

EXAMPLE VIII

New Zealand white rabbits, with induced, long-term diabetes (12.7 ± 3.2 months) are used to study the effects of a test compound on red blood cell (RBC) deformability. If glucose-mediated crosslinking of red blood cell components results in less deformable, more rigid membrane, then therapy should prevent this change in new red cells. Red blood cells already in existence at the initiation of therapy would be unaffected; because RBCs are replaced frequently newly formed red blood cells would be exposed to the therapy. The diabetic rabbits (n=6) had a mean blood sugar of 292.6 ± 84.6 mg/dl before and after a two-month dosage regimen. The test compound is administered at a rate of 100 mg/kg/day by oral gavage.

The deformability index (DI) (filtration rate of buffer/filtrate rate of suspended RBCs; hematocrit = 4.0%) is used as a measure of RBC deformability. Buffer and suspended RBCs are filtered through a 3 μ micropore membrane (Nucleopore Corp., Pleasanton, Calif.) with a pressure of -20 cm H₂ at 37° C. A reference range for RBC deformability (2.67 to 4.84, mean 4.02 ± 0.69) and % hemoglobin glycosylation (1.7 to 3.96, mean 2.60 ± 0.70) is obtained from a group of normal New Zealand white rabbits (n = 14), matched for age, sex and weight.

The results are given in Table III, below for diabetic New Zealand white rabbits dosed with 100 mg/kg/day aminoguanidine.

TABLE III

Red Blood Cell Deformability of Long-Term Diabetic Rabbits on Aminoguanidine HCl	
Duration of Dosing (weeks)	Red Blood Cell Deformability Index
0	18.32 ± 6.03
4	7.17 ± 3.12
8	6.83 ± 2.97
12	4.64 ± 1.58
16	4.44 ± 1.33
20	4.14 ± 1.06

Diabetes causes an increase in the deformability index from 4.0 to 18. The results show that aminoguanidine administration reduces the diabetes-induced decreased deformability (increased deformability index) value to normal after 12 weeks. In a separate experiment, aminoguanidine hydrochloride was administered daily to rabbits at the time of induction of diabetes. The RBC deformability index in these animals never became abnormal. These experiments indicate that aminoguanidine prevents diabetes-induced RBC deformability changes; the rabbits with delayed treatment showed positive effect over time presumably as old red cells, which already had been cross-linked and were less deformable, were replaced with new red cells under the influence of aminoguanidine to inhibit cross-linking.

EXAMPLE IX

Streptozotocin-diabetic male Lewis rats were treated for a 9-month time period with daily doses of aminoguanidine hydrochloride of 0, 6.25, 12.5, 25, and 50 mg/kg/day. After the treatment period, collagen in tail tendon fiber was subjected to solubility determination. Ten mg samples were incubated with gentle rocking for 5 hours at 4° C. in 5 ml of 0.5 M acetic acid containing 1 mg pepsin. The samples were then centrifuged at 50,000 × g for 1 hour, and the soluble and insoluble fraction separated by decanting. Each fraction was

hydrolyzed in 6N HCl and total hydroxyproline measured. The results are set forth in Table IV, below.

TABLE IV

Condition	Aminoguanidine HCl mg/kg/day	Collagen Solubility
Normal	0	80.7 ± 3.4%
Diabetic	0	64.8 ± 0.8%
Diabetic	12.5	66.7 ± 1.2%
Diabetic	50	75.9 ± 0.4%
Normal	50	88.2 ± 0.2%

Diabetes of 9 months duration reduced tail tendon collagen solubility from 91% to 8%. Administration of AG HCl at doses from 6.25 up to 50 mg/kg/day orally significantly prevented this change in a dose-dependent manner, with the highest dose preventing 80% of the diabetes induced cross-linking.

EXAMPLE X

The effect of aminoguanidine administration on collagen cross-linking was examined in a genetically-diabetic animal model, the BB/Worcester rat. Once the animals become diabetic, daily insulin injections are essential for survival. Glucose levels are maintained at a derate level (250-350 mg %), much lower than in the streptozotocin-diabetic animals (>500 mg %).

Diabetic and non-diabetic controls were treated orally with aminoguanidine hydrochloride at 12.5 and 50 mg/kg/day for 6 months. Control animals were dosed with water only. Tail tendon collagen solubility was then determined by the pepsin method described in Example IX. The results are set forth in Table V, below.

TABLE V

Condition	Aminoguanidine HCl mg/kg/day	Collagen Solubility
Normal	0	80.7 ± 3.4%
Diabetic	0	64.8 ± 0.8%
Diabetic	12.5	66.7 ± 1.2%
Diabetic	50	75.9 ± 0.4%
Normal	50	88.2 ± 0.2%

These data show that the solubility of tail tendon collagen was reduced from 81% to 65% with diabetes of 6 months' duration but prevented significantly, in a dose-dependent manner, by both aminoguanidine treatments. Diabetic BB/Worcester rat collagen showed less decrease in solubility over the 6 month period than streptozotocin-diabetic rats presumably because of the lower degree of hyperglycemia and lower concentration of glucose to which collagen was exposed chronically.

This invention may be embodied in other forms or carried out in other ways without departure from the spirit or essential characteristics thereof. The present disclosure is therefor to be considered as in all respects illustrative and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

What is claimed is:

1. A method for treating an animal to inhibit the formation of advanced glycosylation end products of a target protein within said animal, said method comprising administering to said animal an effective amount of a pharmaceutical composition, said pharmaceutical