Histol Histopathol (2013) 28: 463-471
DOI: 10.14670/HH-28.463

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Hepatic response to chronic hypoxia in experimental rat model through HIF-1 alpha, activator protein-1 and NF-kappa B

Thomas Y.H. Lau[1]*, Jia Xiao[2,7]*, Emily C. Liong[2], Linchuan Liao[4], Tung Ming Leung[2,6], Amin A. Nanji[5], Man Lung Fung[3] and George L. Tipoe[2]

[1]Department of Health Technology and Informatics, Hong Kong Polytechnic University, Hong Kong SAR, [2]Department of Anatomy, The University of Hong Kong, Pokfulam, Hong Kong SAR, [3]Department of Physiology, The University of Hong Kong, Pokfulam, Hong Kong SAR, [4]Department of Analytical Toxicology, School of Basic and Forensic Medicine, Sichuan University, P.R. China [5]Department of Pathology and Laboratory Medicine, Dalhousie University, School of Medicine, Halifax, N.S. Canada, [6]Liver Disease Division, Department of Medicine, Mount Sinai School of Medicine, New York, USA and [7]Center for Gene and Cell Engineering, Shenzhen Institute of Advance Technology, Chinese Academy of Sciences, Shenzhen, China

*These authors contributed equally to this work

Summary. Chronic liver diseases are commonly associated with tissue hypoxia that may cause inflammation, oxidative stress, liver cell injury and increased nuclear transcriptional regulation. The hepatic response to chronic hypoxia at the molecular level has not yet been clearly understood until now. The aim of this study is to investigate whether nuclear transcription factors [hypoxia-inducible factor-1 (HIF-1α), activator protein-1 (AP-1), nuclear factor-kappa B (NF-κB)] exhibit activity changes during hepatic response to chronic hypoxia. Blood and liver samples were collected from adult Sprague-Dawley rats living in atmospheric air or 10% oxygen for four weeks. Levels of serum alanine aminotransferase (ALT), 8-isoprostane and nitrotyrosine were measured. The activities of nuclear transcription factors and the expression of downstream genes (iNOS, eNOS, ET-1 and VEGF) were measured using RT-PCR, Western blotting and Gel shift analysis. Results showed that serum ALT level, 8-isoprostane level and formation of nitrotyrosine were within normal range at all time-points. In the hypoxic liver, DNA-binding activities of HIF-1α, NF-κB and AP-1 increased significantly. Expression levels of iNOS, VEGF and ET-1 progressively increased from day 7 to day 28. eNOS was also elevated in the hypoxic liver. In conclusion, our study suggests that increased activity of HIF-1α, AP-1 and NF-κB may partly play a significant role in the hepatic response to oxidative stress and liver injury under chronic hypoxia. The increased expression of VEGF, ET-1, iNOS and eNOS may be partly due to the compensatory mechanism in the vascular beds of the liver in response to chronic hypoxia.

Key words: Hypoxia, Nitric oxide, Oxidative stress, Nuclear transcription factors

Offprint requests to: Dr. George L. Tipoe, Department of Anatomy, Li Ka Shing, Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong SAR. e-mail: tgeorge@hkucc.hku.hk

Introduction

Hypoxia inducible factors (HIFs) are key mediators of physiological and pathological responses to hypoxia (Semenza, 2000; Heidbreder et al., 2003). HIFs are important mediators of oxygen homeostasis. They have been implicated in pathophysiological and developmental functions. They are also involved in several pathological conditions such as cerebral ischemia (Bergeron et al., 1999), cardiovascular disease (Lee et al., 2000), cancer (Zhong et al., 1999; Zagzag et al., 2000) and chronic lung disease (Yu et al., 1999). Hypoxia has been demonstrated to be associated with chronic liver diseases, such as hepatocellular carcinoma, alcoholic liver disease, steatohepatitis and liver fibrosis. Activation of HIF-1α under hypoxic stress plays an important role in neovascularization in hepatocellular carcinoma through the regulation of vascular endothelial growth factor (VEGF) expression and promotes angiogenesis (Huang et al., 2005; Bozova and Elpek, 2007). A study also shows that hypoxia makes a direct contribution to the progression of liver fibrosis (Corpechot et al., 2002). This may be the result of

enhanced expression of TGF-$ß_1$ in hepatocytes of cirrhotic liver (Jeong et al., 2004).

When activated, HIF-1 is then translocated into the nucleus and regulates the transcription of genes which possess the hypoxia response elements (HRE) in their promoters. There are more than 60 genes that are regulated by HIF-1, such as endothelin-1 (ET-1), inducible nitric oxide synthase (iNOS) and VEGF (Semenza, 2003). The subsequent biophysiological responses induced by HIF-1 include angiogenesis, vascular remodeling, glucose metabolism, cell proliferation and survival (Semenza, 2003). In the human heart, HIF-1α mRNA and protein expressions are induced and preceded by VEGF expression during acute ischemia and early infarction (Lee et al., 2000). The vascular remodeling that occurs in chronic lung disease correlates with the high level of HIF-1 expression. ET-1, which is produced by pulmonary vascular endothelial cells in response to hypoxia, is an HIF-1 target gene (Hu et al., 1998). Angiotensin II, platelet-derived growth factor (PDGF), serotonin and thrombin induce smooth muscle hypertrophy and proliferation and they stimulate HIF-1α expression in vascular smooth muscle cells. This hypoxia-induced vascular remodeling is impaired in mice that are heterozygous for a loss of function of allele at the HIF-1α locus (Yu et al., 1999).

