Alterations in enzymatic antioxidant defence in diabetes mellitus – a rational approach

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Summary

Defence against the reactive oxidants produced during aerobic metabolism is a complex process and is provided by a system of enzymes and antioxidant compounds capable of preventing excess radical production, neutralising free radicals and repairing the damage caused by them. Regulation of the antioxidant system must provide sufficient, properly located, antioxidant compounds and enzymes. Damage to this system has been proved to play a role in various disorders. Long-term complications of diabetes mellitus are supposed to be partially mediated by oxidative stress. The authors summarise experimental and clinical investigations in this field and analyse the possible importance of the changes in the antioxidant system in the development of diabetic vascular complications.

Keywords: antioxidants; diabetes mellitus; oxidative stress

Aerobic metabolism is always accompanied by the production of reactive oxygen species. Therefore, all aerobic organisms possess some sort of antioxidant defence, with enzymatic and non-enzymatic constituents.¹ The quantity and quality of the reactive species is determined by metabolic pathways within the organism, influenced by exogenous factors such as radiation, food, stress, etc. The adverse effects of free radicals are recognised in several disorders,² but care should be taken when assessing their causative role.³ Damage caused by free radicals is possibly involved in beta-cell destruction and in the pathogenesis of diabetes mellitus.⁴ Alterations of metabolic processes in diabetes also influence enzymatic defences, and these changes may be associated with late complications of diabetes. Antioxidant enzymes primarily account for intracellular defence, while several non-enzyme molecules, small molecule weight antioxidants, protect various components against oxidation in plasma. In this review, we summarise knowledge on enzymatic antioxidant defence in diabetes mellitus, with emphasis on the possible role of enzyme dysfunction in the development of diabetic sequelae.

The antioxidant enzymes

Intracellular antioxidant defence is primarily provided by antioxidant enzymes, which catalyse decomposition of reactive oxygen species.

The three major antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (Gpx), and catalase (CAT), differ from each other in structure, tissue distribution, and cofactor requirement. Their substrates are reactive species, according to the following reactions:

SOD catalyses the conversion of superoxide anion to hydrogen peroxide and oxygen. SOD activity was discovered by McCord and Fridovich in 1969; they later proved that the enzyme is required to sustain life in aerobic conditions.⁵ Several classes of the enzyme have since been specified, each containing a transition metal in its catalytic centre. In humans, mitochondrial MnSOD, and extra- and intracellular CuZnSOD have been identified.

Gpx is a selenium-dependent enzyme (selenoprotein). The extracellular form is a glycoprotein; the intracellular and mitochondrial forms also possess different antigenic structures. The substrate of the enzyme is reduced glutathione (GSH), and therefore it depends indirectly on the flavoprotein glutathione reductase (Gred) and cellular NADPH concentration. Gpx uses a specific H donor, GSH, for the reduction of non-specific substrates (hydrogen peroxide, lipid and non-lipid hydroperoxides). The enzyme contains selenocystein in its active centre which is incorporated into the polypeptide chain during translation. Selenium deficiency, both *in vitro* and *in vivo*, leads to enzyme deficiency. Thus, when assessing Gpx function, it may be necessary to examine selenium status and free GSH concentration, at least when looking for the cause of altered activity.

Catalase is a haem-containing ubiquiter enzyme, in eukaryotes it is found in peroxisomes. The enzyme probably serves to degrade hydrogen peroxide produced by peroxisomal oxidases to water and oxygen.

Several other enzymes are also involved in the prevention of oxidative damage or its repair, such as Gred, enzymes of NADPH production, and DNA repair enzymes. Different cell types and cellular compartments contain antioxidant enzymes in varying quantities. Regulation of antioxidant enzyme activity in eukaryote organisms may be influenced by such factors as age, hormonal state, organ specificity, and amount of cofactors present.⁶

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Theoretical causes of antioxidant enzyme activity alterations in diabetes

The metabolic alterations that accompany diabetes can affect pro-oxidant–antioxidant balance in several ways.

INDUCTION: RESPONSE TO OXIDATIVE STRESS

There are several proposed mechanisms of free radical production in diabetes: glycoxidation,^{7,8} change in intracellular NADH/NAD ratio (hyperglycaemic pseudohypoxia),⁹ and effects on prostaglandin biosynthesis.¹⁰ Since expression of antioxidant enzymes may be induced at the transcriptional level by oxidative stress,¹¹ it is possible that the metabolic changes accompanying diabetes may induce these enzymes.

