Effects of Sorbinil Treatment on Erythrocytes and Platelets of Persons with Diabetes

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Thirty-three diabetic subjects were given the aldose reductase inhibitor sorbinil (Pfizer, UK) for 3 wk. There was a significant fall in mean erythrocyte sorbitol concentration over this period. In all subjects erythrocyte sorbitol concentrations after treatment were within or below the range found in normal subjects. No changes in erythrocyte 2,3-diphosphoglycerate (2,3-DPG) or *myo*-inositol concentrations, plasma beta-thromboglobulin (β-TG) concentration, or P_{50}—a measure of the oxygen affinity of hemoglobin—were observed. There was a high incidence of adverse reactions to the drug. DIABETES CARE 1986; 9:36–39.

Aldose reductase, the rate-limiting first enzyme of the polyol pathway, catalyzes the formation of the sugar alcohol sorbitol from glucose. Because the K_m of this enzyme falls within the range of plasma glucose concentrations found in persons with diabetes, the activity of this pathway is highly dependent on substrate availability.[1] In diabetes, therefore, those cells in which glucose uptake is independent of insulin (e.g., erythrocytes, lens, Schwann cells) tend to accumulate high concentrations of sorbitol and fructose. Increased activity of the polyol pathway has been implicated in the pathogenesis of diabetic cataract and, more speculatively, of diabetic peripheral neuropathy.[2]

In diabetic rats, erythrocyte sorbitol concentrations parallel the sorbitol content of both lens and peripheral nerve over a wide range of hyperglycemia.[3] Although the enzymes of the erythrocyte that convert glucose to sorbitol have not been completely characterized and are probably not identical to nerve and lens aldose reductase,[4,5] the erythrocyte may provide a convenient model for assessing the effectiveness of treatments intended to block the formation of sorbitol.[3] The polyol pathway is present in both erythrocytes and platelets but its physiologic function is unknown. It has been postulated that the increased activity of this pathway in conditions of hyperglycemia may cause a secondary change in the redox states of pyridine nucleotides with consequences on other aspects of intermediary metabolism.[6] It seemed possible that increased activity of the polyol pathway might be responsible for some of the abnormalities of erythrocyte and platelet function that occur in diabetes.[7]

We have investigated the aldose reductase inhibitor sorbinil (Pfizer, UK) in a group of metabolically stable diabetic individuals to evaluate its effect on erythrocyte sorbitol and *myo*-inositol concentrations and to determine whether, by reducing activity of the polyol pathway, the increased affinity of hemoglobin for oxygen[8] and increased tendency for platelet aggregation[9] found in diabetic individuals could be returned to normal.

SUBJECTS AND METHODS

Subjects. Forty-five individuals with diabetes were recruited but, because of a high incidence of adverse effects to the drug, only 33 (25 men, 8 women) completed the study. These 33 subjects (mean age 46 yr, age range 25–60 yr; mean duration of diabetes 12 yr) were investigated before and after 18–21 days treatment with sorbinil (250 mg once a day). Twenty-one subjects were treated with insulin; the other 12 were maintained on oral hypoglycemic agents or on diet alone. No subject suffered from nephropathy (plasma creatinine <200 mmol/L in all subjects), proliferative retinopathy or symptomatic peripheral neuropathy. Other treatment for diabetes remained unchanged.

Protocol. Venous blood was obtained at the start and finish of the treatment period in all 33 subjects. Samples were usually taken between 2:00 and 4:00 p.m. and, for each individual, sampling on the two occasions was carried out at the same time of day. Glycosylated hemoglobin and plasma glucose concentrations were estimated immediately. Erythrocytes were separated from plasma, washed, and aliquots transferred to 6% perchloric acid and 8% trichloracetic acid for