There are other transcription factors that are activated in conjunction with HIF-1α or separately during hypoxia, such as activator protein-1 (AP-1) and nuclear factor-kappa B (NF-κB). Endothelial cells transcriptionally activate vasoactive genes, such as PDGF-B, ET-1, thrombospondin-1 (TSP-1) and VEGF through an AP-1 binding site (Hoffmann et al., 2001). Similarly, rapid activation of NF-κB has been shown in Jurkat leukemic cells and NIH-3T3 cells exposed to hypoxia (Koong et al., 1994). Thus, our study aims to determine whether the expressions of HIF-1α, including HRE-contained genes, are altered as part of the hepatic response to chronic hypoxia. We also evaluated other transcription factors like NF-κB and AP-1, which regulate the expression of iNOS and eNOS genes, respectively.

Materials and methods

Animal experiments

Adult male Sprague-Dawley (SD) rats (CH groups) were placed in an acrylic hypoxic chamber at about 10% oxygen concentration for a period of 7, 14, 21 and 28 days. During the duration of hypoxia treatment, chambers were only opened for food and bedding replacement for a very short time every 3-4 days to minimize the disruption to hypoxic treatment. Corresponding normoxic control rats (C groups) were placed in an acrylic chamber with atmospheric air containing about 21% oxygen (Lam et al., 2008b). The number of rats per group at each time point was 6-8. The rats were kept in a 12 hr interval of light-dark cycle schedule throughout the entire period of the experiment with free access to animal chow and water. The model of chronic hypoxia induction in this study has already been shown to induce evident hypoxic injury in the carotid body (Lam et al., 2008a,b). The care of animals and euthanasia procedures were in accordance with guidelines established by The University of Hong Kong and National Institute of Health. The Laboratory Animal Unit of The University of Hong Kong is fully accredited by the Association for Assessment and Accreditation for Laboratory Animal Care (AAALAC International). Animals were killed by decapitation after anesthesia at the respective time points. Blood and liver tissue samples were collected for later analysis.

Determination of alanine aminotransferase (ALT) in serum

A reaction mixture containing L-alanine (80 mM), NADH (0.2 mM) and lactate dehydrogenase (2 units) in potassium phosphate buffer (0.2 mM, pH 7.4) and serum (0.35 ml) were incubated at 37°C for 3 min in the thermostated compartment of the spectrophotometer (Beckman Coulter, Brea, CA). Lastly, α-ketoglutarate (10 mM) was added to measure the rate of NADH utilization at 340 nm by alanine aminotransferase present in the serum (Chen et al., 2004). The activity of the enzyme was expressed in Units/Liter of serum. All chemicals used in this assay were obtained from Sigma.

Enzyme immunoassay (EIA) for total 8-isoprostane

The assay of the total serum 8-isoprostane was carried out by a Cayman EIA 8-isoprostane kit (Cayman Chemical, Ann Arbor, MI). This is a competitive immunoassay that is used to quantify the 8-isoprostane level of the serum sample. It is based on the competition between 8-isoprostane and 8-isoprostane-acetyl-cholinesterase (AchE) conjugate (tracer) for a limited number of 8-isoprostane-specific rabbit anti-serum binding sites. The experimental procedure and the final calculations were performed according to the instructions in the user manual. The amount of total 8-isoprostane was expressed in picogram per milliliter of serum.

RNA extraction and analysis of mRNA by reverse transcriptase polymerase chain reaction (RT-PCR)

To examine the mRNA expressions of VEGF, iNOS, eNOS, and ET-1 in the liver samples, total RNA was extracted using the RNeasy mini kit (QIAGEN Inc., Valencia, CA). The sequences of primer pairs and the predicted size of the amplified PCR products have been previously published (Nanji et al., 1995, 1997). Primer pairs for each gene were verified with cycle number test to guarantee that the cycles applied were in their linear amplifications. Reverse transcription and amplification (Invitrogen, Life Technologies, CA) were done as described previously (Nanji et al., 1997). After subjecting the PCR products to electrophoresis and

ethidium bromide staining, the intensity of the bands were analyzed in terms of optical density (OD) by densitometry (Nanji et al., 1997). The arbitrary units represent the OD of the gene PCR products divided by the OD of the GAPDH. To normalize signals from different RNA samples, 1 μl of the same reverse transcriptase reaction was amplified with specific GAPDH primers.

Western blot analysis

The specificity and the relative differences of nitrotyrosine (NTR), VEGF, ET-1, iNOS and eNOS protein expressions were studied by Western blot analysis. A detailed protocol of this procedure has been described in our previous publication (Tipoe et al., 2006). Briefly, the membrane after transfer was incubated in a blocking buffer (5% non-fat milk in Tween Tris-Buffered Saline (TBST, pH 7.5) (100 mM Tris-HCl, 0.9% NaCl, 0.1% Tween-20) for 1 hr and then incubated with antibodies for NTR (1:500; Millipore, Billerica, MA), VEGF (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA), ET-1 (1:4000; BD Transduction Laboratories, Franklin Lakes, NJ), iNOS (1:500; BD Transduction Laboratories) and eNOS (1:1000; BD Transduction Laboratories) respectively overnight at 4°C. The membrane was then washed and incubated with secondary antibody (1:2000; Amersham Pharmacia Biotech Inc., Buckinghamshire, England) at room temperature for 1 hr. The expression signals were detected using an ECL™ Western Blotting Detection Reagents (Amersham Pharmacia). ß-actin (Monoclonal antibodies, 1:5000, Sigma, St. Louise, MO) was used as a house-keeping internal control for normalization. The arbitrary units represent the optical density of the protein bands.

Evaluation of activity of hypoxia inducible factor-1α (HIF-1α), nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1)

Nuclear protein fractions from liver homogenates were prepared as described previously (Nanji et al., 1999). Electrophoretic mobility shift assays (EMSA) were performed on the nuclear extracts from livers in different experimental groups as described previously (Nanji et al., 1999). Densitometric scanning analysis was performed using laser scan densitometry. The specificity of binding was determined by prior addition of 100-fold excess of unlabeled competitor consensus oligonucleotide (Promega Corporation, Madison, WI). Supershift experiments were performed on 5% non-denaturing gels using antibodies (Santa Cruz Biotechnology) against the p50 and p65 subunits of NF-κB respectively.

