Heme oxygenase: relationship to nitric oxide synthase/ nitric oxide system in murine schistosomiasis

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ABSTRACT

The present work studied the possible relationship between the nitric oxide synthase (NOS)/nitric oxide (NO) production and heme oxygenase-1 (HO-1) expression and HO activity in the acute and chronic phases of murine schistosomiasis. Control and *S. mansoni* infested mice were sacrificed bi-weekly from the 6th to the 20th week postinfestation. The expression of HO-1, inducible (i)NOS and endothelial, (e)NOS were assessed in liver tissues by RT-PCR and gel documentation. In addition, HO activity, cGMP levels, malondialdehyde (MDA) and total nitrite and nitrate levels (Nox) were also estimated. The data showed that HO-1 expression and activity increased in both early and later stages of Schistosomiasis in comparison to normal controls. eNOS expression decreased at the 18th and 20th weeks versus the control. iNOS expression showed continuous induction starting from the 6th week till the 20th week, with a decline in the 18th and 20th weeks versus the 8th and 10th weeks. This was accompanied by an increase in MDA levels (at 16th, 18th and 20th weeks) and a decrease in cGMP in 20th week. A negative correlation was detected between MDA and both Nox and HO-activity. The present results also suggest that HO-1 may be mediated partially by iNOS/NO production, as indicated by the positive correlation between Nox and HO-activity. HO may be important in decreasing the toxic bursts of iNOS/NO production through degrading the hemoprotein iNOS at the protein level. However, in limiting hepatocyte injury, it may contribute to development of the reported portal hypertension, probably by also degrading eNOS which is responsible for maintenance of normal portal perfusion.

RIASSUNTO

Eme-ossigenasi: relazione con il sistema ossido nitrico sintasi /ossido nitrico nella schistosomiasi del topo

Nel presente lavoro sono state studiate le possibili relazioni tra la produzione di ossido nitrico sintasi (NOS) / ossido nitrico (NO) e l'espressione della eme-ossigenasi-1 (HO-1) e l'attività della HO, nelle fasi acuta e cronica della schistosomiasi del topo. Topi di controllo e infestati con S. mansoni sono stati sacrificati a intervalli bisettimanali dalla sesta alla ventesima settimana dopo l'infestazione. L'espressione di HO-1, di NOS(i) inducibile e di NOS(e) endoteliale è stata valutata nel tessuto epatico mediante RT-PCR e dimostrazione in gel. In aggiunta si è anche determinato la attività della HO e le concentrazioni di cGMP, malondialdeide (MDA) e di nitriti e nitrati totali. I risultati hanno evidenziato che l'espressione e l'attività di HO-1 aumentava negli stadi iniziali e successivi della schistosomiasi in confronto ai controlli normali. L'espressione di NOS(e) diminuiva alla 18° e alla 20° settimana nel confronto dei controlli. L'espressione di NOS(i) mostrava continua induzione partendo dalla 6° fino alla 20° settimana, con un declino nella 18° e 20° settimana in confronto alla 8° e alla 10°. Questo era accompagnato da aumentata concentrazione di MDA (16°, 18° e 20° settimana) e da diminuzione del cGMP alla 20° settimana. Si evidenziava una correlazione negativa tra MDA e le attività di sia NOS sia HO. I risultati suggeriscono inoltre che HO-1 possa essere parzialmente mediata dalla produzione di NOS(i) / NO, come indicato dalla correlazione positiva tra NOS e attività di HO. HO può essere importante nel attenuare il carico tossico derivante dalla produzione di NOS(i) / NO tramite degradazione della emoproteina NOS(i) a livello proteico. Tuttavia, nel limitare il danno epatocitario, può contribuire allo sviluppo della documentata ipertensione portale probabilmente anche degradando NOS(e) che è responsabile per il mantenimento di una normale perfusione portale.

