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THE EFFECT OF ALDOSE REDUCTASE INHIBITOR STATIL (ICI 128436) ON THE GLUCOSE OVER-UTILIZATION IN KIDNEY OF DIABETIC RATS

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Abstract—The present study examined the effect of the aldose reductase inhibitor Statil (ICI 128436, ICI, Cheshire, U.K.) on the levels of metabolites and activities of enzymes involved in the glycolysis, polyol pathway and pentose phosphate pathway and on the flux of radioactive glucose through these pathways in kidney of streptozotocin diabetic rats. In kidneys of diabetic rats of 30 days duration the level of sorbitol was increased by +82% and fructose concentration was raised by +42%. After treatment with Statil for 9 days (reversal study) a significant fall in kidney sorbitol concentration and kidney fructose concentration was found. Lactate and UDP-glucose concentrations which were both significantly raised in diabetes by +80% and +23% respectively decreased by 20% after Statil treatment, together with a decline in UDP-glucose dehydrogenase activity. Aldose reductase and sorbitol dehydrogenase activities were also significantly lowered by Statil. In the reversal study there was no significant effect of Statil on the flux of glucose via alternative routes in the kidney cortex. In kidneys of diabetic rats of 9 days duration, the level of sorbitol increased by +61% and the concentration of fructose was raised by +30%. The treatment with Statil (25 mg/kg) from the day of induction of diabetes (prevention study) prevented the accumulation of sorbitol, fructose and UDP-glucose. The increase in the incorporation of radioactive glucose through the pentose phosphate pathway seen in diabetes was less marked in the renal cortex of diabetic rats treated with Statil *ab initio*.

A number of recent publications have shown the importance of glucose over-utilization in diabetes in tissues not requiring insulin for glucose transport and phosphorylation [1–4]. The enhanced metabolism of glucose by the polyol pathway and the accumulation of sorbitol and fructose via the aldose reductase (EC 1.1.1.21) and sorbitol dehydrogenase (EC 1.1.1.14) reactions have been found in the tissues in which diabetic complications occur such as lens, peripheral nerve and kidney [5–7]. The presence of an active aldose reductase and sorbitol dehydrogenase has been demonstrated in these tissues [6, 8, 9].

A number of inhibitors of aldose reductase have been reported to reduce the sorbitol level in lens, kidney and peripheral nerve of diabetic animals [10–12] and to improve diabetic cataract and neuropathy [12, 13].

Statil (ICI 128 436) a novel, orally active, potent inhibitor of aldose reductase, has a beneficial effect on cataract development and on the slowing of motor nerve conduction velocity in diabetic rats [14]. At doses as low as 25 mg/kg/day, it completely prevents the development of cataract and in kidney cortex it significantly reduces the level of sorbitol [14].

The aim of the present study was to investigate further the role of Statil in normalising the changes in the polyol pathway and alternative routes of glucose metabolism in kidney of streptozotocin-diabetic rats by measurement of levels of metabolites, activities of enzymes and the flux of glucose through alternative routes.

MATERIALS AND METHODS

Materials. Substrates, coenzymes and enzymes used in the assay procedures were purchased from Boehringer Corporation Ltd (London, U.K.) or Sigma London (Poole, U.K.). The aldose reductase inhibitor, Statil (ICI 128 436) was a gift from Imperial Chemical Industries plc, U.K. Radioactive compounds were purchased from Amersham International plc, U.K.

Animals. Male albino rats of the Wistar strain were used. The initial weight was approximately 150 g. Diabetes was induced by a single i.v. injection of streptozotocin at a dose of 60 mg/kg body weight. Glycosuria was confirmed by Diastix (Ames Co. Slough, U.K.). The standard laboratory cube diet and water were allowed *ad lib*. up to the time of killing.

Prevention study. In the study of the effect of Statil in preventing the accumulation of metabolites of the polyol pathway, the drug was administered to the animals orally by gavage, as a daily dose of 25 mg/kg body weight commencing on the day of induction of diabetes and continuing thereafter for 9 days. The rats were killed on the tenth day of the experiment.