Statistical analysis

All data from the experimental SD rats in the C and CH groups of each animal and each estimated parameter were pooled to obtain a mean value and standard error of mean (SEM) at all time-points (day 7, 14, 21, 28). The number of animals for each parameter from the C and CH groups was 6-8 SD rats. A non-parametric statistical test was used. Kruskall-Wallis test was used to compare differences among group data sets followed by post-hoc

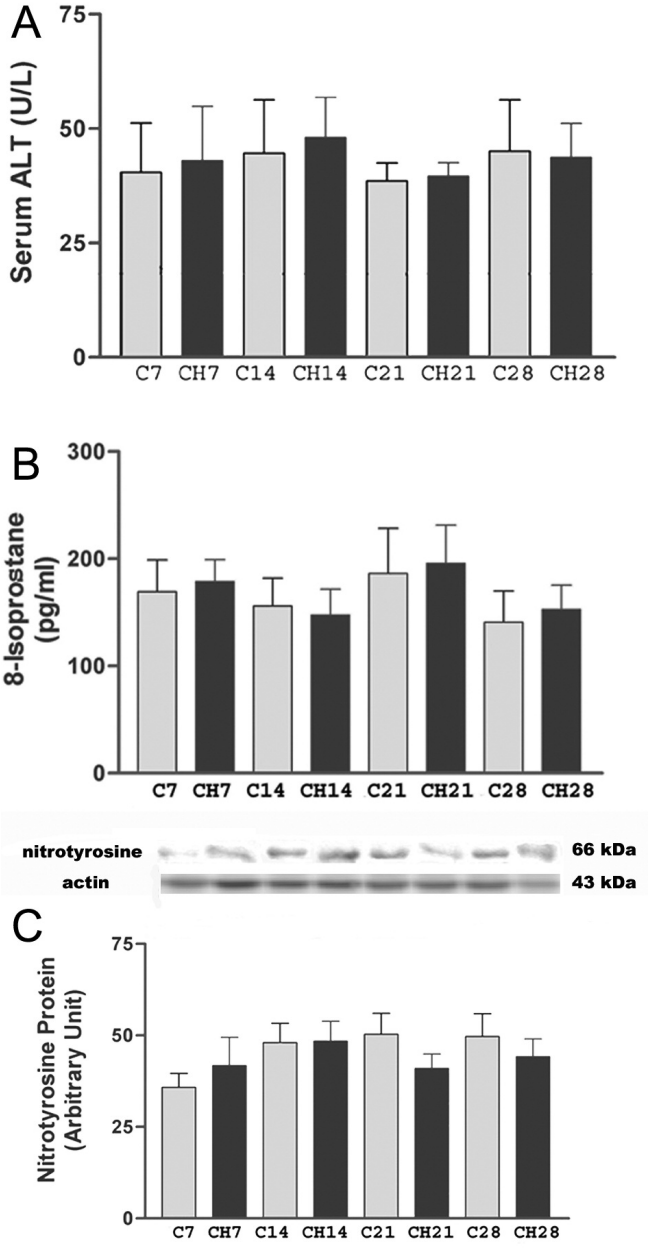

Fig. 1. Levels of serum ALT **(A)**, total 8-isoprostane **(B)** and the formation of nitrotyrosine in the normoxic **(C)** and hypoxic (CH) groups. No differences were detected between groups on the ALT, 8-isoprostane and nitrotyrosine levels. The levels of these parameters remained at the basal level throughout the study.

Dunn's test. A $p<0.05$ was considered statistically significant (Prism 3.0, Graphpad software, Inc., San Diego, CA).

Results

Serum alanine aminotransferase (ALT) level and oxidative stress markers

There were no significant differences between the control and the hypoxic rats on the serum ALT level, as well as the assay on the oxidative stress markers, serum total 8-isoprostane level and the formation of nitrotyrosine in the liver tissues (Fig. 1A-C) across the time points from day 7 to day 28.

Messenger RNA and protein expression levels of VEGF and ET-1

The expressions of VEGF and ET-1 were significantly higher in the hypoxic rats when compared with the respective control groups at all time points at