INTRODUCTION

Schistosomiasis is a chronic disease that can be complicated by portal hypertension and occasionally death. The egg induced inflammatory granulomas that are produced play a protective role against hepatic necrosis because they prevent the toxic effects of soluble antigens on the liver parenchyma. However, granuloma formation and subsequent fibrosis occlude the hepatic portal circulation leading to portal hypertension. In S. mansoni infested livers, a network of new capillaries is produced in the periportal areas i. e. angiogenesis results[1].

The potential regulatory roles of two gases, nitric oxide (NO) and carbon monoxide (CO) in liver disease and portal hypertension have attracted much attention [2,3]. NO, which has diverse physiological and pathophysiological

roles[4] is produced from L-arginine by nitric oxide synthase (NOS). Three isoforms of NOS have been cloned: inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS). All isoforms are dimeric in the active form [4]. Stabilization of the active dimeric form of NOS is suggested to be produced by the action of its cofactor tetrahydrobiopterin [5]. All three isoforms have been found in the liver. The iNOS is synthesized do novo in hepatocytes (and other cells) only after induction by liposaccharides and inflammatory cytokines[6]. In contrast eNOS releases NO for short periods of time in response to several endogenous and exogenous stimulants including physical stimuli[7]. This eNOS-derived NO production is required to maintain perfusion of the hepatic microcirculation. iNOS and eNOS are thought to play critical roles during liver injuries[2]. NO was reported to induce heme oxygenase-1 (HO-1) expression[8] leading to increase in its byproduct carbon monoxide (CO). CO, an endogenous akin to NO, can activate soluble guanylyl cyclase, leading to the production of cyclic guanosine monophosphate (cGMP) which then mediates various physiological functions[9]. Three HO isoforms have been identified: HO-1, HO-2 and HO-3. HO-1 is ubiquitously distributed at a low level in mammalian tissues and can be induced by a variety of stressors as cytokines, reactive oxygen species and heme itself[10]. Accumulating evidence has shown that CO is an important regulator of hepato-biliary function[3]. The relationship between CO/HO system and NO/NOS system is controversial. While some studies reported synergism between NO and CO[11,12], others reported that inhibition of nitric oxide synthesis promoted renal production of CO[13]. Short acting NO donors were reported to elevate HO-activity[14],while in prolonged NO action, HO activity was reported to decrease [15].

Thus, the present study aims to address the possible relation between NO/NOS system and the CO/HO system in acute and chronic phases of murine schistosoma mansoni infection.

MATERIALS AND METHODS

Animals

The present study included forty-two age and weight matched Swiss albino mice bred in the animal unit in Theoder Bilharz Research Institute, according to the National Institute of Health Guide for Care and Use of Laboratory animals[16]. All animals were given the standard mouse chow diet and water for drinking. Food was withheld fifteen hours before they were sacrificed.

Infestation of animals

Thirty six mice were injected intraperitoneally (i. p.) with approximately 60 S. mansoni cercariae obtained from pooled Biomphalaria Alexandrina snails[17]. The rest of the mice were left as normal controls (n=6).

Sample collection

Control and S. mansoni infested mice were sacrificed by cervical dislocation at 6,8 and 10 weeks (stage of granuloma formation) and 16, 18 and 20 weeks (stage of granuloma modulation and fibrosis)[18] post- infestation (p. i). The livers were removed and a part was taken for histopathological assessment of granuloma formation [at 6,8and 10 weeks (ws.)] and periportal fibrosis at 18 and 20 ws. The rest of the liver was divided into portions for RNA extraction, determination of malondialdehyde, cGMP, and total nitrite and nitrates. The tissues for RNA extraction were put in RNA extraction buffer[19] and frozen at -80^{o}C until used. Tissues for cGMP estimation were homogenized in 10 volumes of 0. 1N HCl, centrifuged at 600 x g and then frozen at -80oC till time of assay.

Assay procedures:

1) RNA extraction:

Total RNA from the liver tissues was extracted by the acid guanidinium thiocyanate-phenol-chlorform method[19]. The extracted RNA was quantitated spectrophotometrically at 260 nm.