Reversal study. The reversal study was designed to show if the treatment with Statil will be effective to influence the metabolic changes already well established in kidney after 3 weeks duration of

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diabetes. Statil treatment was given orally, by gavage, once daily, in the dose of 10, 25 or 40 mg/kg body weight, on the 21st day from the induction of diabetes and thereafter for 9 days. The rats were used for experiments on the 30th day after the induction of diabetes. Statil was dissolved in 1% $NaHCO_3$. Age-matched control animals were given 1% $NaHCO_3$ orally.

Metabolite estimations. Rats were killed and the right kidney was quickly removed and freeze clamped within 10 sec. A blood sample was taken from the heart. The frozen tissues were weighed and homogenized in 3 vol. of ice-cold 0.5 N perchloric acid using an Ultra-Turrax homogeniser. The protein was removed by centrifugation at 4°. The supernatant was neutralized and insoluble $KClO_4$ was removed by centrifugation after 10 min standing at 0°. The final concentration of the extract was equivalent to 1:4 (w/v).

Metabolites were assayed according to the standard methods described by Bergmeyer [15], using a Unicam SP 8000 recording spectrophotometer to measure the changes in optical density of the NAD and NADP-linked reactions. Blood glucose was assayed using Beckman Glucose Analyser 2 by the method based on the glucose oxidase reaction.

Enzyme estimations. Tissue homogenates (1:10 w/v) were prepared from the whole kidney using a Potter homogenizer with Teflon plunger in 0.25 M sucrose 0.02 M triethanolamine buffer pH 7.4, containing 0.12 mM dithiothreitol. The supernatant fraction was isolated by centrifugation at 105,000 *g* for 45 min and dialysed against the same buffer for 2 hr at 3°.

Enzymes of glycolytic route, the pentose phosphate pathway and glucuronate-xylulose pathway and glycogen synthetase were measured as previously described [15].

Sorbitol dehydrogenase was assayed using fructose as a substrate as described by Bergmeyer [15]. Aldose reductase was estimated by a method of Kinoshita *et al.* [16] and Pottinger [17] using a high glucose concentration (300 mM), NADPH (0.5 mM) and 50 mM phosphate buffer, pH 7.2. Two major forms of the enzyme aldehyde reductase have been found to be present in kidney [18]. The major component in both these tissues appears to be the aldehyde (high K_m) reductase (EC 1.1.1.2) which shows no reaction with glucose at any concentration, while the aldose (low K_m) reductase (EC 1.1.1.21) reacts with glucose as a substrate [18]. Aldose reductase inhibitors do not appear to discriminate effectively between the aldose reductase and aldehyde reductase [16]. Thus, the use of glucose in the assay of aldose reductase was selected as the best means of distinguishing the activity of the enzyme linked to sorbitol formation from glucose in diabetes.

In all enzyme reactions a unit of enzyme is the amount catalysing the conversion of 1 μmol substrate/min/25°, except glycogen synthetase which catalyses 1 nmol substrate/min/37°.

Flux estimation. The flux of glucose through pathways of glucose metabolism in kidney was measured by conversion of ^{14}C-labelled glucose to $^{14}CO_2$ and ^{3}H-labelled glucose to $^{3}H_2O$. Kidney cortex slices were incubated in 5 ml Krebs–Ringer bicarbonate medium containing 1 μCi of ^{3}H-glucose or 0.5 μCi of ^{14}C-glucose. The glucose concentration was 5 mM for kidney slices from control animals and 20 mM for kidney slices from diabetic animals. The gas phase was O_2/CO_2 (95/5) and the incubation time was 1 hr. The $^{14}CO_2$ was collected by injecting 1 ml 0.5 N hyamine through the rubber cap into centre well and 0.5 ml 5 N HCl into the outer chamber at the end of incubation period. After a further 1 hr incubation the hyamine was transferred to scintillation vials with 2 ml methanol and 12 ml toluene based scintillation medium was added. The yield of $^{3}H_2O$ from ^{3}H-glucose was determined by distillation of a sample of water from the incubation mixture as described by Hutton [19]. The radioactivity was determined using a Beckman scintillation counter.

Statistical analysis. The data are presented as means ± SD. Fisher's P values were calculated using Student's *t*-test.

