Free Rad. Res., Vol. 34, pp. 189-191
Reprints available directly from the publisher
Photocopying permitted by license only

Induction of Heme Oxygenase 1 In Liver of Spontaneously Diabetic Rats

L. COSSO[a,*], E. PESCE MAINERI[a], N. TRAVERSO[a], N. ROSATTO[b], M.A. PRONZATO[a], D. COTTALASSO[a], U.M. MARINARI[a] and P. ODETTI[b]

[a]*Department of Experimental Medicine and* [b]*Department of Internal Medicine, University of Genova, Italy*

Accepted for publication by Prof. R. Tyrrell

(Received 26 November 1999; In final form 1 June 2000)

It has been suggested that diabetes induces an increase in oxidative stress; the increased expression of heme-oxygenase 1 (HO-1) in liver is believed to be a sensitive marker of the stress response. The aim of this study was to examine whether diabetes is able to induce HO-1 expression in liver. The specific mRNA was amplified by RT/PCR and calibrated with amplified β-actin mRNA.

The mRNA HO-1 levels in the liver of spontaneously diabetic rats were increased by 1.8 fold compared with non diabetics; this supports the hypothesis of weak but significant oxidative damage due to chronic hyperglycaemia. This work represents the first in vivo study exploring the semi-quantitative expression of HO-1 in the liver of spontaneously diabetic rats.

Keywords: Oxidative stress, heme oxygenase, RT/PCR, stress protein

INTRODUCTION

Expression of HO-1 in mammalian cells is believed to be a general response to oxidative stress induced by heat, UV radiation, heavy metals, and other oxidising agents, and could be associated with cellular defence systems [1]. HO-1 has been identified as the 32,000-dalton stress protein (HSP 32) and is found ubiquitously in nearly all organs [2] but in liver its expression can be increased by up to 100-fold in response to an oxidative stress [3]. Moreover the induction of HO-1 has been shown to be related to intracellular redox changes, and hence an increase in mRNA levels of the HO-1 gene is considered to be a sensitive marker of oxidative stress [4].

The aim of this study was to evaluate whether hyperglycaemia is able to enhance, in the rat liver, the expression of HO-1 after six months of spontaneous diabetes.

METHODS

Diabetes-prone (BB/Wor/Mol/BB) and diabetes resistant (BB/Wor/Mol/WB) male rats, used as control group, purchased from Mollegaard Ltd. (Skensved, Denmark), were treated as described in Odetti et al. [5]. Here, insulin treatment was used only to avoid extreme hyperglycaemia and death of the animals.

* Corresponding author: L. Cosso, Department of Experimental Medicine (DIMES) Section of General Pathology, Via L.B. Alberti 2, I-16132 Genova, Italy. Fax Number: +39 10 353 88 36, E-mail: lcosso@hotmail.com

TABLE I Blood glucose (mmol/L), glycosylated haemoglobin (%GHb) and expression of HO-1 mRNA (A.U.D.: Arbitrary Units of Density). Diabetic group (n=3) is compared to non diabetic group (n=3). Values are mean±SEM

	Non Diabetics	*Diabetics*	*P*[a]
Blood glucose (mmol/L)	6.0±0.12	21.0±1.4	<0.001
% GHb	4.75±0.15	15.32±0.20	<0.001
HO-1 (Arbitrary Units of Density)	40.23±8.0	74.33±19.37	<0.001

a. p≤0.05.

Both groups of animals were killed at nine months of age (six months of diabetes with continuous hyperglycaemia).

Total RNA was isolated from rat liver by the guanidine isothiocyanate/caesium chloride method as described by Chirgwin et al. [6].

Total RNA was reverse transcribed using AMV (Boehringher Mannheim Italia S.p.A., Milan, Italy) and PCR amplification was performed with specific primers for HO-1 synthesised by TIB MOLBIOL S.r.l. (Genoa, Italy), according to Shibahara et al. [7].

PCR fragments were analyzed by electrophoresis and silver staining. Data are expressed as mean±SEM. Significance was accepted at the p≤0.05 level.

RESULTS

Non-diabetic rats had a normal mean glycaemic value of 6.0±0.12 mmol/L (106.5±2.3 mg/dl) while in diabetic rats the mean (21.0±1.4 mmol/L; 375.2±24.5 mg/dl) was significantly higher (p<0.001). Blood glucose was determined three times per week and glycaemic values, expressed as mean of 159 random determinations, point out the prolonged hyperglycaemia. Glycated haemoglobin values, as integrated value of last 2–4 weeks, were 15.32±0.20% in diabetics, compared to 4.75±0.15% in control rats (p<0.001).

The relative increase of HO-1 mRNA was analysed by a densitometer with the software "Molecular Analyst" (Biorad, Laboratories S.r.l. Milan, Italy). The expression of HO-1 increased by 1.8 fold in diabetic rats compared to the non diabetic ones (74.33±19.37 vs 40.23±8.0 Arbitrary Units of Density; p<0.001) (Table I).

