

Expression and modulatory effects of heme oxygenase in acute inflammation in the rat

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Abstract. Heme oxygenase (HO) is the rate limiting enzyme in the catabolism of heme molecules to the bile pigments which have recently been demonstrated to be strong antioxidants. In this study we analyzed the activity of HO in inflammatory cells isolated from a model of carrageenin induced acute inflammation in the rat. HO activity was significantly higher 24 hours after induction of the inflammation, this increase in activity coincided with the appearance of the highly inducible isoform of HO, Heat Shock Protein 32 kDa (HSP32) as detected by Western Blot analysis. Pre-treatment of animals with Tin protoporphyrin, a HO inhibitor, increased cell exudate at 24 hour in this model by 128% as compared to vehicle control. In comparison pre-treatment with a HO inducer, Ferriprotoporphyrin, decreased inflammatory cell number by 50% and cell exudate by 73% at 24 hours compared to control. These results suggest that HO may represent an endogenous protective mechanism against free radicals in acute inflammation and may be involved in the resolution of acute inflammation. The HSP32 isoform of HO may therefore represent a novel therapeutic target for the modulation of the inflammatory response.

Introduction

The development of an acute inflammatory response involves the sequential production and release of a myriad of biological mediators which act both locally and systemically. The rate limiting enzyme in the catabolism of heme molecules is heme oxygenase (HO), the products of this reaction are the bile pigment biliverdin, carbon monoxide (CO) and iron. Two isoforms of HO have at the present time been characterised, the constitutive form heme oxygenase-2 and a second inducible form, heme oxygenase-1 [1]. This second isoform which is highly expressed after heat and oxygen free radical treatment has also been classified as a heat shock protein/stress protein (HSP32) [2]. Interest in HO has been recently stimulated by the demonstration that it may play a protect role against oxidative stress [3]. In the present study we examine the activity of HO and the expression of the inducible form HSP32 in the rat

carrageenin-induced pleurisy model of acute inflammation. The effect of modulating HO on acute inflammation was also investigated.

Materials and methods

Animals and drugs

Male Wistar rats 200 ± 20 grams (Tuck & Sons Ltd., Essex, UK) were used for inflammation. Animals were administered either with 15 mg/kg Ferriprotoporphyrin IX chloride (FePP) by intravenous injection -18 hours before inflammation or with 2 doses of 40 μ moles/kg Tin protoporphyrin dichloride (SnPP) by subcutaneous injection -18 hours and at the time of inflammation induction (porphyrins were obtained from Porphyrin Products Inc., Logan, Utah). Drugs were prepared in 0.1 N NaOH and mixed 1:1 with saline, these were then adjusted to pH 7.4, drug vehicle. The total volume injected was 0.2 ml. Control animals received vehicle only.

Induction of pleurisy

Carrageenin pleurisy was induced in rats and cell pellet prepared as previously described [4].

HO activity

HO activity in the cell pellets was measured in post mitochondrial supernatant by quantifying the generation of bilirubin [5], biliverdin reductase was substituted with rat hepatic cytosol (3 mg/ml). Protein was estimated by the Bradford method using BSA as standard [4].

Western blot analysis

Cell pellets were lysed by the addition of protease inhibitory buffer containing 1% Triton X100 and boiled (10 min) with gel loading buffer (Tris, 50 mM; SDS, 10%; glycerol, 10%; 2-mercapthoethanol, 10%; bromophenol blue, 2mg/ml) in a ratio of 1:1 and centrifuged at 10,000 g, for 10 min. The protein concentrations of supernatants were determined as above, and total protein-equivalents (20 μ g) for each sample separated on 10% sodium dodecyl sulfide-polyacrylamide mini-gels (Hofer; Staffordshire, UK) using the Laemmli buffer system and transferred to polyvinylidene difluoride membranes (Millipore, Hertfordshire, UK). Nonspecific IgGs were blocked with 5% dried milk protein