RESULTS

There was a significant decrease in body weight and increase in kidney weight in diabetic rats 9 days and 1 month after induction of diabetes. Statil treatment given at the onset of diabetes for 9 days did not prevent the renal hypertrophy, neither did it affect the body weight or blood glucose; similarly it had no effect in reversing the kidney hypertrophy seen after 1 month of diabetes (Table 1). Bayer-Mears [20] reported that the aldose reductase inhibitor sorbinil, when given in large dose (60 mg/kg) for 25 days to galactose fed rats, protected the kidney against hypertrophy, conditions very different from the present experiment. The effect of longer-term treatment with high doses of Statil have not been studied here.

The polyol pathway

The effect of diabetes on the kidney content of sorbitol and fructose 9 days and 1 month after induction of diabetes is shown in Tables 2 and 3; there was a 60% and 77% increase in sorbitol level and 40% and 30% increase in fructose level at these times respectively. Statil given at the onset of diabetes, in the dose of 25 mg/kg, decreased sorbitol level by 30% and also caused a marked lowering of accumulated sorbitol when given after a three week period of untreated rats.

Renal fructose accumulation was partially prevented by Statil treatment (Table 3); reversal of fructose accumulation was seen when diabetic rats were given a high dose of Statil for 9 days.

The activity of kidney aldose reductase increased by 25% above the control value in diabetes of 30 days duration, and by 26% in diabetes of 9 days duration (Tables 4 and 5). After treatment with Statil the activity of aldose reductase was decreased to the value of controls. The low doses of Statil did not affect the sorbitol dehydrogenase; a significant decrease in the activity was found only after treatment with the higher dose (40 mg/kg body weight) of the inhibitor.

Table 1. Body weight, kidney weight and blood glucose values for control and diabetic rats

	Control	Control + Statil 25 mg/kg	Diabetic	Diabetic and Statil 10 mg/kg	Diabetic and Statil 25 mg/kg	Diabetic and Statil 40 mg/kg
Reversal study	(N = 16)	(N = 6)	(N = 20)	(N = 8)	(N = 6)	(N = 6)
Body weight (g)	301 ± 20***	290 ± 7***	221 ± 20	222 ± 25	232 ± 22	209 ± 24
Kidney weight (g)	2.40 ± 0.24***	2.35 ± 0.10***	3.20 ± 0.44	3.07 ± 0.50	3.11 ± 0.39	3.05 ± 0.44
Kidney weight/100 b.w. (g)	0.79 ± 0.08***	0.81 ± 0.05***	1.45 ± 0.31	1.38 ± 0.17	1.34 ± 0.12	1.45 ± 0.19
Blood glucose (mM)	5.7 ± 1.0***	6.1 ± 0.5***	27 ± 4	30 ± 5	29 ± 2	29 ± 2
Prevention study	(N = 8)	(N = 6)	(N = 10)		(N = 10)	
Body weight (g)	241 ± 16***	231 ± 7**	196 ± 15		195 ± 15	
Kidney weight (g)	2.09 ± 0.16***	2.05 ± 0.09***	2.51 ± 0.25		2.53 ± 0.20	
Kidney weight/100 b.w. (g)	0.87 ± 0.08***	0.90 ± 0.07***	1.28 ± 0.15		1.30 ± 0.12	
Blood glucose (mM)	5.9 ± 0.3***	6.1 ± 0.5***	25 ± 6		24 ± 3	

Values are given as means ± SD, the number of animals used are given in parentheses. P values: ** $P < 0.01$; *** $P < 0.001$; as compared to diabetic groups.

Table 2. Reversal study: the level of metabolites in kidney of control, 30 days diabetic and Statil treated diabetic rats

Kidney metabolites (nmol/g)	Control (N = 6)	Control + Statil 25/mg/kg (N = 6)	Diabetic (N = 20)	Diabetic + Statil 10 mg/kg (N = 8)	Diabetic + Statil 25 mg/kg (N = 6)	Diabetic + Statil 40 mg/kg (N = 6)
Sorbitol	226 ± 44***	206 ± 34***	412 ± 60	352 ± 15***	333 ± 16***	279 ± 25***
Fructose	166 ± 44***	150 ± 20***	236 ± 40	217 ± 36	210 ± 30	188 ± 36*
Glucose 6-P	31 ± 8***	30 ± 4***	65 ± 8	61 ± 12	61 ± 8	59 ± 8
UDP-glucose	169 ± 20***	156 ± 8***	208 ± 20	184 ± 12**	180 ± 12**	180 ± 12***
Lactate	595 ± 52***	505 ± 40***	1075 ± 62	1030 ± 90	913 ± 97*	888 ± 90*
Glucose (μmol/g)	2.42 ± 0.30***	2.08 ± 0.20***	24 ± 2.8	27 ± 7.5	32 ± 3.9*	28 ± 3.0
Glycogen (μmol/g)	2.35 ± 0.80***	—	10.8 ± 1.0	—	8.9 ± 1.6	—

Treatment was given orally once daily and begun on the 21st day from the induction of diabetes and continued for 9 days and the rats were used for experiments on the 30th day after induction of diabetes. Values are given as means ± SD.