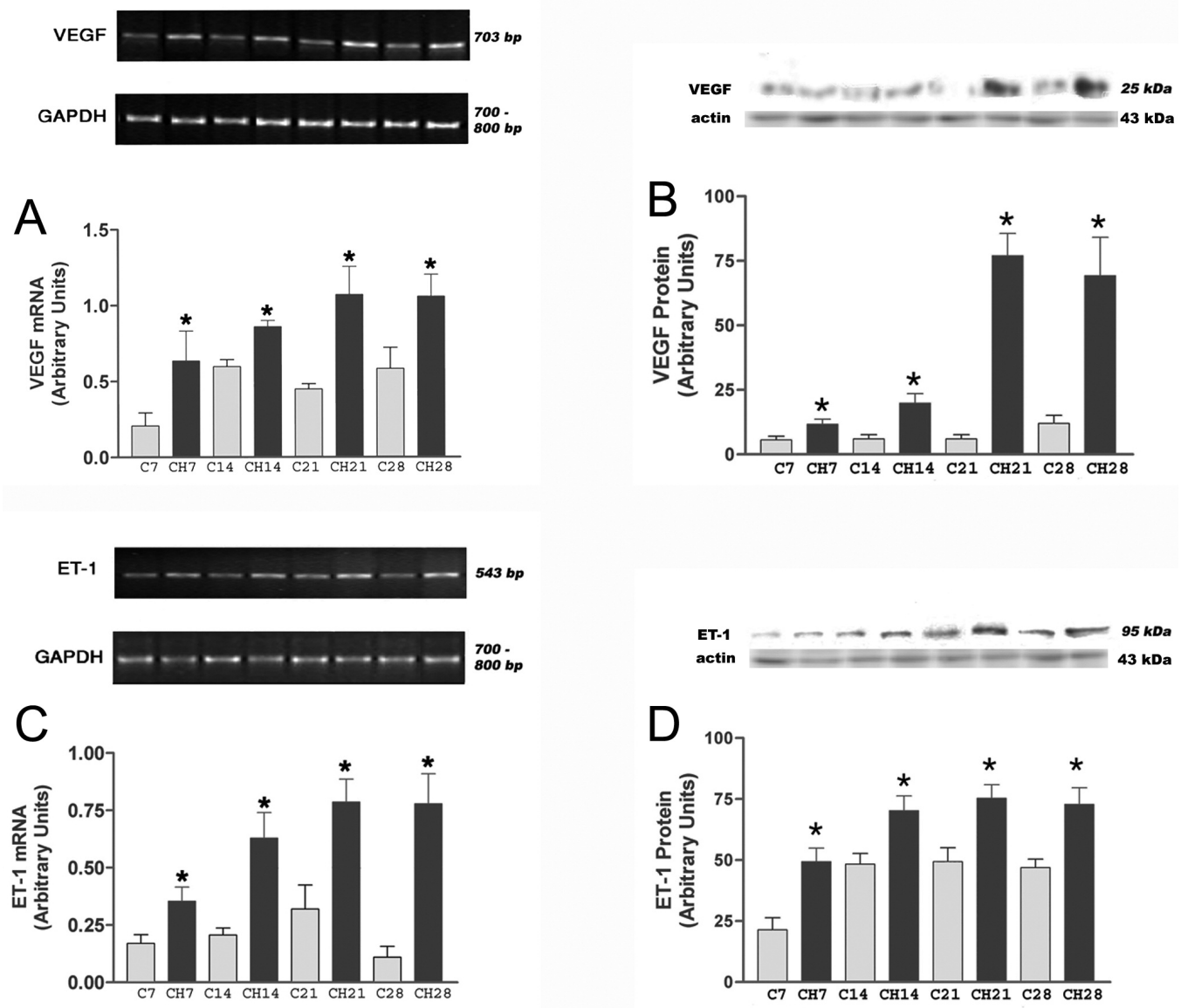

Fig. 2. mRNA and protein expression levels of VEGF and ET-1. **A and B.** A progressive increase in the mRNA and protein expression levels of VEGF in hypoxic (CH) group from day 7 to day 21 and level-off from day 21 to day 28. The hypoxic group has a significantly higher expression level than that of the normoxic (C) control at all time points. **C and D.** The hypoxic (CH) group also shows a progressive increase in the expression level of ET-1 from day 7 to day 21 and maintains a high level at day 28. The expression level of ET-1 is higher in the hypoxic group than the normoxic (C) group at all time points. (*$p< 0.05$= C vs. CH).

both transcriptional and translational levels (Fig. 2A-D). A gradual increase in the mRNA and protein levels of both VEGF and ET-1 were observed in the hypoxic groups from day 7 to day 21 and stayed at this level until day 28.

Messenger RNA and protein expression levels of iNOS and eNOS

iNOS mRNA expression progressively increased in the hypoxic rats from day 7 to day 21 and decreased at day 28, but were significantly higher at days 21 and 28 when compared with the respective normoxic controls (Fig. 3A). iNOS protein expression showed a similar trend with the levels of iNOS mRNA which progressively increased throughout all time points (Fig. 3B). eNOS mRNA expression also increased in the hypoxic rats at days 7, 14, and 28 when compared to corresponding controls. At day 21, there was no significant difference in the eNOS mRNA level between

