PLACENTAL ALDOSE REDUCTASE INHIBITION BY Silybin (preliminary communication)

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SUMMARY
Silybin, a flavonoid obtained from Silymarin is a powerful inhibitor of aldose reductase. It is suggested that it might be beneficial in the therapy and prevention of diabetic complications.

RESUMO
Inibição da aldose reductase placentária pela silibina.

A silibina, um flavonoide obtido a partir da silimarina é um potente inibidor da aldose reductase. Sugere-se que seria interessante experimentar a sua ação terapêutica e preventiva nas complicações da Diabetes mellitus.

INTRODUCTION
Aldose reductase (alditol-NADP oxidoreductase, EC 1.1.1.21) is an enzyme of the polyol pathway, the other being sorbitol dehydrogenase (L-iditol-NAD oxidoreductase, EC 1.1.1.14) which transforms sorbitol in fructose. Aldose reductase is not specific for glucose, and accepts as a substrate any one possessing an aldehyde group. Aldose reductase is one of the isoenzymes of aldose reductase. The polyol pathway is especially active in the testis, placenta, brain, nerve, kidney, lens, pancreatic islets, and is practically absent in other tissues. In the red blood cell sorbitol accumulates during incubation with elevated concentrations of glucose, and it has been suggested that the determination of sorbitol in the erythrocyte might serve as an index of diabetic compensation.

The intracellular accumulation of sorbitol in different tissues which possess aldose reductase was held responsible for the development of cataracts, retinopathy, peripheral neuropathy and macrovascular complications. The organs which do not depend on insulin for the transport of glucose are the most seriously affected, since intracellular non phosphorylated glucose is metabolized into sorbitol, whereas insulin controls free glucose concentration in the other cells, through the activation of its phosphorylation.

Several inhibitors of aldose reductase have been utilized as an attempt to stop this damage. Sorbinil and Alrestatin are powerful inhibitors, but their use in diabetic patients is difficult since they are very toxic. Silybin is a flavonoid which has been employed in the treatment of intoxications due to mushrooms of the Amanita phalloides type and in the toxic syndrome due to ingestion of rapeseed oil. It is practically nontoxic and may be employed continually for long periods.

In the present paper we demonstrate the Silybin is an inhibitor of placental aldose reductase.

Received: December 12, 1983
MATERIAL AND METHODS

Aldose reductase has been purified from fresh human placenta, according to the method described by Clements et al, slightly modified.19 Enzyme activity was determined at 30 °C using DL-glyceraldehyde as the substrate, according to O’Brien and Schofield.20 The reaction mixture contained 0.1M sodium phosphate buffer, pH = 6.2, DL-glyceraldehyde, 0.1M NADPH, 30μl of enzyme solution and Silybin, offered by Madaus Laboratories, Germany. The reaction was followed at 340nm. The method of Lowry was used for protein determination in the enzyme purification procedures.21 The kinetic constants were determined from Eisenthal plots.22 All determinations have been made in quadruplicate.

RESULTS

Figure 1 shows the kinetic parameters obtained from the Eisenthal plots Km = 0.1225mM and V = 0.1214μmol/min/mg of protein.

The inhibition by Silybin shows an increase of Km and a decrease of V, compatible with a linear mixed type of inhibition (Fig. 2). The value of the inhibition constant (K_i) is of 0.0120mM (Fig. 3).

DISCUSSION

Several reports indicate that the utilization of aldose reductase inhibitors in both human and experimental diabetes has beneficial effects,23 such as cataract prevention24 and improvement of nerve conduction.25

The only objection seems to be the degree of toxicity of the compounds employed.

In the present paper we demonstrate that Silybin is a powerful inhibitor of the enzyme. Due to its lack of toxicity in the usual doses, it might be worth to do a therapeutic trial in diabetic patients.

ACKNOWLEDGEMENTS

The Authors wish to thank Prof. Pratas Ferreira for permission to use Placentae from obstetric patients of Hospital de Santa Maria. Dr. Alda Pereira da Silva collaborated in obtaining them. The AA also thank the technical help of Gabriela Fontes.

REFERENCES


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