P values: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; compared with diabetic group.

The number in parentheses indicates the number of animals used.

Table 3. Prevention study: the level of metabolites in kidney of control, 9 days diabetic and Statil treated diabetic rats

Kidney metabolites (nmol/g)	Control (N = 6)	Control + Statil 25 mg/kg (N = 6)	Diabetic (N = 6)	Diabetic + Statil 25 mg/kg (N = 6)
Sorbitol	256 ± 40***	239 ± 24***	413 ± 38	334 ± 30**
Fructose	168 ± 36*	152 ± 20**	218 ± 24	180 ± 22*
Glucose 6-P	34 ± 8*	30 ± 4*	52 ± 6	43 ± 6
UDP-glucose	188 ± 20	163 ± 10*	218 ± 26	181 ± 18*
Lactate	560 ± 58**	448 ± 60**	1070 ± 110	1090 ± 110
Glucose (μmol/g)	3.6 ± 0.4***	3.8 ± 0.3***	28 ± 3	33 ± 5

Statil treatment was given orally once daily and begun on the day of induction of diabetes and continued for 9 days. The rats were used for experiments on the 10th day after induction of diabetes. Values are given as means ± SD. P values: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$; compared with diabetic group. Number in parentheses indicates the number of animals.

Pentose phosphate pathway and glucuronate-xylulose pathway

Significant increases were found in metabolites of the pentose phosphate pathway and glucuronate-xylulose pathway in both short-term (9 days) and long-term (1 month) diabetes with a 50–100% increase in glucose 6-phosphate and 23% increase in the UDP-glucose content (Tables 2 and 3). These changes are in accord with previous observations [4, 21]. The massive increase in glycogen content in diabetes has been reported previously [22]. The enzyme profile of the diabetic rat kidney showed a number of early changes relevant to "glucose over-utilization", namely an increase in hexokinase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and phosphoglucomutase in short term diabetes (9 days) and in UDP-glucose pyrophosphorylase, glycogen synthetase and enzymes of pentose phosphate pathway in long term diabetes (30 days) (Tables 4 and 5). It has been reported previously, that the increase in the activity of hexokinase is found only in early diabetes during the rapid growth of kidney [23, 24]. In later stages of diabetes, 6 weeks after streptozotocin administration, the major growth phase of kidney is complete and the activity of hexokinase remains unchanged [25]. Statil significantly reduced the activity of glucose 6-phosphate dehydrogenase in prevention study, but did not affect the activity of hexokinase. The level of glucose 6-phosphate also remained unchanged. Increased UDP-glucose content in diabetes was significantly prevented and reversed by Statil together with a 25% decrease in UDP-glucose dehydrogenase activity. Glucuronate-xylulose pathway enzymes and metabolites are involved in complex-carbohydrate synthesis of glycogen and components of basement membrane. The reduced level of metabolites and lower activity of enzymes of this pathway by Statil could be an important factor in prevention of kidney hypertrophy and dysfunction in diabetes [26].

Lactate formation

The results in Tables 2 and 3 show the changes in the pattern of lactate utilization by kidney in diabetes. There was a nearly twofold increase in renal lactate content in diabetes and the higher doses of Statil (25 and 40 mg/kg) significantly lowered its level in reversal study. There was a 50% increase in renal lactate dehydrogenase activity in diabetic kidney, but this enzyme was not affected by Statil treatment.

Glucose flux

The effect of streptozotocin diabetes and treatment with Statil on the flux of glucose through the different pathways of metabolism in kidney cortex slices is shown in Table 6.

Prevention study

(a) In diabetes, $^{14}CO_2$ formation from [1-^{14}C]glucose is increased by 60% in kidney cortex and significantly decreased after treatment with Statil.