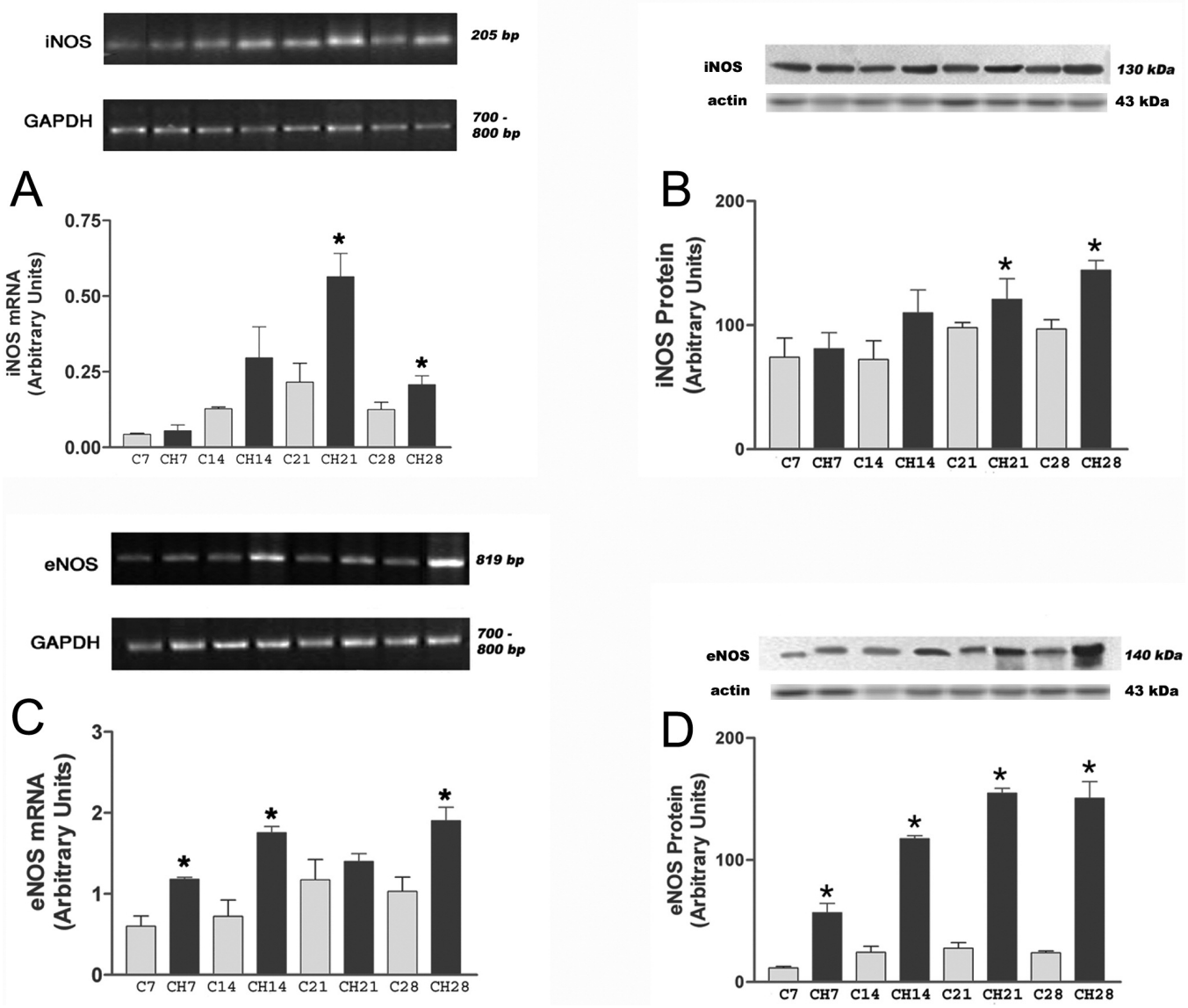

Fig. 3. mRNA and protein expression of iNOS and eNOS. **A and B.** A gradual increase in the level of iNOS is observed from day 7 to day 21 in the hypoxic group. A drop in the mRNA level of iNOS is observed from day 21 to day 28. The expression of iNOS shows a significant difference between the hypoxic (CH) and normoxic (C) groups at day 21 and day 28 (*p< 0.05). **C and D.** The hypoxic (CH) group shows an elevated level of eNOS mRNA and protein when compared with the normoxic (C) control, with a progressive increase from day 7 to day 21 and level-off from day 21 to day 28. (* p< 0.05 = C vs. CH)

control and hypoxic groups. eNOS protein levels of the hypoxic groups were also significantly higher than those of the respective control groups (Fig. 3C,D).

DNA-binding activity of HIF-1α, NF-κB and AP-1

The activity of HIF-1α increased gradually (from 4-fold at day 7 to 6-fold at day 28) in a time-dependent manner and was significantly higher in hypoxic rats when compared to the control rats (Fig. 4A). NF-κB activity was also significantly higher in hypoxic rats than normoxic rats, with a peak at day 21 and a slight decrease at day 28 (Fig. 4B). The activity levels of AP-1 in hypoxic rats increased from day 7 to day 14 and remained at the same levels from day 14 to day 28 (Fig. 4C). The AP-1 value was statistically different between hypoxic and normoxic rats at all time points.

Discussion

The physiological and pathological responses in pulmonary hypertension have been well documented, particularly the events that are involved in vascular remodeling (Jeffery and Wanstall, 2001). One of the factors that stimulate vascular remodeling is hypoxia. In our animal model, we sought to document the response of the liver in a prolonged, severe oxygen deprived environment. This has not been demonstrated by others, apart from a report of altered gene profile under chronic hypoxia (Baze et al., 2010) and a study investigating the expressional changes of HIF-1α from different organs under hypoxia (Bianciardi et al., 2006). The significance of this work lies in two main aspects. Firstly, hypoxia is an underlying event in chronic liver disease of non-tumoral origin which is characterized by inflammation and/or fibrosis. This hypoxic condition is found in alcoholic liver disease, hepatitis, steatohepatitis and liver cirrhosis. Secondly, understanding the molecular events that occur in chronic hypoxia would provide us with new understanding in the treatment of these diseases and diseases in other systems. Unlike other organs with sufficient oxygen supply, the liver is relatively "hypoxic" due to relatively low partial pressure of oxygen (pO_2) even under normoxic conditions. Therefore, studying the pathophysiological changes of the liver under normoxic conditions to chronic severe hypoxia may also provide significant knowledge to the compensatory responses of the liver in this microenvironment.

DNA binding activity of HIF-1α in chronic liver hypoxia

The expression of HIF-1α progressively and markedly increased from day 7 to day 28 in the hypoxic rats, with mild basal expression in the normoxic control, as shown by EMSA (Fig. 4A). The four-fold increase of HIF-1α in the hypoxic rats on day 7 when compared