2) Primer synthesis

Different primer sequences (table 1) were prepared at the Unit of Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University using the Oligo-1000 DNA synthesizer (Beckman, California, USA).

Table1
Oligonucleotide primer sequences:

Parameter	Primer sequence	PCR product (bp)
HO-1	F: 5'AAATAATTCTTTCCCACCCACC3' R: 5'CCATGTTGACTGACCACGAC3'	206
eNOS	F: 5'-GCAGAAGAGTCCAGCGAACA-3' R: 5'-GGCAGCCAAACACCAAAGTC-3'	110
iNOS	F:5'GGCTTGCCCCTGGAAGTTTCTCTTCAAAGTC3' R:5'AAGGAGCCATAATACTGGTTGATG-3'	441
β-actin	F:5'TCACCCTGAAGTACCCCATGGAG3' R:5'TTGGCCTTGGGGTTCAGGGGG3	150

3) RT-PCR for HO-1

RNA was reverse transcribed using 12. 5 μL oligo (dT)18 primer, denatured at 70°C for 2 minutes was done. The denatured RNA was placed on ice and 6. 5 μL of reverse transcription mixture containing 50 mM Tris HCl, pH 8. 3, 50 mM KCl, 1. 5 mM Mg Cl_2, 0. 5 mM of each dNTP, 1u/μL RNase inhibitor and 200 units of MMLV reverse transcriptase were added. The reaction tube was placed at 42°C for 1 h followed by heating to 92°C to stop the reaction. Then PCR reaction was performed by adding the PCR-mix to a final volume of 100 μL. The PCR mix contained 10 mM/L Tris-HCl, pH 8. 3, 50 mM KCl, 1. 5 mM $MgCl_2$, 0. 001% gelatin, 250 μM dNTPs mix, 2. 5u Taq polymerase, and 100 μM of each primer. The reaction mixture was then subjected to 40 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min. After the last cycle, final extension at 72°C for 10 min was done.

2) RT-PCR for eNOS

Total RNA (1 μg), 6μL of 10 mM each dNTPs, 5 x buffer and 30 pmol specific downstream primer were brought up to a total volume of 28 μL. The mixture was heat shocked for 5 min at 65°C and chilled on ice for 5 min. Then, 0. 5 μL RNase inhibitor (40 u/μL) and 1 μL AMV(10 U / μL) were added and the mixture was incubated at 42°C for 2 hrs. Five microlitres of cDNA was added to the following PCR mixture: 0. 5 μL of dNTPs (100 mM each), 5 μL of 10 x PCR buffer containing 25 mM $MgCl_2$, primers (30pmol each) and Taq DNA polymerase (2. 5 U/μL). The final volume of the PCR mix was 50 μL.

The thermocycling conditions were an initial denaturation for 3 min at 94°C, followed by 30 cycles of: 94°C for 30 sec, 58°C for 30 sec and 72°C for 30 sec followed by a final elongation of 5 min. at 72°C.

3) RT-PCR for iNOS

Reverse transcription was carried out on 1 μg of RNA applying 0. 25 μg random primers, 0. 1 mM/L dNTPs mixture, 40 units of RNase inhibitor, 200 units of Superscript II reverse transcriptase in 1 x PCR buffer (10 mM/L Tris-HCL, 1. 5 mM/L $MgCl_2$ and 50 mM/L KCl, pH 8. 3). The reaction was carried out at 37°C for 1 hr followed by 5 min. at 95°C to destroy the enzyme. Five microlitres of cDNA were added to the following PCR mixture: 2. 5 L of dNTPs (10 mM each), 5 μL of 10x PCR buffer containing 25 mM $MgCl_2$, 1 μL of forward and reverse primers (30pmol each), 2. 5U of Taq DNA polymerase and completed with nuclease free water to a final volume of 50 μL. Samples were denatured at 94°C for 3 min. followed by 30 cycles of amplification, each consisting of 1 min at 94°C, 1 min at 55°C and at 72°C for 1 min with a final elongation of 10 min. at 72°C.

