African Journal of Biotechnology Vol. 8 (20), pp. 5169-5172, 19 October, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Nrf2 transcription factor gene regulates basal transcription of mitochondrial superoxide dismutase enzyme in mouse brain

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Accepted 9 July, 2009

Evidence suggests that the Nrf2 transcription factor participates in the regulation of expression of genes that contain functional antioxidant responsive elements (AREs) in their promoter regions. Previous studies have shown that induction of glutathione-S- transferases (GST) and NADPH quinone reductase 1 (NQO1) by t-butylated hydroxy anisole (BHA) is impaired in the livers of Nrf2$^{(-/-)}$ mice. Basal expression of certain antioxidant enzymes is also lower in the livers of Nrf2 $^{(-/-)}$ mice. Results indicate that Nrf2 contributes to basal expression but not inducible expression of mitochondrial superoxide dismutase. SOD2 level was affected in the Nrf2$^{(-/-)}$ and about 2-fold lower than the Nrf2$^{(+/+)}$ mouse control. The dietary additives caused a small induction of SOD2 in the Nrf2$^{(-/-)}$ mouse brain, ethoxyquin and kahwoel palmitate each induced SOD2 marginally, while oltipraz and indole-3-carbinol caused 1.5 fold induction in the Nrf2$^{(-/-)}$ mouse brain. In contrast, there was no obvious effect on SOD2 in the Nrf2$^{(+/+)}$ mouse brain by any of the chemicals used .

Key words: Nuclear factor-erythroid 2-related factor-2 (Nrf2), antioxidant response element (ARE), mitochondrial superoxide dismutase (SOD2), Nrf2 mutant mice, chemopreventive agents.

INTRODUCTION

Chemopreventive agents exhibit co-ordinate induction of a variety of endogenous antioxidant proteins. Promoter analysis of the genes of these endogenous protein show the commonality of the presence of a cis-acting anti-oxidant response element (ARE) (Rushmore and Pickett, 1990a; Rushmore et al., 1990b, 1991) or electrophilic response elements (EpRE) (Friling et al., 1990). It was demonstrated that the EpRE mediates basal expression of mouse GSTA1 and is activated by phenolic anti-oxidants (Friling et al., 1990; Favreau and Pickett, 1995). Specific transcriptional factors called basic-region leucine zipper (bZIP) transcription factors were identified to bind to the EpRE (Yoshioka et al., 1995; Prestera and Talalay, 1995). Furthermore ARE consensus sequence shows high similarity to the erythroid nuclear factor gene regulatory elements (Itoh et al., 1991). The DNA binding sequence of Nrf2 (5_-TGA(C/G) TCA-3_) (Motohashi et al., 1997) is very similar to the ARE core sequence (5_TGACnnnGC-3_) (Rushmore et al., 1991). Several lines of evidence suggest that Nrf2 binds to the ARE sequence, leading to transcriptional activation of down-stream genes encoding GSTs (Lee et al., 2002) and glutamate-cysteine ligase (Ishii et al., 2000; Alam et al., 1999).

Hence it was thought possible that nuclear factor erythoid-2 related factors 1, 2 and 3 (Nrf1, Nrf2 and Nrf3) that recognize such elements might also stimulate transcription from a reporter gene containing an ARE (Venugopal and Jaiswal, 1996). It was demonstrated that transfection of HepG2 cells with increasing amount of Nrf1 and Nrf2 was dose dependent in the activation of ARE-driven transcription (Venugopal and Jaiswal, 1996). This study also showed that treatment with tBHQ and βNF enhanced the ability of Nrf1 and Nrf2 to activate ARE driven gene expression. It was demonstrated also that mice with a targeted deletion of the gene encoding Nrf1 has lethal effect while Nrf2 mutant had impaired ability to respond to BHA by inducting GST and NQO1 in the liver (Hayes et al., 2000). Basal expression of certain enzymes is also lower in the liver of Nrf2$^{(-/-)}$ mice (Hayes et al., 2000) lending support to the hypothesis that Nrf2 was an important player in ARE-mediated gene induction.