TABLE 1
Effects of 18–21 days treatment with sorbinil in 33 diabetic subjects

	Before treatment (mean ± SD)	After treatment (mean ± SD)		Normal range
Plasma glucose (mmol/L)	11.1 ± 5.8	11.0 ± 5.4	NS	<8
Glycosylated hemoglobin (%)	11.8 ± 2.3	11.6 ± 2.3	NS	<8%
Erythrocyte sorbitol (nmol/ml RBC)	8.8 ± 4.8	2.6 ± 1.6	$P < 0.001$	5.8 ± 1.6*
Erythrocyte *myo*-inositol (nmol/ml RBC)	39.6 ± 10.4	40.3 ± 13.8	NS	28.8 ± 4.2*
Erythrocyte 2,3 DPG (nmol/ml RBC)	2.26 ± 0.6	2.30 ± 0.7	NS	1.6–2.6
P_{50} (mm Hg)	24.8 ± 0.9	25.1 ± 0.9	NS	25.7 ± 0.4†
Plasma β-thromboglobulin (ng/ml)	75.3 ± 23.4	86.7 ± 39.4	NS	10–50

*Mean ± SD obtained from 13 normal subjects.
†Mean ± SD obtained from 6 normal subjects.

subsequent estimation of sorbitol, *myo*-inositol, and 2,3 diphosphoglycerate (2,3-DPG) concentrations. Plasma separated from venous blood obtained at separate venipuncture was used for estimation of beta-thromboglobulin (β-TG).[10] Samples were stored at −20°C for analysis after the study was completed. In 12 subjects venous blood was also taken for measurement of P_{50} (the partial pressure of oxygen at which hemoglobin is 50% saturated) before and after sorbinil treatment. This estimation was performed within 2 h of blood collection. All samples were coded so that assays were performed blind.

Assays. Glycosylated hemoglobin was measured by the Corning Glytral procedure. Plasma glucose was estimated using an automated glucose-oxidase method. Erythrocyte sorbitol and *myo*-inositol concentrations were measured in duplicate by a capillary gas chromatographic method.[11] This method was developed specifically for the assay of sorbitol and in about 15% of measurements, *myo*-inositol did not appear as a clearly separated peak on the chromatogram. We have used data concerning *myo*-inositol concentrations only where the chromatographic separation was adequate. Plasma concentrations of β-TG were measured by radioimmunoassay.[12] Erythrocyte 2,3-DPG concentrations were estimated by enzymatic assay.[13] P_{50} was estimated by applying the Hill equation to data obtained by in vitro tonometry of whole blood.[14]

Statistical analysis. Results before and after treatment were compared using the paired *t*-test.

RESULTS

The group mean values for all measurements before and after treatment with sorbinil are summarized in Table 1.

No significant change in glycemic control in the subject who completed the trial occurred during the treatment period, as shown by measurement of both glycosylated hemoglobin and random plasma glucose concentrations.

A large and statistically highly significant fall in mean erythrocyte sorbitol concentration was detected after treatment with sorbinil. Before treatment, erythrocyte sorbitol concentrations in the diabetic subjects varied over a wide range, correlating well with the prevailing plasma glucose concentration ($r = 0.69$). After treatment, erythrocyte sorbitol concentrations fell to normal or subnormal levels. The correlation with plasma glucose concentrations was maintained ($r = 0.60$), but the slope of the regression line was much less steep. A decrease in erythrocyte sorbitol concentration occurred in 32 of the 33 subjects (Figure 1). In the one subject who failed to show a decrease, pretreatment erythrocyte sorbitol concentration was low. There was no change in erythrocyte *myo*-inositol concentrations. No significant differences in response to sorbinil were detected between insulin-dependent and non-insulin-dependent diabetic subjects or between men and women.

Mean erythrocyte 2,3-DPG concentrations did not show any change during treatment with sorbinil; nor was any increase in P_{50} detected. There was a slight rise in plasma β-TG concentration over the treatment period but this did not reach statistical significance.