N. B: RT-PCR for ß-actin, a house keeping gene, was performed to confirm integrity of RNA.

4) Agarose gel electrophoresis:

All the PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide and visualized by UV transilluminator.

5) Gel documentation:

The PCR products were semi-quantitated using the gel documentation system (BioDoc Analyze) supplied by Biometra.

6) Assay for heme oxygenase activity:

Liver tissues were sliced and homogenized in 2. 5 volume Tris-HCl buffer (10mM, pH7. 6) containing 250 mM sucrose and 0. 4 mM phenylmethylsulfonyl fluoride (PMSF). The homogenate was centrifuged at 800 xg for 10 minutes and then for 13,500 xg for 20 minutes to produce the mitochondrial pellet. The supernatant was withdrawn[20]. The protein content in supernatant was determined using the method of Lowry et al[21]. The activity of heme oxygenase in the supernatant was determined as described by Abraham et al. [22]. Briefly, the supernatants were incubated at 37°C for one hour with: heme (50 μM), rat liver cytosol (1mg/mL), $MgCl_2$ (1mM), glucose 6 phosphate dehydrogenase (3 units), glucose 6 phosphate (1mM) and $NADP^+$ (2 mM) in 0. 5 mL of 0. 1M potassium phosphate buffer pH (7. 4). The reaction was stopped by placing the tubes on ice. The bilirubin generated was extracted by chloroform and its amount was defined as the difference in absorbance between 463 and 520 nm using a standard bilirubin curve.

7) Estimation of total nitrite and nitrate (Nox): (as an index for NO production):

Frozen liver tissues were minced and homogenized in 1mL of Tris HCl (10mM, pH 7. 5); NaCl,(150mM), triton X-100,((1% (v/v)), and PMSF (10mM). The homogenized tissue was centrifuged at 900xg. The supernatant was used for the assays. Protein concentration was determined [21]. NO levels were determined indirectly by quantification of their oxidized products of degradation using nitrate reductase and the Greiss reagent by the method of Moshage et al. [23].

8) Estimation of malondialdehyde (MDA) levels:

Liver tissues were homogenized as in the Nox assay. Lipid peroxidation was determined by measuring MDA in liver homogenates using the thiobarbituric acid method according to Ohkawa et al. [24].

9) Estimation of cGMP levels:

Liver tissues level of cGMP was determined using the ELISA kit provided by R& D systems, Inc. Minneapolis, USA.

RESULTS

Results are presented in tables 2-3 and in figures 1 to 4. A significant increase was found in HO-1 expression at all weeks (ws) in S. mansoni infested mice in comparison to control mice. However, a significant decrease in HO-1 expression was detected in the 18 and 20th ws p. i in comparison to the 8th, 10th and 16th ws p. i (Table 2 and Fig. 1).

The HO-activity was also increased significantly in the 6th, 8th, 10th,16th, and 18th week p. i. in comparison to the control. Although still remaining above normal control levels, HO activity showed a significant decline in the 18th w. p. i. versus the 8th week and a significant decline in the 20th w. p. i versus the 8th and 10th week p. i. (Table 3). Thus HO-1 expression more or less paralleled HO-activity especially in the stage of granuloma formation.

The eNOS gene expression did not significantly change in the 6th to the 16th week p. i. in comparison to the control levels. However, eNOS band density as assessed by gel documentation decreased significantly in the 18th and 20thws. p. i in comparison to control (Table 2 and Fig. 2).

The iNOS gene expression was induced in all weeks p. i. but a decrease in the band density was detected in the 18th and 20th ws. p. i. in comparison to the 6th, 8th and 10th ws. p. i (Table 2 and Fig. 3).