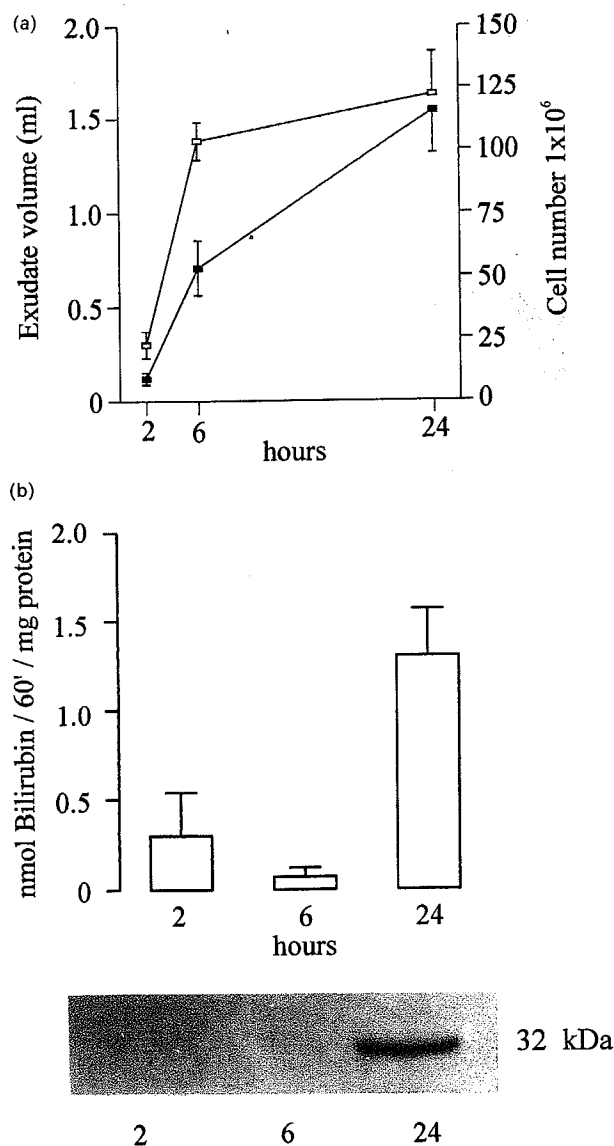


Fig. 1. a) The development of an acute inflammation after injection of carrageenin into the rat pleural cavity. □ Cell exudate, ■ Total cells, $n=10$ each time point. b) The measurement of HO activity and HSP32 expression by western blot analysis during acute inflammation, $n=6$ each time point b).

and incubated with a polyclonal antibody to HSP32 1:1000 dilution (Stressgen Corp., Victoria, Canada). Bands were detected with an amplified alkaline phosphatase kit (Sigma Co. Poole, UK) and developed with nitroblue tetrazolium (NBT)-5-bromo-4-chloro-3-indonyl phosphate (BCIP). Rainbow marker and pre-stained blue protein markers were used for molecular weight determinations.

Statistics

Results are expressed as the mean \pm s.e.mean. Statistical analysis was determined by Mann-Whitney U-test, with a p value of < 0.05 considered significant.

Results

Figure 1a shows the development of exudate volume and cell number during inflammation in the pleural cavity,

Table. The effect of Heme oxygenase inhibitor Tin protoporphyrin dichloeid (SnPP) and inducer Ferritoporphyrin IX chloride (FePP) on acute inflammation. Treatment animals received either 2 dose of 40 μ moles/kg SnPP s.c -18 and 0 hours before induction of inflammation, or 1 dose of 15 mg/kg FePP i.v -18 before inflammation. Treatment animals were compared to control animals which had received vehicle only by the appropriated route. *** $P < 0.001$.

Treatment groups	Exudate volume (ml)	Total cells 10^6
6 hours		
Vehicle injected s.c (n=27)	1.26 \pm 0.10	72 \pm 7
SnPP injected s.c (n=27)	1.59 \pm 0.12	67 \pm 6
Vehicle injected i.v. (n=23)	1.29 \pm 0.15	69 \pm 8
FePP injected i.v. (n=26)	0.99 \pm 0.11	50 \pm 8
24 hours		
Vehicle injected s.c (n=28)	0.79 \pm 0.12	63 \pm 6
SnPP injected s.c (n=24)	1.80 \pm 0.21***	58 \pm 6
Vehicle injected i.v. (n=22)	1.37 \pm 0.16	90 \pm 6
FePP injected i.v. (n=25)	0.37 \pm 0.07***	45 \pm 6***

these were both maximal 24 hours after injection of the carrageenin. A significant increase in HO activity in the exudate cell pellet, Figure 1b and the detection of HSP32 protein by western blot analysis, Figure 1b photo, coincided with this peak in inflammation. The effects of the HO inhibitor SnPP and inducer FePP on carrageenin inflammation were then investigated (Table). Exudate volumes and cell numbers at 6 hours were not significantly modified by pre-treatment with HO inhibitor or inducer. However, 24 hours after initiation of the inflammation SnPP increased exudate volume by 128% as compared to vehicle control ($p < 0.001$) whereas FePP decreased exudate volume by 73% and cell number by 50% compared to vehicle control (both $p < 0.001$).

Discussion

These results demonstrate for the first time the induction of HSP32 expression and HO activity in inflammatory cells from a model of acute inflammation. The increase in HO activity reported above is associated with an increase in macrophages population in the pleural cavity [4]. Immunohistochemistry demonstrated strong positive immunoreactivity of inflammatory macrophages but not peripheral monocytes for HSP32 in this model (data not shown). The effect of pre-stimulating or inhibiting HO activity significantly decreases or increases inflammation respectively at 24 hours. This would indicate that the major effects of HO modulators is on inflammatory macrophages at 24 hours, with no significant effect at 6 hours when the inflammatory site is dominated by polymorphonuclear leucocytes. However, other possible mechanisms of action cannot be discounted. Bile pigments have been shown to display potent antioxidant activity [6], consequentially the expression of HO by macrophages may represent an endogenous protective mechanism against free radicals which are generated during inflammation [7]. It is also intriguing to speculate on the involvement of CO in inflammation, following the demonstration of the importance of nitric oxide in

physiological and pathophysiological processes [8]. Recently we have shown the involvement of the inducible isoforms of cyclooxygenase and nitric oxide synthase in the development of the carrageenin induced pleural inflammation in the rat [4]. HSP32 may therefore represent a further inducible enzyme which has an important modulatory effect in acute inflammation. It is suggested that an increase in HO activity may represent a novel therapy for the treatment of chronic inflammatory disease, whereas a decrease in activity could be of benefit in those individuals with an impaired inflammatory response.

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