ALLOSTERIC EFFECTS: INFLUENCE OF GLYCATION ON ENZYME ACTIVITY

The characteristic feature of diabetes, hyperglycaemia, enhances non-enzymatic binding of glucose to proteins: the phenomenon, glycation, causes structural and functional changes in the proteins like haemoglobin, albumin, lens crystalline proteins, basal membranes of glomeruli, etc. Thus, high extra- and intra-cellular concentrations of glucose could cause glycation and consequently functional changes of the antioxidant enzymes.

ACTIVATION–INACTIVATION: CHANGES IN THE CONCENTRATION OF COFACTORS

SOD requires manganese or copper and zinc, Gpx needs selenium, and catalase contains haem as cofactor. Diabetes may influence antioxidant enzyme activity through disturbances in micronutrient status.¹² The distribution and function of ions of vital importance may change in diabetes, and the potassium and calcium channels may work differently. Changes in iron metabolism lead to changes at the cofactor level.

Experimental results

Studies on experimental diabetes are carried out using certain spontaneously diabetic in-

bred animals or animals with viral or chemically induced diabetes.¹³ Streptozotocin is a toxin which destroys beta-cells selectively; a single adequate dose produces lasting hyperglycaemia and insulin deficiency. It has been established that reactive free radicals have a role in this damaging effect. Although the results of the experimental animal studies cannot be extrapolated directly to human disease, this finding has raised questions on the aetiological role of free radicals in human diabetes.

We have summarised the results of clinical and experimental observations in the table.

SUPEROXIDE DISMUTASE

In a long-term experiment Wohaieb observed a decrease of SOD activity in the liver and kidney and an increase in the pancreas of streptozotocin-treated diabetic rats.¹⁴ He proposed that the increase in enzyme activity might be an adaptive response in the otherwise SOD-poor pancreas, while the reduction of SOD activity in liver and kidney might be due to the direct damaging effect of free radicals on the enzyme. Dohi found no difference in the SOD activity of the kidneys in streptozotocin-treated diabetic rats after 4 months of diabetes.¹⁵ Matkovics *et al* observed decreased SOD activities in all examined organs (liver, kidney, spleen, brain, heart, muscles, pancreas) except lungs of streptozotocin- and alloxan-treated diabetic rats.¹⁶ Loven observed a decrease in CuZnSOD activity in liver, kidney, and erythrocytes after 10 days of streptozotocin-induced diabetes.¹⁷ Sukalski described the decrease of liver mitochondrial SOD activity.¹⁸ A significant decrease of CuZnSOD activity in diabetic rabbit aorta endothelium was reported by Tagami,¹⁹ although others have not found any difference in aortic SOD activity between diabetic and control rats.²⁰

Red blood cell SOD activity is frequently measured in humans as an index of defence against superoxide in blood. In diabetics, activity of erythrocyte SOD has been shown to be

Table Summary of the observed changes of activity of antioxidant enzymes in some tissues of diabetic animal models and human diabetic patients. The numbers refer to the relevant references.

	SOD		Gpx		GSH		Gred		CAT	
<i>Animal models</i>										
Erythrocyte	↓	17	↑	21			↑	21	=	14, 16, 21
Whole blood			↓	26			↓	26		
Endothelium	↓	19	↓ ↓ ^b	15, 19	↓	19	=	19	↓	19
	=	20	=	20					↓ ^b	15
									↑	20
Liver	↓ ↓ ^c	14,16–18	↓ ↓ ^c	14, 18	↓	14, 17, 26	↓ ↓ ^c	18, 26	↓	14, 21, 35
									↑	15, 16
Kidney	↓	14, 16, 17	↑	14			↓	26	↓	14, 21, 35
	=	15							↑	15, 16
Heart	↓	16							↑	14, 21, 35
Pancreas	↓	16							↑	14, 21, 35
	↑	14								
<i>Human diabetes</i>										
Plasma	= ^a	25	↑	23, 34						
Erythrocyte	↓	16	↓	33	↓	22, 30, 32,	↓	28, 30	=	21–23
	=	22, 23	↑	23		33	↑	21		
	↑	21	↑	16						
Whole blood			=	29			=	29		