The mean level of liver Nox was significantly increased in the 6th and 8th weeks p. i. and declined progressively thereafter. Liver cGMP mean levels were increased significantly at the 6th week p. i in comparison to the control, non-significantly increased at the 8th and 10th ws. p. i and non-significantly decreased at the 16th and 18th ws. p. i However, a significant decrease in cGMP level was detected in the 20th week p. i in comparison to the control level as well as to the levels at the 6th, 8th and 10th ws. p. i.

Liver MDA mean levels were non-significantly increased in the 6th, 8th and 10th ws. p. i. while they were significantly increased in the 16th, 18th and 20th ws. p. i. in comparison to control level. Also MDA level was significantly higher in the 18th and 20th week p. i in comparison to the 6th, 8th and 10th ws p. i.

Positive correlations were detected between Nox levels and each of cGMP levels (r=0. 714, p<0. 0001) and HO activity levels (r=0. 34, p=0. 027), respectively. A highly significant negative correlation was found between Nox levels and MDA levels (r=-0. 802, P< 0. 0001).

HO-activity levels showed non-significant correlations with cGMP levels while it was negatively correlated with MDA levels (r = -0. 38, p< 0. 02).

Statistical Analysis

For comparison of means between groups ANOVA with posthoc test was used. Simple correlation (r) was performed to correlate various parameters.

DISCUSSION

The present work aimed at studying the possible relation between the NOS/NO system and HO system in acute

Table 2
DNA concentration (g/mL) of the PCR products of HO-1, eNOS and iNOS by gel documentation in control and at different weeks post-infestation (n=6).

Parameter	Control (n=6)	Weeks post-infestation					
		6 (n=6)	8 (n=6)	10 (n=6)	16 (n=6)	18 (n=6)	20 (n=6)
HO-1	256±40.15	750±49.42*	3144±141.87*	2990±403.64*	2400±276.45*	1211±237.57*	800±76.94*
eNOS	844.6±186.05	799.5±95.5	821±101.8	818.8±115.66	783.16±115.13	625±59*	500±63.24*
iNOS	0	1585.66±172.2*	1803±127.05*	1885.6±107.59*	1833.5±174.03*	1231.83±74.07*	1175.66±96.6*

*Denotes significant difference between the control group and the other groups.
N . B. (1): significant decrease in HO-1 band density was detected in the 18th and 20th weeks p. i versus the 8th and 10th and 16th weeks postinfestation (p<0. 001).
N. B. (2): significant decrease in iNOS band density was detected in the 18th and 20th weeks p. i versus the 6th, 8th and 10th weeks postinfestation (p<0. 001).

Figure 1
RT-PCR products of HO-1. Lane 1: Molecular weight marker (100bp each). Lane1: product of control; Lanes 2to 7: RT-PCR product of HO-1 from mRNA extracted from liver tissues of S. mansoni infected mice at 6, 8, 10, 16, 18, and 20 weeks postinfestation respectively.

Table 3
Heme oxygenase (HO) activity (nmol bilirubin / mg protein / hour) and concentration of Liver total nitrite and nitrate (Nox), cyclic guanosine monophosphate (cGMP), and malondialdehyde (MDA) in control and at different weeks post-infestation (n=6).

Parameter	Control (n=6)	Weeks post-infestation					
		6 (n=6)	8 (n=6)	10 (n=6)	16 (n=6)	18 (n=6)	20 (n=6)
HO activiry nmol bilirubin / mg protein / hour	1.04±0.22	1.88±0.25*	2.71æ0.50*	2.65±40.50*	2.11±0.38*	1.95±0.41*	1.71±0.50*
Nox μmol/ mg protein	39.7±2.30 (a)	50.13±3.61 (b)	47.35±3.69 (b)	43.29±5.84 (a,b)	32.50±4.44 (c)	27.95±4.07 (c)	26.96±2.88 (c)
cGMP pmol/mg protein	5.35±1.29 (a)	8.07±1.76 (b)	7.7±1.52 (a,b)	7.41±1.44 (a,b)	4.76±1.12 (a,c)	3.21±0.92 (a,c)	2.78±0.92 (c)
MDA nmol/mg protein	0.11±0.03 (a)	0.16±0.04 (a,b)	0.17±0.04 (a,b)	0.17±0.06 (a,b)	0.23±0.08 (b,d)	0.27±0.08 (d)	0.3±0.05 (d)