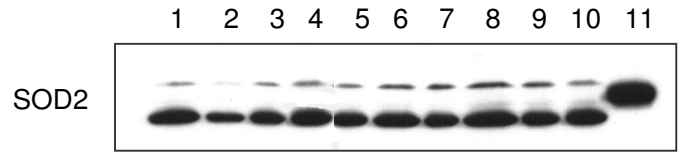

Figure 1. The Regulation of mitochondrial superoxide dismutase (SOD2) by ethoxyquin, oltipraz, kahweol cafesol palmitate and indole-3- carbinol in Nrf2 wild and null mice brain. Basal and inducible expression of antioxidant enzymes were examined by western blotting. 20 µg protein was from Nrf2(+/+) and Nrf2(-/-) mouse brain extracts was subject to SDS/PAGE before transfer to nitro-cellulose membrane. The blots were stained with ponceau S to ensure even transfer and equal loading. Lane 1, Nrf2(+/+) control; lane 2, Nrf2(-/-) control; lane 3, Nrf2(+/+) ethoxyquin; lane 4, Nrf2(-/-) ethoxyquin; lane 5, Nrf2(+/+) oltipraz; lane 6, Nrf2(-/-) oltipraz; lane 7, Nrf2(+/+) kahweol cafesol palmitate; lane 8, Nrf2(-/-) kahweoll cafesol palmitate; lane 9, Nrf2(+/+) indole-3-carbinol; lane 10, Nrf2(-/-) indole-3-carbinol; lane 11, standard Recombinant SOD2 human protein.

It is however, unknown whether Nrf2 contributes to either basal or inducible expression of mitochondrial superoxide dismutase and other endogenous antioxidant proteins in mouse brain. In order to investigate this possibility, cytosols from the brains of $Nrf2^{(-/-)}$ and $Nrf2^{(+/+)}$ mice treated with chemopreventive agents ethoxyquin, oltipraz, kahweol palmitate or indole-3-carbinol were analyzed for glutathione content and expression and activity of certain antioxidant enzymes.

MATERIALS AND METHODS

$Nrf2^{(+/+)}$ and $Nrf2^{(-/-)}$ mice

$Nrf2^{(+/+)}$ and $Nrf2^{(-/-)}$ mice are a cross breed between 129 and ICR (129 x ICR). The mice were fed, maintained and treated at Ninewells Hospital Animal House, University of Dundee. The mice were housed in cages of 3 with 12 h light and dark cycle. They were allowed free access to standard drinking water, powdered control diet RM1 for 2 weeks of acclimatization. 5 separate groups (3 animals per group) were set up each for $Nrf2^{(+/+)}$ and $Nrf2^{(-/-)}$ mice. Group 1 were fed on control diet RM1 for 2 weeks. Group 2 on RM1 containing 0.25% (w/w). Group 3 were fed on control diet RM1 containing oltipraz 0.075% (w/w). Group 4 were fed on control diet RM1 containing indole-3-carbinol 0.5% (w/w). Group 5 were fed on RM1 diet containing 0.0025% (w/w) kahweol palmitate. They were allowed free access to water and weight gain was monitored daily. They were killed by exposure to carbondioxide to make them unconscious, before killed by neck dislocation. The brain was snap frozen in liquid nitrogen. Soluble extracts prepared by homogenizing tissues in 4 volume ice-cold 80 mM Tris/HCl pH7.4 buffer containing 250 mM sucrose and 0.25 mM KCl. The homogenates were centrifuged at 10,000 x g for 20 min and the soluble fraction retained. The soluble fractions were subject to further centrifugation at 100,000 x g and supernatants retained as the cytosolic fractions.

Immunoblotting

Proteins were analysed by SDS-PAGE according to Laemmli (1970) using a Bio-Rad mini-protean vertical electrophoresis kit. Typically, 20 µg proteins from mouse cytosol were resolved in a 12% (w/v) polyacrylamide gel with an applied electromotive force (EMF) of 200 V.

Western blotting was carried out using a modified method of Towbin et al. (1979). 20 µg protein of Nrf2 null and wild type mouse brain extracts was subject to SDS/PAGE before transfer to nitro-cellulose membrane. Proteins that had been resolved by SDS-PAGE were electrotransferred to nitro-cellulose membrane (Millipore, Watford, Herts, U.K). Even loading was determined by staining the nitrocellulose with ponseou S prior to blocking with defatted milk. The blots were then probed with the human SOD2 antiobody raised in rabbit. Band intensities were determined using a molecular dynamics model 300A computing densitometer.

Protein estimation

Estimation of protein concentration was performed by the method of Bradford (1976) adapted for the use on Cobas Fara centrifugal analyzer (Roche Diagnostics, Welwyn Garden city, Herts, U.K.) (Galloway et al., 1999).

