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.

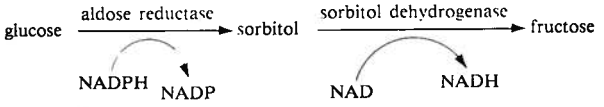
RESUMO

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 acção 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:[1, 2]



Aldose reductase is not specific for glucose, and accepts as a substract any ose possessing an aldehyde group.[3, 4] More recently it has been demonstrated that aldose reductase is one of the isoenzymes of aldehyde reductase.[5]

The polyol pathway is especially active in the testis, placenta, brain, nerve, kidney, lens, pancreatic islets, and is practically absent in other tissues.[6] 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.[7, 8]

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.[4, 6]

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,[9] whereas insulin controls free glucose concentration in the other cells, through the activation of its phosphorylation.[10, 11] In the presence of hyperglycemia, glucose is deviated to sorbitol synthesis in the first group of organs. The accumulation of sorbitol is responsible for the entry of excess water in the cells and subsequent damage of the tissues.[12]

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.[13, 14, 18]

Silybin is a flavonoid which has been employed in the treatment of intoxications due to mushrooms of the Amanita phalloides type [15, 16] and in the toxic syndrome due to ingestion of rapeseed oil.[17] 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.

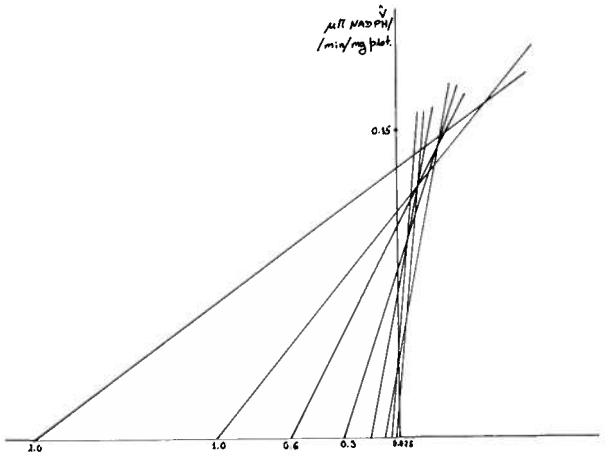


Figure 1: Eisenthal plot for determination of Km using DL-glyceraldehyde as substract.

Received: December 12, 1983

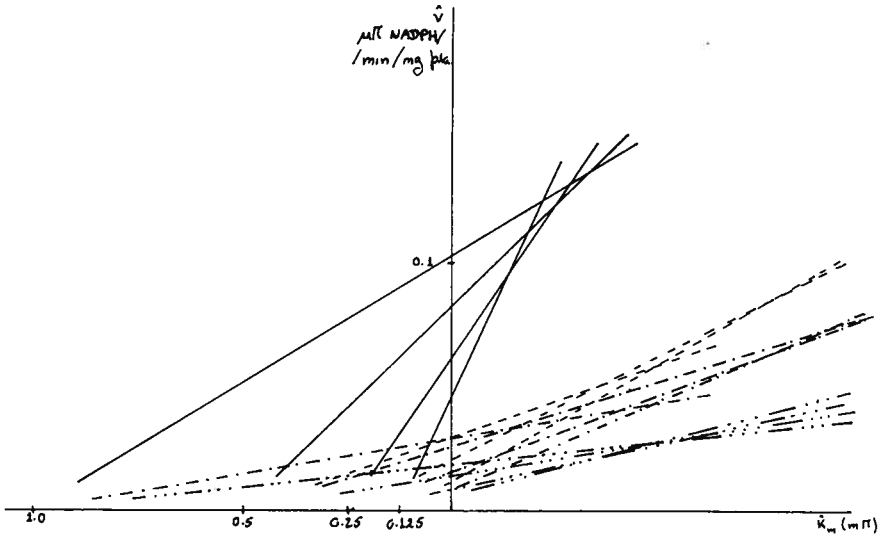


Figure 2: Eisenthal plot using DL-glyceraldehyde as substract, for different concentrations of Silybin (I = 0 ——; I = 0.025 ---; I = 0.050 ---; I = 0.075 mM ·····)

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 substract, according to O'Brien and Schofield.[20] The reaction mixture contained 0.1 M sodium phosphate buffer, pH = 6.2, DL-glyceraldehyde, 0.1 M NADPH, 30 μl of enzyme solution and Silybin, offered by Madaus Laboratories, Germany. The reaction was followed at 340 nm. 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.

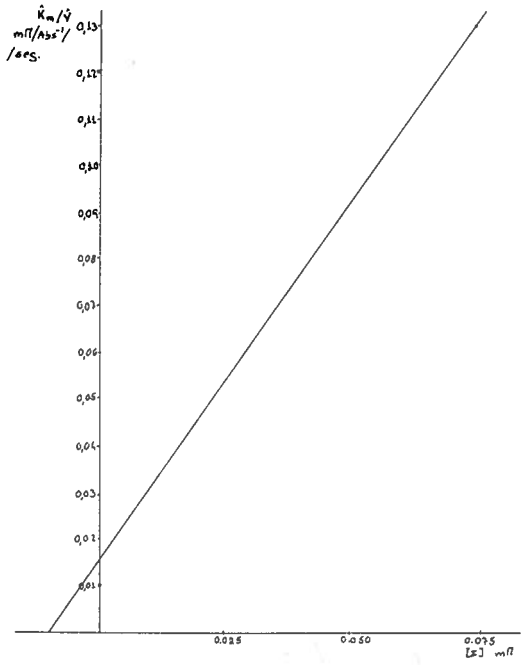


Figure 3: Determination of Ki of the inhibitor (Silybin)

RESULTS

Figure 1 shows the kinetic parameters obtained from the eisenthal plots $Km^{ap} = 0.1225$ mM and $V^{ap} = 0.1214$ μmol/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 (Ki) is of 0.0120 mM (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 prevention[24] 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

1. McCORKINDALE, J.; EDSON, N.: Polyol dehydrogenases. I - The specificity of rat liver polyol dehydrogenase. *Biochem J.* 1954; 52: 518.
2. GABBAY, K.; CATHCART, E.: Purification and immunologic identification of aldose reductases. *Diabetes.* 1974; 23: 460.