ADVERSE REACTIONS

Possible adverse reactions to sorbinil occurred in 16 of the 45 subjects exposed to the drug. These were significantly more frequent in women (chi-square, $P < 0.05$). A hypersensitivity reaction of the type occasionally seen with other hydantoins[15] occurred between 6 and 24 days after starting treatment in 11 subjects; it took the form of a febrile illness variably associated with myalgia, lymphadenopathy, and a maculopapular rash over the trunk, face, and limbs. A transient neutropenia, thrombocytopenia, and mild derangement of liver function tests together with a worsening of glycemic control accompanied the symptoms in most cases. Circulating immune complexes were detected in the serum of four subjects at the time of the rash. Symptomatic recovery was complete within 10 days of stopping the drug but abnormalities of liver function persisted. Two subjects were asymptomatic but showed transient abnormalities of liver function tests. These reactions were similar to those observed in a previous trial using this drug.[16]

One subject developed cervical lymphadenopathy without other symptoms, one complained of epigastric pain similar to

what he had experienced before with a peptic ulcer, and one developed an itchy erythematous rash over shins and forearms 1 wk after completing the treatment.

There is no placebo group in this study with which to compare the incidence of adverse effects reported by the subjects receiving sorbinil. Some of the minor symptoms may well be unrelated to the drug, but we think it almost certain that the syndrome of fever, rash, myalgia, and lymphadenopathy was caused by the treatment.

DISCUSSION

Sorbinil in a dose of 250 mg once a day is clearly a very effective inhibitor of erythrocyte sorbitol synthesis in both insulin-dependent and non-insulin-dependent diabetic individuals. These results are similar to those reported by other workers.[3,17] In animal models of diabetes, concentrations of sorbitol in erythrocytes parallel those found in peripheral nerve and lens and inhibition of aldose reductase by sorbinil causes a simultaneous decrease of sorbitol concentrations in all three tissues. It seems likely that the drug will be equally effective in these tissues in human beings.

The oxyhemoglobin dissociation curve is shifted to the left in diabetic subjects, largely as a result of an increased proportion of glycosylated hemoglobin. This shift results in impaired oxygen release in peripheral tissues and may contribute to ischemia.[8] It has been suggested that increased activity of the polyol pathway results in a decrease in the free NAD^+/free NADH ratio within the erythrocyte and that this in turn leads to a decrease in the concentration of 2,3-DPG, which is an important modulator of oxyhemoglobin dissociation.[7] Inhibition of polyol pathway activity might be expected to restore free NAD^+/free NADH ratios, thus favoring 2,3-DPG synthesis and allowing normalization of the oxyhemoglobin dissociation curve.[6] Despite the considerable reduction in erythrocyte sorbitol concentration achieved, we observed no effect on erythrocyte 2,3-DPG concentrations or on the oxyhemoglobin dissociation curve as assessed by measurement of P_{50}. The initial values for P_{50} in the diabetic subjects were not grossly abnormal, although they were significantly lower than those obtained from a small group of normal subjects ($P < 0.05$). Values for 2,3-DPG were not outside the normal range either before or after treatment. It is possible that our failure to observe changes in these variables is due to the absence of initial abnormality. A more likely explanation is that variations in polyol pathway activity have little effect on the ability of the erythrocyte to synthesize 2,3-DPG.

Peripheral nerves in persons with diabetes are known to be depleted of *myo*-inositol. In experimental diabetic animals, inhibition of aldose reductase is associated with an increase in nerve *myo*-inositol and an increase in nerve conduction velocity.[18,19] By contrast, in erythrocytes we have found that reducing sorbitol concentrations has no effect on *myo*-inositol levels. Erythrocyte *myo*-inositol metabolism is qualitatively different from that of nerve cells. There is no evidence for active uptake of *myo*-inositol by the erythrocyte and it is probable that the intraerythrocyte concentration of *myo*-inositol merely reflects the prevailing plasma concentration.[20] Erythrocyte *myo*-inositol concentrations in the diabetic subjects in this study tended to be higher than those found in normal subjects. This is in keeping with the finding that plasma concentrations of *myo*-inositol are raised in persons with diabetes.[21] Failure of the peripheral nerve to maintain its high intracellular concentration of *myo*-inositol may be an important part of the mechanism of the development of diabetic peripheral neuropathy.[22] One must conclude that for investigating the role of the polyol pathway in the pathogenesis of the neuropathic complications of diabetes the erythrocyte is not a perfect model.