RESULTS

Evidence suggests that the Nrf2 transcription factor participates in the regulation of expression of genes that contain the functional AREs in their promoter regions. Previous studies indicated that induction of GST and NQO1 by BHA is impaired in the livers of $Nrf2^{(-/-)}$ mice (Hayes et al., 2000). Basal expression of certain antioxidant enzymes is also lower in the livers of $Nrf2^{(-/-)}$ mice (Hayes et al., 2000). It is, however, unknown whether Nrf2 contributes to either basal or inducible expression of superoxide dismutase in mouse brain. In order to investigate this possibility, cytosols from the brains of Nrf2 mice treated with the chemopreventive agents ethoxyquins, oltipraz, kahweol palmitate or indole-3-carbinol were analysed by immunoblotting to study the transcriptional regulation of mitochondrial superoxide dismutase enzyme in mouse brain.

Interestingly, the basal level of mitochondrial superoxide dismutase (SOD2) was affected in the $Nrf2^{(-/-)}$ and about 2 fold lower than the $Nrf2^{(+/+)}$ mouse control (Figure 1). The dietary additives caused a varying degree of induction of SOD2 in the $Nrf2^{(-/-)}$ mouse brain. Ethoxyquin and KP each induced SOD2 marginally, while oltipraz and indole-3-carbinol caused 1.5-fold induction in the $Nrf2^{(-/-)}$ mouse brain. In contrast, there was no obvious effect on SOD2 in the $Nrf2^{(+/+)}$ mouse brain by any of the chemicals used (Figure 1).

DISCUSSION

Superoxide dismutase (SOD) is considered as the first line of defence against reactive oxygen speices (van Loon et al., 1986) and SOD2 is the far the most important member of the SOD family in aerobic organisms, because superoxide radicals are mainly generated on the matrix side of the inner mitochondrial membrane (Balzan

et al., 1999). Thus it is conceivable that increase in SOD2 activity may provide increased protection against reactive oxygen species (ROS). SOD2 is inducible by various stimuli, such as Tumour Necrosis Factor-α (TNF-α), interleukin-1 (Ilu-1), lipopolysaccharides (LPS) and interferon-γ and NF-κB (Visner et al., 1990; Hirose et al., 1993; Akashi et al., 1995; Maehara et al., 1999). In the present study, Nrf2 has been shown to regulate basal expression of SOD2. There was 50% reduction in the level of SOD2 in $Nrf2^{(-/-)}$. However, dietary additives caused induction of SOD2 in the $Nrf2^{(-/-)}$ mouse brain to various magnitude. However, the chemopreventive agents were not effective inducers of SOD2 in the $Nrf2^{(+/+)}$ mouse brain.

SOD1 was not induced in any in the mouse brain, which is in agreement with most previous work. Like SOD2, SOD1 has a housekeeping function. Despite the reduction of brain SOD2 in $Nrf2^{(-/-)}$ mice no changes in the SOD1 protein level was observed (Figure 1).

Disruption of Nrf2 gene has been shown to enhance upregulation of nuclear factor-*κ*B activity, proinflammatory cytokines and Intercellular adhesion molecule-1 in the Brain after traumatic brain injury (Jin et al., 2008). In this study, SOD2 was down regulated in $Nrf2^{(-/-)}$ mouse brain. However, induction with chemopreventive agents restored the level of SOD2 in Nrf2 null to the base levels of the wild. Basal expression of SOD2 was reduced by 50% in the $Nrf2^{(-/-)}$ mouse brain. However, treatment with oltipraz, I3C or KP restored the level to that of the wild type. Thus it may be possible that upreugulation of nuclear factor-*κ*B activity and proinflammatory cytokines, as a consequence of Nrf2 disruption in the null contributed to the induction of mitochondrial SOD on treatment of with chemopreventive agents seen in this study. It may also be possible that CNC bZip family displays functional degeneracy and other members of the family may contribute to the expression of the antioxidant proteins in the tissue upon induction by xenobiotics with or without cooperativity with nuclear factor-*κ*B and proinflammatory Cytokines. However, future work needs to evaluate cooperativity between degenerate members of Nrf proteins, and proinflammatory cytokines or NF kB in the induction of SOD2 in Nrf2 null mice in the presence of chemopreventive agents.

ACKNOWLEDGEMENTS

I would like to thank Professor J. D. Hayes of Biomedical Research Centre, Faculty of Medicine, Ninewells Hospital, University of Dundee for kindly giving me the Nrf2, null and wild mice as part of my PhD work. I wish also to acknowledge the generous gift of Dr L.I. McLellan of SOD2 human antibody and provision of laboratory space for the project. Dr L.I. McLellan is from of Biomedical Research Centre, Faculty of Medicine, Ninewells Hospital, University of Dundee. Currently She is in the Department of Surgery, University of Dundee.

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