3. BEUTLER, E.; GUINTO, T.: The reduction of glyceraldehyde by human erythrocytes. L-hexonate dehydrogenase activity. *J. Clin. Invest.* 1974; 53: 1258.

4. MORRISON, A.; CLEMENTS, R.; TRAVIS, S.; OSKI, F.; WINEGRAD, A.: Glucose utilization by the polyol pathway in human erythrocyte. *Biochem Biophys Res Comm.* 1970; 40: 199.

5. BOGHOSAN, R. A.; McGUINESS, E.: Pig brain aldose reductase: a kinetic study using the centrifugal fast analyzer. *Int. J. Biochem.* 1981; 13: 909.

6. KINOSHITA, J.; FUTTERMAN, S.; SATOH, K.; MEROLA, L.: Factors affecting the formation of sugar alcohols in ocular lens. *Biochem Biophys Acta.* 1963; 74: 394.

7. TRAVIS, S.; MORRISON, A.; CLEMENTS, R.; WINEGRAD, A.; OSKI, F.: Metabolic alteration in human erythrocyte produced by increases in glucose concentration. *J. Clin. Invest.* 1971; 50: 2014.

8. MALONE, J.; KNOX, G.; BENTROD, S.; TEDESCO, T.: Red cell sorbitol. An indicator of diabetic control. *Diabetes.* 1980; 29: 861.

9. YALCIN, S.; WINEGRAD, A.: Defect in glucose metabolism in aortic tissue from alloxan diabetic rabbits. *Am J Physiol.* 1963; 205: 1253.

10. CROFFORD, O.; RENOLD, A.: Glucose uptake by incubated rat epididymal adipose tissue. *J Biol Chem.* 1965; 240: 14.

11. LEVER, J.: Modulation of glucose uptake in animal cells. *J Biol Chem.* 1979; 254: 2961.

12. GABBAY, K.: The sorbitol pathway and the complications of diabetes. *New Engl J Med.* 1973; 288: 831.

13. VARMA, S.; KINOSHITA, J.: Inhibition of lens aldose reductase by flavonoids. Their possible role in the prevention of diabetic cataracts. *Biochem Pharmacol.* 1976; 25: 2505.

14. O'BRIEN, M.; SCHOFIELD, P.; EDWARDS, M.: Inhibition of human brain aldose reductase and hexonate dehydrogenase by Alrestatin and Sorbinil. *J Neurochem.* 1982; 39: 810.

15. TUCHWEBER, B.: Modificacion del daño experimental en el hígado por la Silimarina. *Symposium sobre la Farmacodinamia de la Silimarina.* 1974; p. 39.

16. BRAATZ, R.: Effecto de la Silibina sobre ratones pretratados com Faloidina. *Symposium sobre la Farmacodinamia de la Silimarina.* 1974; p. 62.

17. Comunicação pessoal. Laboratório Madaus.

18. YOUNG, R.; EWING, D.; CLARKE, B.: A controled trial of Sorbinil and aldose reductase inhibitor in chronic painful diabetic neuropathy. *Diabetes.* 1983; 32: 938.

19. CLEMENTS, R.; WINEGRAD, A.: Purification of Alditol: NADP oxireductase from human placenta. *Biochem Biophys Res Comm.* 1972; 47: 1473.

20. O'BRIEN, M.; SCHOFIELD, J.: Polyol pathway enzymes of human brain. Partial purification and properties of aldose reductase and hexonate dehydrogenase. *Biochem J.* 1980; 189: 21.

21. LOWRY, O.; ROSENBROUG, M.; FARR, A.; RENDALL, R.: Protein measurements with the Folin Phenol Reagent. *J Biol Chem.* 1951; 193: 265.

22. EISENTHAL, R.; CORNISH-BOWDEN, A: The direct linear plot. A new graphical procedure for estimating enzyme kinetic parameters. *Biochem J.* 1974; 139: 715.

23. VARMA, S.; MIKUNI, I.; KINOSHITA, J.: Flavonoids as inhibitors of lens aldose reductase. *Science.* 1975; 188: 1215.

24. MEARS, A.; CRUZ, E.; ALEXANDRE, J.; VARAGIANNIS, E.: Sorbinil Protection of lens. Protein components and cell hydration during diabetic cataract formation. *Pharmacology.* 1982; 24: 193.

25. KIKKAWA, R.; HATANAKA, I.; YASUDA, H.; KOBAYASHI, N.; SHIGETA, Y.; TERASHIMA, H.; MORIMURA, T.; TSUBOSHIMA, M.: Effect of a new aldose reductase inhibitor, (E)-3-carboxymethyl-5 (2E)-methyl-3-phenylpropenylidene Rhodanin (ONO-2235) on peripheral nerve disorders in Streptozotocin-diabetic rats. *Diabetologia.* 1983; 24: 290.

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