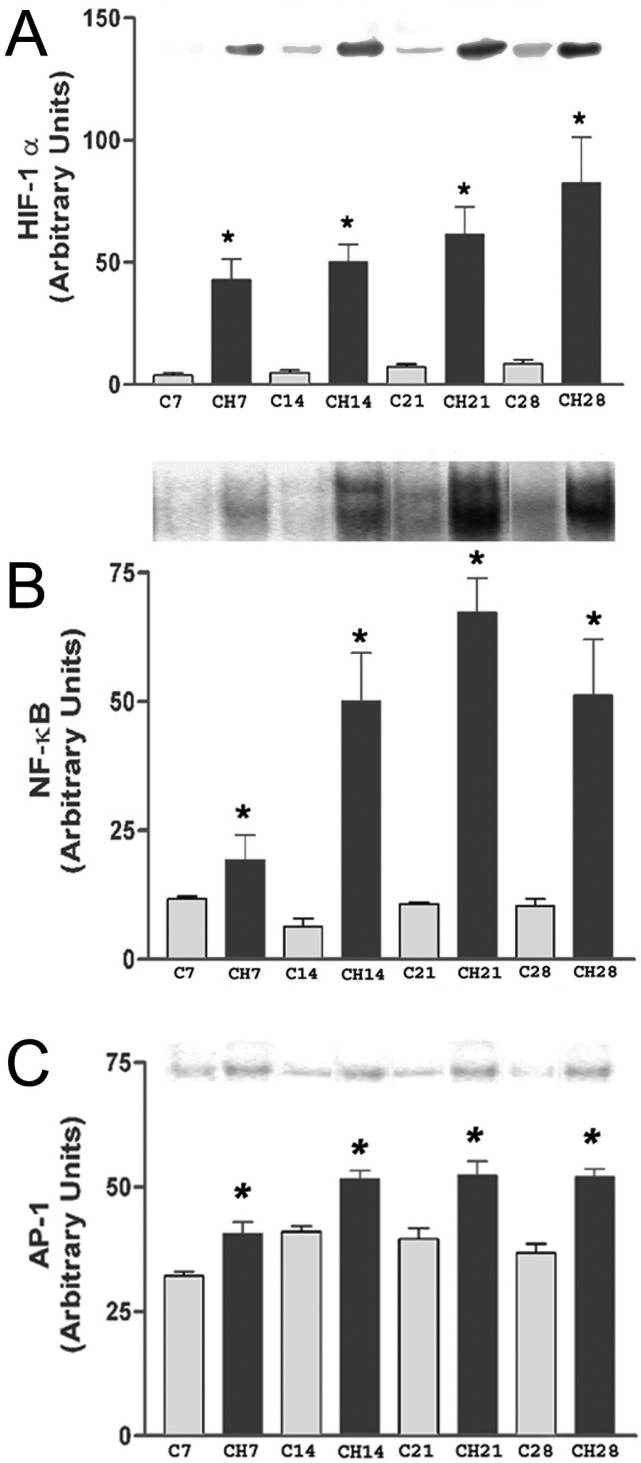

Fig. 4. DNA-binding activity of transcription factors HIF-1α **(A)**, NF-κB **(B)** and AP-1 **(C)**. The activity of HIF-1α is progressively increased from day 7 to day 28 in the hypoxic (CH) group. An increase in the activation of NF-κB and AP-1 is observed in the hypoxic group from day 7 to day 21 and the activities decrease and level off from day 21 to day 28 respectively. The hypoxic group shows a significantly higher activation level of HIF-1α, NF-κB and AP-1 than the corresponding normoxic (C) control. (*p< 0.05 = C vs. CH)

with corresponding normoxic controls, indicated that HIF-1α was activated a few days after the initiation of hypoxia because of the magnitude of the increase and the relatively low basal expression of HIF-1α in the normoxic control. The HIF-1α obtained by EMSA also indicated the amount of HIF-1α that dimerized with HIF-1ß and translocated into the nucleus for binding with the HRE of VEGF, ET-1 and iNOS. The HIF-1α activity in this model has shown that the liver cells could compensate when subjected to severe prolonged hypoxia. The liver cells also demonstrated no evidence of necrosis and oxidative stress, as shown by the low levels of serum ALT, 8-isoprostane and nitrotyrosine formation (Fig. 1-C). Another study has shown that after 2-week chronic hypoxia treatment, the protein expression of HIF-1α was undetectable in the rat liver (Bianciardi et al., 2006). The inconsistent results may be due to the sensitivity of the detection method. Our study applied radioisotope EMSA to detect the binding activity of HIF-1α, which exhibited much higher sensitivity and specificity than routine Western blotting and immunohistochemistry.

DNA binding activities of NF-κB and AP-1 in chronic liver hypoxia

Both NF-κB and AP-1 are stimulated during acute and moderate hypoxia (Cummins and Taylor, 2005). Whether these nuclear transcription factors have a sustained expression in the liver during chronic and severe liver hypoxia are not yet known. Our data showed that NF-κB was activated relatively early (i.e. first week) in the time course of the experiment and was sustained up to day 28. NF-κB is one of the major nuclear transcription factors that activate inducible nitric oxide synthase (iNOS). Our result showed that iNOS was upregulated during the course of chronic liver hypoxia both at the transcriptional and translational levels. Although the expression of iNOS mRNA did not sustain at the peak level at day 28 but it stayed above the basal level. The pattern of NF-κB activity was similar to the iNOS mRNA levels.

AP-1 is the transcriptional factor that partly activates the eNOS gene (Cummins and Taylor, 2005). AP-1 progressively increased from day 7 to day 21 and remained at a high level. However, the magnitude of increase was about 25% when compared with the normoxic controls. This response was also relatively early (i.e. first week) but not as high as NF-κB and HIF-1α activity. Our data also demonstrated that AP-1 response, in conjunction with the other two transcription factors, plays a vital role in chronic severe hypoxia, particularly for HIF-1α. This "adaptive" response is important in stimulating the HRE and eNOS genes, since they are vital in the production of nitric oxide (NO), thus could partly regulate the sinusoidal resistance and could possibly improve the hepatic perfusion to cope with prolonged oxygen deprivation conditions.