(b) The flux of glucose through pentose phosphate pathway, as shown by the difference in $^{14}CO_2$ yield from [1-^{14}C]glucose and [6-^{14}C]glucose [C_1–C_6], increased 2.5-fold in kidney cortex and was markedly reduced by Statil treatment.

(c) The amount of ^3H_2O formed from [2-^{3}H]-glucose is the measure of total glucose phosphorylation and conversion to fructose 6-phosphate [27, 28]. Table 6 shows that the amount of ^3H_2O from [2-^{3}H] glucose is increased in diabetic rat kidney by 70% and significantly reduced by Statil treatment.

Reversal study

A significant increase in the rate of pentose phosphate pathway and the amount of total glucose phosphorylation by glycolysis in kidney cortex can be seen again when the rats were diabetic for 30 days (Table 6). In contrast to the prevention study, only a slight reduction of this rate by Statil treatment for 9 days was achieved. It seems that after a long-term diabetes the metabolic changes in kidney are well established and possibly the longer treatment with Statil may result in more significant changes.

Table 4. Reversal study: the activity of enzymes in kidney of control, 30 days diabetic and Statil treated diabetic rats

Enzyme (u/g)	Control (N = 16)	Control + Statil 25 mg/kg (N = 6)	Diabetic (N = 20)	Diabetic + Statil 10 mg/kg (N = 8)	Diabetic + Statil 25 mg/kg (N = 6)	Diabetic + Statil 40 mg/kg (N = 6)
Glycolytic route						
Hexokinase I + II	0.87 ± 0.10	0.98 ± 0.12	0.95 ± 0.08	0.97 ± 0.08	0.99 ± 0.08	0.87 ± 0.04
Phosphoglucomutase	15.7 ± 1.0	15.2 ± 0.8	15.7 ± 0.4	16.0 ± 0.6	16.0 ± 0.6	15.7 ± 1.0
Lactate dehydrogenase	80 ± 4***	87 ± 4***	100 ± 8	107 ± 4	111 ± 6	111 ± 10
Pentose phosphate pathway						
Glucose 6-phosphate dehydrogenase	1.43 ± 0.14*	1.40 ± 0.10*	1.62 ± 0.06	1.57 ± 0.06	1.81 ± 0.12*	1.74 ± 0.10*
6-phosphogluconate-dehydrogenase	1.17 ± 0.08***	1.14 ± 0.08***	1.52 ± 0.18	1.54 ± 0.08	1.44 ± 0.14	1.49 ± 0.06
Glucuronate-xylulose pathway						
UDP-glucose pyrophosphorylase	5.08 ± 0.36***	5.20 ± 0.40**	5.93 ± 0.34	6.55 ± 0.52*	5.73 ± 0.32	5.37 ± 0.70
UDP-glucose dehydrogenase	0.170 ± 0.024	0.168 ± 0.022	0.182 ± 0.028	0.197 ± 0.030	0.156 ± 0.014	0.129 ± 0.018*
[a]Glycogen synthetase Active	26.3 ± 3.8**	—	38.5 ± 6.0	—	43.5 ± 6.5	—
[a]Inactive	14.4 ± 2.2	—	16.4 ± 3.0	—	17.5 ± 3.1	—
[a]Total	40.7 ± 2.8**	—	54.8 ± 6.5	—	56.7 ± 8.2	—
Polyol pathway						
Sorbitol dehydrogenase	6.15 ± 0.81	5.80 ± 0.81	6.75 ± 0.54	6.50 ± 0.66	6.47 ± 0.64	5.87 ± 0.52*
Aldose reductase	0.150 ± 0.018***	0.148 ± 0.016***	0.182 ± 0.008	0.141 ± 0.008**	0.145 ± 0.020**	0.149 ± 0.19*

Treatment was given orally once daily and begun on 21st day from the induction of diabetes, continued for 9 days and the rats were used for experiments on the 30th day after induction of diabetes. Values are given as means ± SD. P values: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with diabetic group.

[a] Units: nmol/g/min/37°.

