 where they are stated to possess secretolytic activity, in addition to cough-relieving, monoamine oxidase inhibiting and anti-pyretic activity, when administered in dosages of from 0.5 to 10 mg, preferably 2.0 to 4.0 mg. The antiulcus activity of these compounds when administered in higher dosages of from 30 to 100 mg, preferably 35 to 60 mg, has also previously been described in British Patent Specification No. 1,464,082.

According to one feature of the present invention there are provided pharmaceutical compositions in the form of dosage units comprising, as active ingredient, N-(2-amino-3,5-dibromo-benzyl) -N-methyl-cyclohexylamine and/or a physiologically compatible acid addition salt thereof in association with a pharmaceutical carrier or excipient, each dosage unit containing at least 15 but less than 30 mg of active ingredient. Particularly preferred compositions according to the invention are those wherein each dosage unit contains about 24 mg of active ingredient.

The active ingredient, preferably N-(2-amino-3,5-dibromo-benzyl) -N-methyl-cyclohexylammonium chloride (known under the non-systemaitic name Bromhexine) may be incorporated in the usual pharmaceutical preparations especially those for oral administration such as, for example, tablets, coated tablets, and capsules. The carriers

and excipients may be those customarily employed in pharmaceutical compositions such as, for example, talc, gum arabic, lactose, starch, magnesium stearate, aqueous or non-aqueous vehicles, fatty substances of animal or vegetable origin, paraffin derivatives, glycols, various wetting, dispersing or emulsifying agents and/or preservatives.

The compositions according to the invention may conveniently be presented in a container together with written or printed directions to use the compositions in the treatment of diabetic nephropathy. The said container may, for example, be a bottle, an ampoule or a vial. If desired, the container may itself bear printed or written directions for the intended use of the product. Alternatively, or additionally, the container may be accompanied by separate written or printed directions for use such as, e.g. a leaflet describing the intended medical use of the product concerned. Such leaflets are frequently referred to as "package inserts" and may, for example, recommend daily doses, mention possible side effects and provide other information useful to a medical practitioner.

According to a further feature of the present invention there is provided a method of treating non-human animals suffering from diabetic nephropathy which comprises administering to the animal an effective amount of N-(2- amino-3,5- dibromo-benzyl) -N-methyl-cyclohexylamine or a physiologically compatible acid addition salt thereof.

The effect of N-(2-amino -3,5-dibromo-benzyl) -N-methyl-cyclohexylammonium chloride (Bromhexine) on glycoprotein metabolism has been illustrated as follows:

24 hour urine collections were made from 8 normal healthy humans (4 women and 4 men) and 8 diabetics (1 women and 7 men) before and after a 3 to 4 week period of treating each subject orally 3 times daily with 24 mg of Bromhexine. A 20% by volume aliquot was taken from each urine specimen and freeze-dried.

The dried material (ca 1 g) was dissolved in the minimum quantity of distilled water and chromatographed with water on a "Sephadex" [registered Trade Mark] G-50 column (length: 148 cm, diameter: 3.4 cm). The carbohydrate-containing fraction was collected and subject to ultrafiltration using a UM-05 membrane (Amicon Ultrafiltration Cell-Model 202). The filtrate obtained was again freeze-dried.

The dried material (ca 1 mg) was analysed for carbohydrate using gas liquid chromatography. In this procedure, the sample, together with internal standards, was first subjected to methanolysis with 1.0 M HCl in dry methanol (0.5 ml) for 24 hours at 85°C. The resultant solution was neutralised and then treated with acetic anhydride (0.05 ml) for 6 hours. On completion of the treatment the solution obtained was centrifuged and the supernatant transferred to a clean flask, dried and stored in a desiccator. When required for gas chromatography, the dried material was triturated with a trimethylsilylating mixture and the resultant trimethylsilyl ethers of the methyl glycosides were subjected to gas chromatography (140-200°C at 0.5°C/min).

The 8 diabetics has a mean age of 42.5 yr (24-66) and an average duration of diabetes of 11.75 yr (4-19 yr). 5 patients were maintained on insulin, 2 on oral hypoglycaemic agents and 1 on diet alone. Diabetic treatment was not changed during the study. 3 patients suffered varying degrees of retinopathy, 1 has ischaemic heart disease and 1 had intermittent proteinuria. The normal subjects had a mean age 25.28 yr. (23-47 yr) The following table illustrated the results obtained:

| | Healthy Subjects | | Diabetics | |
|---|---|---|---|---|
| | before treatment | after treatment | before treatment | after treatment |
| 24 - Hour Volume (ml) | 1281 | 1406 | 1694 | 1686 |
| Total dry weight (g) | 36.3 | 44.0 | 102.6 | 110.7 |
| Fraction dry weight (g) | 4.2 | 4.4 | 8.7 | 6.1 |
| Carbohydrate content ($\mu$Mol/24 hours) | | | | |
| Fucose | 121 | 224 | 259 | 203 |
| Glucuronic acid | 1085 | 1573 | 1854 | 987 |
| Mannose | 121 | 169 | 205 | 121 |
| Galactose | 258 | 364 | 616 | 383 |
| Glucose | 329 | 425 | 12 609 | 7 241 |
| N-Acetylglucosamine | 204 | 287 | 313 | 181 |
| N-Acetylgalactosamine | 139 | 196 | 264 | 176 |
| N-Acetylneuraminic acid | 94 | 147 | 195 | 145 |

1,581,912

As may be seen from the Table, before treatment, the diabetic subjects excreted significantly more carbohydrate-containing material than the normal subjects. After treatment the normal subjects showed an insignificant rise in the urinary excretion of carbohydrate-containing material whereas the diabetic subjects showed a considerable fall in the excretion of such material.