Upregulation of HRE genes and eNOS in chronic liver hypoxia

As of now, there are approximately more than sixty genes targeted by HIF-1 which contain the hypoxia response element (Semenza, 2003, Wenger, 2002). They are related to cellular survival, proliferation, angiogenesis and vascular regulation and metabolism. We investigated those HIF-1 target genes that are involved in vascular regulation, namely, VEGF, iNOS and ET-1. Our results showed that these genes were activated by HIF-1α with a relatively early, persistent and sustained response, both at the mRNA and protein levels. VEGF is involved in cell survival and angiogenesis (Ferrara, 1999). iNOS is an enzyme directly involved in the production of NO. NO regulates vascular tone and cell survival. A study has been shown that hypoxia is one of the important factors that causes pulmonary vasoconstriction (Jeffery and Wanstall, 2001). Furthermore, hypoxia can also increase the production of mitogens such as VEGF and ET-1. ET-1 has been shown to stimulate proliferation of fibroblasts (Peacock et al., 1992) during hypoxia in the lungs, and induces pulmonary smooth muscle cells to produce type IV collagen (Mansoor et al., 1995). Thus, ET-1 plays a key role in vascular remodeling. It has also been shown to be a potent constrictor of the hepatic sinusoids (Sato et al., 1983). Our data showed that there was a persistent and sustained production of ET-1 mRNA and protein during the four weeks of hypoxia treatment to the rats. In the pathogenesis of alcoholic liver disease, Sato et al. showed that the liver oxygen tension is very low among chronically ethanol-fed rats as compared with their normal counterpart (Zhang et al., 1994). In another animal model, ET-1 antiserum infusion inhibits ethanol-induced vasoconstriction (Oshita et al., 1993). In our model, the effect of ET-1 may be counteracted by iNOS and VEGF. Moreover, eNOS also contributed to this counter-effect, although eNOS is not a HRE gene regulated by HIF-1α but is partly regulated by AP-1 (Kumar et al., 2008). Apart from the angiogenic effect of VEGF, it promotes cell survival, possibly through its ability to stimulate NO formation with resultant vasodilation (Jozkowicz et al., 2004; Wei et al., 2004). Furthermore, there was a progressive and sustained increased in eNOS expression at both mRNA and protein levels. Overall, the end effect of VEGF, iNOS and eNOS is probably to produce a sustained level of NO in order to reduce sinusoidal resistance and increase hepatic blood flow, which may be partly through HIF-1α, NF-κB and AP-1, as possible compensatory responses to chronic severe hypoxia.

In summary, our chronic hypoxic model showed that in prolonged oxygen deprived conditions, the liver was capable of inducing relatively early high expression and activation of HIF-1α which promoted gene expression, such as VEGF, iNOS and ET-1 (Tipoe and Fung, 2003). Furthermore, severe and prolonged hypoxia activated

other nuclear transcription factors, such as NF-κB and AP-1. All these changes allow the liver to maintain its normal function and keep vital homeostasis under chronic severe hypoxia.

Acknowledgements. This study was supported by a grant from Small Project Funding, University Research Committee, The University of Hong Kong, Hong Kong.

References

Baze M.M., Schlauch K. and Hayes J.P. (2010). Gene expression of the liver in response to chronic hypoxia. Physiol. Genomics 41, 275-288

Bergeron M., Yu A.Y., Solway K.E., Semenza G.L. and Sharp F.R. (1999). Induction of hypoxia-inducible factor-1 (HIF-1) and its target genes following focal ischaemia in rat brain. Eur. J. Neurosci. 11, 4159-4170.

Bianciardi P., Fantacci M., Caretti A., Ronchi R., Milano G., Morel S., von Segesser L., Corno A. and Samaja M. (2006). Chronic *in vivo* hypoxia in various organs: hypoxia-inducible factor-1alpha and apoptosis. Biochem. Biophys. Res. Commun. 342, 875-880.

Bozova S. and Elpek G.O. (2007). Hypoxia-inducible factor-1alpha expression in experimental cirrhosis: correlation with vascular endothelial growth factor expression and angiogenesis. APMIS 115, 795-801.

Chen J.H., Tipoe G.L., Liong E.C., So H.S., Leung K.M., Tom W.M., Fung P.C. and Nanji A.A. (2004). Green tea polyphenols prevent toxin-induced hepatotoxicity in mice by down-regulating inducible nitric oxide-derived prooxidants. Am. J. Clin. Nutr. 80, 742-751.

Corpechot C., Barbu V., Wendum D., Kinnman N., Rey C., Poupon R., Housset C. and Rosmorduc O. (2002). Hypoxia-induced VEGF and collagen I expressions are associated with angiogenesis and fibrogenesis in experimental cirrhosis. Hepatology 35, 1010-1021.

Cummins E.P. and Taylor C.T. (2005). Hypoxia-responsive transcription factors. Pflugers Arch. 450, 363-371.

Ferrara N. (1999). Molecular and biological properties of vascular endothelial growth factor. J. Mol. Med. 77, 527-543.

Heidbreder M., Frohlich F., Johren O., Dendorfer A., Qadri F. and Dominiak P. (2003). Hypoxia rapidly activates HIF-3alpha mRNA expression. FASEB J. 17, 1541-1543.

Hoffmann A., Gloe T. and Pohl U. (2001). Hypoxia-induced upregulation of eNOS gene expression is redox-sensitive: a comparison between hypoxia and inhibitors of cell metabolism. J. Cell Physiol. 188, 33-44.

Hu J., Discher D.J., Bishopric N.H. and Webster K.A. (1998). Hypoxia regulates expression of the endothelin-1 gene through a proximal hypoxia-inducible factor-1 binding site on the antisense strand. Biochem. Biophys. Res. Commun. 245, 894-899.

Huang G.W., Yang L.Y. and Lu W.Q. (2005). Expression of hypoxia-inducible factor 1alpha and vascular endothelial growth factor in hepatocellular carcinoma: Impact on neovascularization and survival. World J. Gastroenterol. 11, 1705-1708.

Jeffery T.K. and Wanstall J.C. (2001). Pulmonary vascular remodeling: a target for therapeutic intervention in pulmonary hypertension. Pharmacol. Ther. 92, 1-20.

Jeong W.I., Do S.H., Yun H.S., Song B.J., Kim S.J., Kwak W.J., Yoo S.E., Park H.Y. and Jeong K.S. (2004). Hypoxia potentiates transforming growth factor-beta expression of hepatocyte during the cirrhotic condition in rat liver. Liver Int. 24, 658-668.