DISCUSSION

Accumulating evidence suggests that oxidative stress plays a central role in the pathogenesis of diabetic complications [8].

Glucose is responsible for the release of reactive oxygen species [9]. In healthy conditions the defensive cellular system efficiently buffers the active and potentially dangerous reactive species. On the contrary, it is conceivable that, in diabetic tissue, cells try to adapt themselves by increasing the inducible defensive network. However, it has also been claimed that oxidative stress is not significantly increased in diabetes [10] and unanimity of interpretation has not been attained yet.

The present work was designed to evaluate if, after a long chronic hyperglycaemia, the expression of liver HO-1 gene was induced; this would indirectly confirm the presence of an excessive formation of reactive oxygen species during diabetes. The data described herein clearly demonstrate that the expression of the HO-1 gene was increased in vivo in response to a chronic high blood glucose after six months of diabetes.

Nishio et al. found that in 24-week streptozotocin-diabetic rats the mRNA content of HO-1 was increased by 3.1 fold compared with that of control rats [11], while we evidenced a 1.8 fold

increase; this discrepancy could be due on the one hand to the different tissue studied and on the other to the different experimental model used: Nishio used streptozotocin, a molecule able to liberate NO, which in vitro activates heme-containing enzymes [12]; instead, we preferred to use spontaneously diabetic rats.

This work supports the hypothesis that chronic hyperglycaemia is able to induce an oxidative stress; moreover, the hepatic level of HO-1 was increased after 6 months of diabetes; this confirms that the chronic activation of the gene was not exhausted after several months of high blood glucose.

Acknowledgements

We thank Prof. Roberto Ravazzolo for helpful discussion and PCR analysis. The technical assistance of Mr. Giuseppe Catalano in animals care is recognised. This work has been supported by grants from CNR (Prog. Strategico n°9604995.ST74), MURST (Cofin. 1997; Biomarkers of ageing: 9706247467.005) and University of Genova (ex 60%).

References

[1] R.M. Tyrrell and S. Basu-Modak (1994) Transient enhancement of heme oxygenase 1 mRNA accumulation: a marker of oxidative stress to eukariotic cells. *Methods in Enzymology*. 234, 224–235.

[2] M.D. Maines (1988) Heme-oxygenase: function, multeplicity, regulatory mechanism and clinical application. *FASEB Journal* 2, 2557–2568.

[3] M.D. Maines (1997) The heme oxygenase system: a regulator of second messenger gases. *Annual Reviews of Pharmacology and Toxicology*, 37: 517–54.

[4] G.M. Trakshel and M.D. Maines (1989) Multiplicity of heme oxygenase isozymes. HO-1 and HO-2 are different molecular species in rat and rabbit. *Journal of Biological Chemistry*, 264, 1323–1328.

[5] P. Odetti, N. Traverso, L. Cosso, G. Noberasco, M.A. Pronzato, U.M. Marinari (1996) Good glycaemic control reduces oxidation and glycation end-products in collagen of diabetic rats. *Diabetologia* 39, 1440–1447.

[6] J.M. Chirgwin, A.E. Przybyla, R.J. MacDonald and W.J. Rutter (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*, 18, 5294–5299.

[7] S. Shibahara, R. Muller, H. Taguchi and T. Yoshida (1985) Cloning and expression of cDNA for rat heme oxygenase. *Proceeding of the National Academy of Sciences of the USA* 82, 7865–7869.

[8] J.W. Baynes (1991) Role of oxidative stress in development of complications in diabetes. *Diabetes*, 40, 405–412.

[9] S.P. Wolff and R.T. Dean (1987) Glucose autoxidation and protein modification: the potential role of oxidative glycosylation in diabetes. *Diabetes*, 245, 243-250.

[10] M.C. Wells-Knecht, T.J. Lyons, D.R. McCance, S.R. Thorpe, J.W. Baynes (1997) Age-dependent increase in ortho-tyrosine and methionine sulfoxide in uman skin collagen is not accelerated in diabetes. *Journal of Clinical Investigation*, 100, 839–846.

[11] Y. Nishio, A. Kashiwagi, H. Taki, K. Shinozaki, Y. Haeno, H. Kojimo, H. Haegawa, M. Haneda, H. Hidaka, H. Yasudo, K. Horiike and R. Kikkawa (1998) Altered activities of transcription factors and their related gene expression in cardiac tissue of diabetic rats. *Diabetes* 47: 1318–1325.

[12] J. Turk, J.A. Corbett, S. Ramanadham, A. Bohrer, M.L. Mc Daniel (1993) Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islets. *Biochemical and Biophysical Research Communications* 197, 1458–64.