↑increase of activity; ↓decrease in activity; =no difference when compared to controls; ^bheparin affinity of the enzyme decreased;

^aaorta homogenate; ^cmitochondrial enzymes.

decreased,¹⁶ increased²¹ and unchanged.^{22–23} Kawamura *et al* showed that red blood cell CuZn-SOD is glycosylated both *in vitro* and *in vivo*, leading to its inactivation, and the percentage of this glycosylated SOD is higher in IDDM children than in healthy individuals.²⁴ The percentage of extracellular glycosylated SOD has also been found to be higher in diabetics,²⁵ but its activity was comparable to that of the unmodified enzyme. Glycation was shown to affect the C-terminal end of the enzyme, reducing its heparin-binding affinity. Thus, protection against extracellular radicals by cell-surface attached SOD may be impaired in diabetes, leaving the endothelium more susceptible to damage by superoxide anion.

GLUTATHIONE PEROXIDASE

Mukherjee observed a significant reduction of GSH content after 15 days, and reduction of Gred activity after 3 weeks in liver, kidney, brain and blood of streptozotocin-treated diabetic rats.²⁶ Others have reported reductions in mitochondrial Gpx and Gred activity in liver of diabetic rats.¹⁸ Loven studied intestinal mucosa and liver GSH content after 10 days of streptozotocin-induced diabetes¹⁷; there was a significant decrease of GSH in the liver while no change was noticed in the mucosa. Orally administered GSH restored, and intramuscular insulin even raised liver GSH above normal levels. Abnormal GSH synthesis was thought to be responsible for the changes. Wohaieb found that in the liver, which normally contains high amounts of GSH and strong Gpx activity, induction of diabetes caused a decrease in both, while in the kidney, which is relatively poor in Gpx activity, diabetes led to an increase in activity.¹⁴ Insulin reversed these alterations.

Gred activity was shown to be increased in erythrocytes of spontaneously diabetic BB rats, while in alloxan-treated animals Gpx activity was also increased.²¹ The same group found similarly elevated erythrocyte Gred levels and resistance to peroxide-induced GSH reduction in type I and type II diabetic patients. They supposed that elevated glucose levels could increase NADPH production resulting in a more effective GSH reduction. Dohi noticed significant reduction in Gpx activity in aorta homogenates of rats 4 and 8 months after the induction of diabetes.¹⁵ He found higher serum selenium concentrations in diabetic rats. Tagami found reduced GSH content and Gpx activity and unchanged Gred activity in aortic endothelial cells of diabetic rabbits.¹⁹ Langenstroer found no alteration in Gpx activity.²⁰

Blakytyn observed that incubation of cow erythrocytes with glucose, glucose-6-phosphate and fructose results in a time-dependent reduction of Gred activity. The experiment suggested that glycation of the enzyme was responsible for the observed decrease.²⁷ Decreased Gred activity in erythrocytes of diabetic children was reported by Stahlberg,²⁸ but Walter *et al* found no difference between Gpx and Gred activity of diabetics and non-diabetics.²⁹ Murakami examined erythrocytes of diabetics (fasting glucose > 140 mg/ml) and concluded that GSH reduction and glutathione elevation in erythrocytes was

caused by defective functioning of gamma-glutamyl-cystein synthase due to its glycation, decrease in Gred activity and defect in glutathione transport.³⁰ Yoshida confirmed that in erythrocytes of poorly controlled diabetics (HbA1c = 10.6±1.3%) GSH synthesis and thiol transport is impaired, and cells become susceptible to oxidative damage.³¹ Jain found that erythrocyte GSH content was negatively correlated to HbA1c, a good estimate of long-term hyperglycaemia, in diabetics.³² In diabetic patients with explicit hyperglycaemia (HbA1c = 11.5±1.9) Uzel found impaired Gpx activity and lower erythrocyte GSH, in addition to elevated lipid peroxidation products, the alterations being more pronounced in patients with retinopathy.³³ Reduced GSH and protein-SH content in erythrocytes of diabetics was reported by Bono.²² Kaji *et al* reported no difference in erythrocyte Gpx activity but an increase in plasma Gpx activity of diabetic compared to non-diabetic women.²³ Elevation of serum selenium levels and Gpx activity in diabetic children was reported by Cser *et al*.³⁴ Similarly, higher Gpx activity has been reported in erythrocytes of diabetics.¹⁶