Jozkowicz A., Dulak J., Nigisch A., Funovics P., Weigel G., Polterauer P., Huk I. and Malinski T. (2004). Involvement of nitric oxide in angiogenic activities of vascular endothelial growth factor isoforms. Growth Factors 22, 19-28.

Koong A.C., Chen E.Y. and Giaccia A.J. (1994). Hypoxia causes the activation of nuclear factor kappa B through the phosphorylation of I kappa B alpha on tyrosine residues. Cancer Res. 54, 1425-1430.

Kumar S., Sun X., Wedgwood S. and Black S.M. (2008). Hydrogen peroxide decreases endothelial nitric oxide synthase promoter activity through the inhibition of AP-1 activity. Am. J. Physiol. Lung Cell Mol, Physiol, 295, L370-377.

Lam S.Y., Tipoe G.L., Liong E.C. and Fung M.L. (2008a). Chronic hypoxia upregulates the expression and function of proinflammatory cytokines in the rat carotid body. Histochem. Cell Biol. 130, 549-559.

Lam S.Y., Tipoe G.L., Liong E.C. and Fung M.L. (2008b). Differential expressions and roles of hypoxia-inducible factor-1alpha, -2alpha and -3alpha in the rat carotid body during chronic and intermittent hypoxia. Histol. Histopathol. 23, 271-280.

Lee S.H., Wolf P.L., Escudero R., Deutsch R., Jamieson S.W. and Thistlethwaite P.A. (2000). Early expression of angiogenesis factors in acute myocardial ischemia and infarction. N. Engl. J. Med. 342, 626-633.

Mansoor A.M., Honda M., Saida K., Ishinaga Y., Kuramochi T., Maeda A., Takabatake T. and Mitsui Y. (1995). Endothelin induced collagen remodeling in experimental pulmonary hypertension. Biochem. Biophys. Res. Commun. 215, 981-986.

Nanji A.A., Griniuviene B., Sadrzadeh S.M., Levitsky S. and McCully J.D. (1995). Effect of type of dietary fat and ethanol on antioxidant enzyme mRNA induction in rat liver. J. Lipid Res. 36, 736-744.

Nanji A.A., Zakim D., Rahemtulla A., Daly T., Miao L., Zhao S., Khwaja S., Tahan S.R. and Dannenberg A.J. (1997). Dietary saturated fatty acids down-regulate cyclooxygenase-2 and tumor necrosis factor alfa and reverse fibrosis in alcohol-induced liver disease in the rat. Hepatology 26, 1538-1545.

Nanji A.A., Jokelainen K., Rahemtulla A., Miao L., Fogt F., Matsumoto H., Tahan S.R. and Su G.L. (1999). Activation of nuclear factor kappa B and cytokine imbalance in experimental alcoholic liver disease in the rat. Hepatology 30, 934-943.

Oshita M., Takei Y., Kawano S., Yoshihara H., Hijioka T., Fukui H., Goto M., Masuda E., Nishimura Y. and Fusamoto H. (1993). Roles of endothelin-1 and nitric oxide in the mechanism for ethanol-induced vasoconstriction in rat liver. J. Clin. Invest. 91, 1337-1342.

Peacock A.J., Dawes K.E., Shock A., Gray A.J., Reeves J.T. and Laurent G.J. (1992). Endothelin-1 and endothelin-3 induce chemotaxis and replication of pulmonary artery fibroblasts. Am. J. Respir. Cell. Mol. Biol. 7, 492-499.

Sato A., Nakajima T. and Koyama Y. (1983). Interaction between ethanol and carbohydrate on the metabolism in rat liver of aromatic and chlorinated hydrocarbons. Toxicol. Appl. Pharmacol. 68, 242-249.

Semenza G.L. (2000). HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J. Appl. Physiol. 88, 1474-1480.

Semenza G.L. (2003). Targeting HIF-1 for cancer therapy. Nat. Rev. Cancer 3, 721-732.

Tipoe G.L. and Fung M.L. (2003). Expression of HIF-1alpha, VEGF and VEGF receptors in the carotid body of chronically hypoxic rat.

Respir. Physiol. Neurobiol. 138, 143-154.

Tipoe G.L., Leung T.M., Liong E., So H., Leung K.M., Lau T.Y., Tom W.M., Fung M.L., Fan S.T. and Nanji A.A. (2006). Inhibitors of inducible nitric oxide (NO) synthase are more effective than an NO donor in reducing carbon-tetrachloride induced acute liver injury. Histol. Histopathol. 21, 1157-1165.

Wei W., Jin H., Chen Z.W., Zioncheck T.F., Yim A.P. and He G.W. (2004). Vascular endothelial growth factor-induced nitric oxide- and PGI2-dependent relaxation in human internal mammary arteries: a comparative study with KDR and Flt-1 selective mutants. J. Cardiovasc. Pharmacol. 44, 615-621.

Wenger R.H. (2002). Cellular adaptation to hypoxia: O2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2-regulated gene expression. FASEB J. 16, 1151-1162.

Yu A.Y., Shimoda L.A., Iyer N.V., Huso D.L., Sun X., McWilliams R., Beaty T., Sham J.S., Wiener C.M., Sylvester J.T. and Semenza G.L. (1999). Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. J. Clin. Invest. 103, 691-696.

Zagzag D., Zhong H., Scalzitti J.M., Laughner E., Simons J.W. and Semenza G.L. (2000). Expression of hypoxia-inducible factor 1alpha in brain tumors: association with angiogenesis, invasion, and progression. Cancer 88, 2606-2618.

Zhang J.X., Pegoli W. Jr and Clemens M.G. (1994). Endothelin-1 induces direct constriction of hepatic sinusoids. Am. J. Physiol. 266, G624-632.

Zhong H., De Marzo A.M., Laughner E., Lim M., Hilton D.A., Zagzag D., Buechler P., Isaacs W.B., Semenza G.L. and Simons J.W. (1999). Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer Res. 59, 5830-5835.

Accepted October 5, 2012