*Denotes significant difference between the control group and the other groups.
N. B (1): A significant decrease in HO activity was found in the 20th week versus the 8th and 10th week.
N. B. (2): A significant decrease in HO activity was found in the 18th week versus the 8th week.
For each parameters, different letters (a, b, c or d) under each group indicate significance difference between groups. Same letter writer for each group indicate non-significant difference between groups.

Figure 2
RT-PCR products of eNOS. M: DNA marker (50 bp). Lane1: product of control; Lanes 2 to 7: RT-PCR product of eNOS from mRNA extracted from liver tissues of S. mansoni infected mice at 6, 8, 10, 16, 18, and 20 weeks postinfestation respectively.

Figure 3
RT-PCR products of iNOS. Lane M: DNA marker (100bp). Lane1: product of control; Lanes 2 to 7: RT-PCR product of iNOS from mRNA extracted from liver tissues of S. mansoni infected mice at 6, 8, 10, 16, 18, and 20 weeks postinfestation. respectively.

Figure 4
RT-PCR products of ß-actin. LaneM: Molecular DNA marker, Lane1: product of control; Lanes 2 to 7: RT-PCR product of ß-actin from mRNA extracted from liver tissues of S. mansoni infected mice at 6,8,10,16,18,and 20 weeks postinfestation respectively.

and chronic schistosomiasis. The results showed a significant increase in HO-1 expression and HO-activity in murine schistosomiasis especially in the stage of egg deposition and granuloma formation with a progressive decline with the increasing chronicity of the condition. In accordance with these results, several studies reported increased HO-1 expression and/or HO activity in portal hypertensive [25] and cirrhotic rats [26]. Wei et al. [26] reported an early increase in HO-1 expression and activity which dropped in the late stages of cirrhosis. Also, Maines et al. [27] stated that during the stage of egg deposition the worm catabolizes hemoglobin to heme which is a potent inducer of HO-1.

In addition adult schistosoma which reside in the hepatic portal system are reported to be exposed to reactive oxygen species (ROS) as a result of the host immune response [28]. ROS are reported to be strong inducers of HO-1[8]. Cytokines produced during granuloma development and in the stage of granuloma modulation [20] are potent inducers of HO-1[29]. The cause of the decline in HO-1 expression in the 16^{th}, 18^{th} and 20^{th} ws. p. i. may be explained by the change in the cytokine pattern which occurs in the stage of granuloma modulation and fibrosis. Interferon gamma (reported to decrease HO-1 induction) [30] was found to increase at the beginning of the 17^{th} week [18].

The decline in HO-activity in later stages of schistosomiasis in the present study can be explained by that cytochrome P450 is a good substrate for HO, and is decreased when HO is induced. It should be noted that the enzymatic reaction mediated by HO requires reducing equivalents supplied via NADPH cytochrome P450 reductase. Competition for this electron pool from other P450 reactions may limit cogeneration from HO activity [3]. In the present study, increased oxidative stress (although an inducer of HO-1 gene expression) may lead to decrease in HO-activity at protein level. It was reported that increased peroxynitrite production lead to a concentration dependant decrease in HO-activity in the rat spleen and brain [31]. This is supported by the negative correlation between HO activity and MDA detected in the present study.

In the present study, iNOS expression and NO production (as measured by Nox levels) were higher during the stage of granuloma development. NO is reported to be a potent inducer of HO-1[8, 32]. However, the present study showed a decline in iNOS expression in the 18^{th} and 20^{th} ws p. i. in comparison to the stage of granuloma formation. This is in accordance with the results of Hirata et al. [33] who found extensive iNOS expression during the stage of granuloma development which decreased progressively with time. This may be explained by the results of Wei et al. [26] who reported that induction of HO-1 in cirrhotic rats may be mediated partially by iNOS/NO production. However, once it is induced, HO-1 may be an important signal in down-regulating iNOS.