CATALASE

Observations regarding catalase activity in the vasculature are rather controversial: a decrease, reversible by insulin, in aortic endothelial cell catalase activity in diabetic rabbits was reported by Tagami.¹⁹ Dohi observed no alteration in catalase activity of rat aorta homogenate,¹⁵ while Langenstroer reported its elevation.²⁰ The activity of catalase in the liver and kidneys of diabetic animals is generally believed to decrease,^{14–21–35} although there are also reports of its increase.^{15–16} On the other hand, heart and pancreas tissues show increased catalase activity in the diabetic state.^{14–21–35} Erythrocyte catalase activity seems not to be altered either in diabetic animals or in type 1 and 2 diabetic patients.^{14–16–21–23}

It is apparent from the table that contradictory changes in the activities of particular enzymes in particular organs have been observed in many cases. These discrepancies may be partly explained by the variability in the diabetes models used, including the strain and sex of the animals, their age at the induction of diabetes, the severity of the resulting insulin deficiency, and the duration of diabetes. For the clinical observations similar confounding factors exist, such as the type and duration of diabetes, mode of treatment, presence or absence of complications, which are not revealed by routine laboratory tests.

Changes in enzyme activity (increased, impaired, unchanged) may depend on the above-mentioned factors to a large extent. In our experience, type I diabetes, when blood glucose is strictly controlled by intensive insulin therapy, is not accompanied by remarkable changes in the prooxidant-antioxidant balance. Antioxidant enzyme activities (erythrocyte SOD and catalase, whole blood Gpx) do not differ from those of healthy controls.³⁶ In an investigation of the relationship between the duration of diabetes and various measures of

Figure Suspected interactions between diabetes and antioxidant balance

antioxidant activity in blood, we found that erythrocyte SOD was reduced in patients who had had diabetes for more than 10 years compared to patients whose disease was not so long-standing, while whole blood Gpx and erythrocyte catalase activities did not differ.³⁷

In recent years it has been definitely shown that the worse the diabetic metabolic control, the higher the frequency of late complications.³⁸ Should oxidative stress play a role in the development of these complications, we would expect adaptive changes to be observed in the antioxidant defence system. Presumably these changes would be of different magnitudes in different states of metabolic control and depend on the duration of the unfavourable metabolic state.

It is well-known that syndromes characterised by disturbances of carbohydrate metabolism are not a homogenous disease; the only common basis is that in all forms of diabetes there is an absolute or relative insulin deficiency. Previous studies have suggested that the clinical forms of late complications seem to be the same, although it has not been established whether the pathogenic factors involved in the development of the late complications are

common and, if so, to what extent. If it is true that the oxidative stress has a role in the development of late complications, it is still not clear whether it has the same importance in the different forms of diabetes. Further, changes in carbohydrate metabolism may influence oxidative stress, the function of antioxidant defence and the damage caused. Keeping this in mind, investigations of the antioxidant system should include different types and degrees of disturbance of carbohydrate metabolism.

Consequences of antioxidant-prooxidant imbalance in diabetic vessels

Oxygen-derived free radicals may potentially play an important role in the pathophysiology of vascular complications associated with diabetes. Superoxide anion and hydroxyl radicals appear to block endothelium-derived nitric oxide (EDNO)-mediated relaxation by inactivating EDNO. The addition of exogenous SOD restores normal or unmasks an even greater acetylcholine-induced relaxation in diabetic aorta,^{20 39 40} while catalase has a similar effect.⁴¹ Thus, in diabetic conditions, 'normal' levels of antioxidant enzymes may be insufficient to preserve physiological contractile responses. An abnormal glutathione redox cycle is observed in human endothelial cells exposed to high glucose concentrations, resulting in an impairment of reduced GSH-dependent H₂O₂-degradation.⁴²

Defence against free radical damage therefore seems to be important in maintaining structural and functional integrity of the endothelium. However, at present we cannot decide whether or not there is relevant dysfunction of the antioxidant enzymes and what role any such dysfunction would play in the development of diabetic complications. Further studies, using standardised methodologies, molecular biological techniques, better defined diabetic models or subjects with better understood pathophysiology, will be necessary to bring us closer to the understanding of the role of oxidant species and defence against them in diabetes mellitus.

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