Table 5. Prevention study: the activity of the enzymes in kidney of control, 9 days diabetic and Statil treated diabetic rats

Enzyme (u/g)	Control (N = 6)	Control + Statil 25 mg/kg (N = 6)	Diabetic (N = 6)	Diabetic + Statil 25 mg/kg (N = 6)
Glycolytic route				
Hexokinase I + II	0.92 ± 0.02***	0.97 ± 0.10*	1.09 ± 0.04	1.10 ± 0.12
Phosphoglucomutase	16.2 ± 0.6**	15.0 ± 0.6***	18.0 ± 1.0	16.7 ± 1.0
Lactate dehydrogenase	88 ± 12***	99 ± 10**	134 ± 12	128 ± 12
Pentose phosphate pathway				
Glucose-6-phosphate dehydrogenase	1.22 ± 0.08***	1.20 ± 0.08***	1.43 ± 0.04	1.25 ± 0.08**
6-Phosphogluconate dehydrogenase	1.10 ± 0.10*	1.25 ± 0.16	1.45 ± 0.22	1.52 ± 0.31
Glucoronate-xylulose pathway				
UDP-glucose pyrophosphorylase	5.15 ± 0.26	5.55 ± 0.40	5.73 ± 0.52	5.63 ± 0.62
UDP-glucose dehydrogenase	0.162 ± 0.022	0.158 ± 0.024	0.175 ± 0.026	0.159 ± 0.010
Polyol pathway				
Aldose reductase	0.130 ± 0.015*	0.130 ± 0.010*	0.164 ± 0.013	0.128 ± 0.007*
Sorbitol dehydrogenase	8.0 ± 0.8	7.8 ± 0.8	9.1 ± 1.0	9.0 ± 1.0

Statil treatment was given daily on the day of induction of diabetes and thereafter for 9 days. The rats were used for experiments on the 10th day after induction of diabetes. Values are given as means ± SD. P values: * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$; compared with diabetic group. Number in parentheses indicates number of subjects.

Table 6. Effect of streptozotocin diabetes and Statil treatment on the flux of radioactive glucose through alternative pathways in kidney cortex

	Kidney cortex		
	Control	Diabetic	Diabetic + Statil 25 mg/kg
	Prevention study		
Conversion of ^{14}C-glucose to $^{14}CO_2$ (μmol/g wet wt/hr)			
[1-^{14}C]glucose (PPP and TCA cycle)	4.23 ± 0.70***	6.72 ± 0.80	5.60 ± 0.70*
[6-^{14}C]glucose (TCA cycle)	2.90 ± 0.30	3.23 ± 0.35	3.22 ± 0.40
[C_1–C_6] (flux via PPP)	1.33 ± 0.40	3.49 ± 0.22	2.38 ± 0.22***
Conversion of ^3H-glucose to ^3H_2O (μmol/g wet wt/hr)			
[2-^3H]glucose (glycolysis)	5.02 ± 0.50***	8.56 ± 0.82	7.18 ± 0.40*
	Reversal study		
Conversion of ^{14}C-glucose to $^{14}CO_2$ (μmol/g wet wt/hr)			
[1-^{14}C]glucose (PPP and TCA cycle)	4.50 ± 0.32***	6.39 ± 0.50	6.01 ± 0.55
[6-^{14}C]glucose (TCA cycle)	3.37 ± 0.40	3.06 ± 0.32	3.24 ± 0.30
[C_1–C_6] (flux via PPP)	1.13 ± 0.15***	3.33 ± 0.30	2.77 ± 0.30
Conversion of ^3H-glucose to ^3H_2O (μmol/g wet wt/hr)			
[2-^3H]glucose (glycolysis)	6.47 ± 0.50***	8.39 ± 0.60	8.11 ± 0.44

The results are given as mean ± SD of at least 6 values. P values: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with diabetic group. PPP—pentose phosphate pathway, TCA cycle—tricarboxylic acid cycle.