In the present study, NO production (measured as Nox) decreased below control levels in the stage of granuloma modulation and fibrosis although iNOS was still induced. This suggests that iNOS may be also regulated at the protein level. Because of the hemoprotein nature of NOS, HO-1 may be important in modulating iNOS activity in reverse by accelerating NOS degradation, or by competing for the cofactor NADPH [34]. . This inactivation of iNOS by HO-1 seems to be potentially beneficial. This is because, following cellular injury, cells express high outputs of iNOS that are capable of generating cytotoxic amounts of NO [35]. NO-induced HO-1 may also provide cytoprotection from oxidative stress associated with elevated rates of NO production [36]. HO-1 can act both as a sensor to, and target of redox based mechanisms involving NO, and thus may serve as a signaling molecule in modulation of tissue stress response [37]. This is through synthesis of bilirubin and biliverdin and induction of ferritin. It was noticed (in the present study) that when HO-activity declined in the later stages of S. mansoni infection, lipid peroxidation (as measured by MDA) increased.

In the present study, although iNOS remained induced at the 16^{th}, 18^{th} and 20^{th} ws p. i, the mean levels of Nox were found to be significantly decreased. The decrease in Nox may be due to a decrease in NO bioavailability rather than production. Produced NO may be degraded by the progressing oxidative stress that occurs in schistosomiosis. NO interacts with superoxide to produce peroxynitrite which can induce lipid peroxidation [38]. This is supported by the strong negative correlation found between Nox and MDA.

The decrease in NO bioavailability did not affect cGMP levels till the 18^{th} week p. i. as it may be temporarily compensated for by the increased HO-activity. However, when HO activity declined in the 20^{th} week the cGMP

levels also significantly decreased. We cannot disregard the possibility of an increased activity of cGMP phoshodiesterase which was reported to occur in the renal tissues of cirrhotic rats[39]. Also since NO was hypothesized to pave the way for CO action[14,15], decreased NO bioavailability may decrease the capacity of CO in stimulation of guanylate cyclase. This offers a mechanism for cross-talk between NOS and HO systems.

Reactive oxygen species (ROS) production by infilterating neutrophils was reported to occur in the early phases of liver fibrosis [40, 41]. It was reported that, NO in physiological doses can directly alter extra cellular matrix generation and decrease collagen synthesis [42]. Therefore it is reasonable to speculate that the decreased NO bioavailability (by ROS) may be involved in enhanced deposition of collagen in the periportal areas of the liver.

From these results we deduce that: up regulation of HO-1 can be mediated by NO synthesized by iNOS. HO-1 as a pro-survival mediator may provide anti-inflammatory, and anti-apoptotic effects [43] to protect hepatocytes against the potential toxicities of excess NO. This may be through the increase in bilirubin (antioxidant) and CO which might help in protection of the hepatic microcirculation and limit liver dysfunction [44, 45]. Increased oxidative stress probably plays a key role in both fibro genesis and portal hypertension. ROS by decreasing the bioavailability of NO, may cause the endothelial dysfunction and portal hypertension associated with fibrosis. Considering that NO is the major endogenous vasodilator, it becomes apparent that any reduction in NO release can lead to unopposed effect of vasoconstrictor stimuli (esp. endothelin [46]), which is detrimental to liver perfusion.

In schistosomiasis, there is a minimal hepatocyte injury which may be due to the cytoprotective effect of HO-1. In protecting the liver from increased iNOS activity HO may have the same negative effect on eNOS activity. eNOS is responsible for the maintence of the normal hepatic microcirculation. Thus in protecting hepatocytes from apoptosis by its antiapoptotic properties [43], HO-1 may contribute to the development of fibro genesis and portal hypertension.

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