As described in British Patent Specification No. 968,254 the acute oral toxicity of Bromhexine has been shown to be >3g/Kg in mice (0 out of 10 animals died).

The following non-limiting Examples serve to illustrate the present invention.

*Example 1*

*Tablets containing 24 mg of N-(2-amino- 3,5-dibromo-benzyl) -N-methyl- cyclohexylammonium chloride*

Composition:

1 tablet contains:

| | |
|---|---|
| Active ingredient | 24.0 mg |
| lactose | 148.0 mg |
| corn starch | 60.0 mg |
| gelatin | 6.0 mg |
| magnesium stearate | 2.0 mg |
| | 240.0 mg |

*Method of preparation*:

The active ingredient was mixed with the lactose and corn starch and then homogeneously moistened with a 10% aqueous solution of the gelatin. The mixture obtained was granulated through a screen of 1.5 mm mesh size, dried and again passed through the same screen. The granulate thus obtained was mixed with the magnesium stearate and pressed into tablets.

| | |
|---|---|
| Weight of tablet: | 240 mg |
| Punch | 9 mm, Ø |

*Example 2*

*Coated tablets containing 24 mg of N-(2-amino-3,5-dibromo-benzyl)-N-methyl-cyclohexyl-ammonium chloride*

*Method of preparation*

The coated tablet cores were prepared analagously to Example 1. The cores were then covered with a coating consisting essentially of sugar and talcum.

*Example 3*

*Capsules containing 24 mg of N-(2-amino-3,5-dibromo-benzyl)-N-methyl-cyclohexyl-ammonium chloride*

1 capsule contains:

| | |
|---|---|
| Active ingredient | 24.0 mg |
| lactose | 61.0 mg |
| corn starch | 34.0 mg |
| magnesium stearate | 1.0 mg |
| | 120.0 mg |

*Method of preparation*:

The substances were intimately mixed and filled into hard gelatin capsules of appropriate size.

| | |
|---|---|
| Capsule contents: | 120 mg |

WHAT WE CLAIM IS:-

1. Pharmaceutical compositions in the form of dosage units comprising, as active ingredient, N-(2-amino-3,5-dibromo-benzyl) -N-methyl-cyclohexylamine and/or a physiologically compatible acid addition salt thereof in association with a pharmaceutical carrier or excipient, each dosage unit containing at least 15 but less than 30 mg of active ingredient.
2. Compositions as claimed in claim 1 wherein each dosage unit contains about 24 mg of active ingredient.
3. Compositions as claimed in claim 1 or claim 2 wherein the active ingredient is N-(2-amino-3,5-dibromo-benzyl) -N-methyl-cyclohexylammonium chloride.
4. Compositions as claimed in any preceeding claim in a form suitable for oral administration.
5. Compositions as claimed in any preceeding claim in the form of tablets, coated tablets or capsules.
6. Pharmaceutical compositions as claimed in claim 1 substantially as herein described.
7. Pharmaceutical compositions substantially as herein described in any one of Examples 1 to 3.
8. A method of treating non-human animals suffering from diabetic nephropathy which comprises administering to the animal an effective amount of N-

As may be seen from the Table, before treatment, the diabetic subjects excreted significantly more carbohydrate-containing material than the normal subjects. After treatment the normal subjects showed an insignificant rise in the urinary excretion of carbohydrate-containing material whereas the diabetic subjects showed a considerable fall in the excretion of such material.

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*Method of preparation:*

The active ingredient was mixed with the lactose and corn starch and then homogeneously moistened with a 10% aqueous solution of the gelatin. The mixture obtained was granulated through a screen of 1.5 mm mesh size, dried and again passed through the same screen. The granulate thus obtained was mixed with the magnesium stearate and pressed into tablets.

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WHAT WE CLAIM IS:-

1. Pharmaceutical compositions in the form of dosage units comprising, as active ingredient, N-(2-amino-3,5-dibromo-benzyl) -N-methyl-cyclohexylamine and/or a physiologically compatible acid addition salt thereof in association with a pharmaceutical carrier or excipient, each dosage unit containing at least 15 but less than 30 mg of active ingredient.
2. Compositions as claimed in claim 1 wherein each dosage unit contains about 24 mg of active ingredient.
3. Compositions as claimed in claim 1 or claim 2 wherein the active ingredient is N-(2-amino-3,5-dibromo-benzyl) -N-methyl-cyclohexylammonium chloride.
4. Compositions as claimed in any preceeding claim in a form suitable for oral administration.
5. Compositions as claimed in any preceeding claim in the form of tablets, coated tablets or capsules.
6. Pharmaceutical compositions as claimed in claim 1 substantially as herein described.
7. Pharmaceutical compositions substantially as herein described in any one of Examples 1 to 3.
8. A method of treating non-human animals suffering from diabetic nephropathy which comprises administering to the animal an effective amount of N-

(2-amino-3,5-dibromo-benzyl) -N-methyl-cyclohexylamine or a physiologically compatible acid addition salt thereof.

9. A method as claimed in claim 8 which comprises administering a composition as claimed in any one of claims 1 to 7.

10. A method as claimed in claim 8 which comprises administering from 15 to 30 mg of N-(2-amino-3,5-dibromo-benzyl) -N-methyl-cyclohexylamine or a physiologically compatible acid addition salt thereof 3 or 4 times daily.

11. A method as claimed in claim 10 which comprises administering about 24 mg of N-(2-amino-3,5-dibromo-benzyl) -N-methyl-cyclohexylamine or a physiologically compatible acid addition salt thereof 3 or 4 times daily.

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