An increased tendency for platelet aggregation has been observed in diabetic individuals[10,23] and it is known that platelets accumulate sorbitol when incubated in media of high

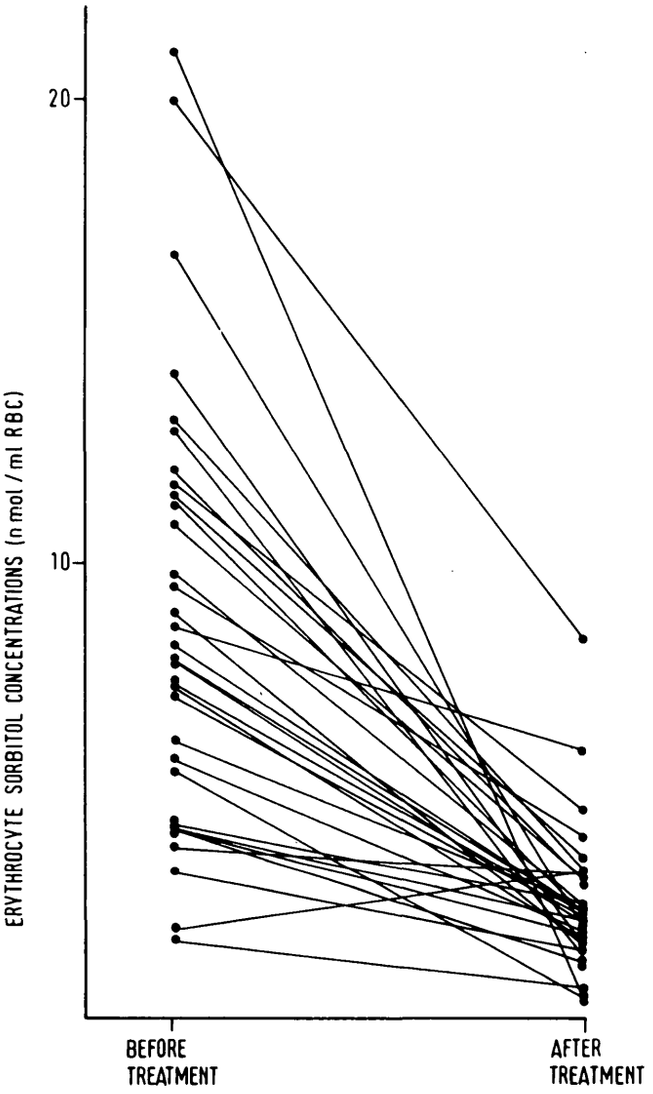

FIG. 1. Effect of 18–21 days of sorbinil treatment on erythrocyte sorbitol concentrations in 33 diabetic subjects.

glucose concentration.[24] β-TG is a platelet-specific protein released from alpha granules at aggregation.[25,26] Plasma concentrations of β-TG are thought to reflect the prevailing level of platelet aggregation within the vascular system.[27] We found no evidence from measurement of plasma concentrations of β-TG that reduction of polyol pathway activity decreases platelet aggregation.

This study has confirmed that sorbinil is a potent inhibitor of erythrocyte sorbitol production in man and there is reason to hope that it may be an effective treatment for some of the complications of diabetes. Near-complete inhibition of polyol pathway activity did not alter the oxyhemoglobin dissociation curve, intracellular concentrations of 2,3-DPG, or plasma concentrations of β-TG and this study provides no clues as to the function of this pathway in the normal erythrocyte and platelet. We encountered a high incidence of adverse reactions to the drug and this is likely to limit its clinical usefulness at a dose of ≥250 mg/day. The efficacy of treatment using lower doses of sorbinil is being evaluated.

ACKNOWLEDGMENTS: We are grateful to Pfizer UK Ltd for supplying sorbinil. D. J. Ewing is a Wellcome Trust Senior Lecturer.

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