DISCUSSION

Effect of Statil on the polyol pathway

The present study established that administration of Statil to diabetic animals limited the rise in the content of sorbitol which normally follows the induction of the diabetic state (Tables 2 and 3) and also reduced the sorbitol level and lowered the level of fructose when the drug treatment was initiated after the rise had occurred. The activity of aldose reductase and sorbitol dehydrogenase was also reduced. The polyol pathway can influence metabolic changes in the cell by a number of mechanisms: (a) by accumulation of sorbitol to a level sufficient to cause osmotic damage; (b) by alterations in the redox state of $NADP^+$/NADPH and NAD^+/NADH with effects

on pathways of glucose metabolism and on the maintenance of glutathione and protein sulfhydryl groups in the reduced form [11, 16, 27, 29]. In diabetic rat lens the concentration of sorbitol rises approximately 40-fold and is sufficient to cause the osmotic damage [5, 11]. In the diabetic rat kidney, the overall concentration of sorbitol appears to be too low to initiate osmotic effects. There is a rapid growth of kidney during the first week of hyperglycaemia and a link between kidney hypertrophy and aldose reductase activity can be postulated from the following observations: (a) kidney hypertrophy is positively correlated with the blood glucose level [23] and with the increased activity of pentose phosphate pathway [30]; (b) the increased rate of the pentose phosphate pathway in kidney produces excess levels of NADPH which has to be reoxidized [31]; (c) NADPH is reoxidized by aldose reductase reaction; (d) kidney hypertrophy caused by galactose feeding can be protected by treatment with high doses of aldose reductase inhibitor [20]. The failure of Statil treatment to slow down the early renal hypertrophy in diabetes in the present prevention study suggests: (a) that the ribose 5-phosphate supply from the oxidative and non-oxidative pentose phosphate pathway is adequate to sustain phosphoribosyl pyrophosphate, nucleotide and nucleic acid synthesis even in the presence of Statil in the dose tested, (b) that the higher doses of, and longer treatment with aldose reductase inhibitor may be required to sever the links between aldose reductase and the pentose phosphate pathway [20]. Beyer-Mears [32] had shown, that aldose reductase inhibitor, administered daily for 10 weeks, effectively diminished proteinuria in long-term diabetes. These results suggest that polyol pathway is implicated in kidney dysfunction in untreated diabetic state and inhibition of aldose reductase may represent a therapeutical approach.

Increased rate of polyol pathway in diabetes could also lead to excessive production of lactate via intermediate formation of fructose 1-phosphate from fructose via fructokinase [24, 33], which would then enter the glycolytic sequence. In reversal study inhibition of polyol pathway by Statil treatment results in the reduction of lactate level (Table 2). The higher dose of Statil treatment shows the greater reduction of lactate level.

Effect of Statil on other metabolic routes

Glucose over-utilization in kidney in diabetes leads to the raised level of glucose-6-phosphate and UDP-glucose [3]. The increase in glucose-6-phosphate content has a significant effect on the flux through the pentose phosphate pathway [4, 34].

Activation of the pentose phosphate pathway which supplies ribose-5-phosphate used for nucleotide and nucleic acid synthesis and NADPH for reductive biosynthesis, may contribute to the compensatory mechanism occurring in acid–base and electrolyte imbalance in diabetes [21, 35]. An increased pentose phosphate pathway is found in tissues, in which the polyol pathway is activated in diabetes as it supplies NADPH for the conversion of glucose to sorbitol [25, 29]. Statil treatment inhibits aldose reductase, thus reducing the level of sorbitol and fructose in diabetic kidney. As less glucose is utilized by this pathway, the flux of glucose via pentose phosphate pathway is reduced. A marked and sustained increase in the UDP-glucose level was demonstrated by our work and other laboratories [26]. This enhancement is associated with decreased tissue concentration of phosphoribosyl pyrophosphate [36] increased activity of the pentose phosphate pathway and increased bioavailability of ribose-5-phosphate for the *de novo* synthesis in purine and pyrimidine nucleotides [37]. It can be assumed that the change in uracil ribonucleotide metabolism is due to a demand for an increased RNA formation in the hypertrophying cell [21]. The increased levels of UDP-sugars, as demonstrated by their increased synthesis and pool expansion [26] and by the increased activity of enzymes involved in UDP-glucose synthesis and utilization [38] may be of pathogenic significance in increased extracellular deposition of glycoproteins in diabetes. The rapid increase in basement membrane synthesis in short term diabetes probably necessitates increased supply of UDP derivatives for protein glycosylation. Therefore, the effect of Statil in lowering the polyol pathway rate and then the activity of pentose phosphate pathway, can indirectly affect the formation of UDP-sugars.

It can be concluded that Statil is not only an effective inhibitor of the polyol pathway, but appears to influence metabolites distal to its primary role of action. This mechanism may be important in the improvement of diabetic nephropathy.

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