Expression of CXC Chemokines Gro/KC and SDF-1α in Rat H4 Hepatoma Cells in Response to Different Stimuli

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ABSTRACT

Background: It is now well established that several environmental stress factors cause activation of p38 MAP kinase and JNK in various cell types to produce chemokines. **Objective:** To investigate the expression of CXC chemokines Gro/KC and SDF-1α in rat's H4 hepatoma cells in response to heat shock, hyperosmolarity and oxidative stress. **Methods:** Hepatoma cells were maintained in MEM medium. Cells were subjected to different stresses [(H_2O_2 0.15% (w/v), manitol and NaCl (160 mM) and heat shock (42 °C for 20 minutes)]. Cells were harvested and RNA was extracted, purified and the CXC chemokine Gro/KC and SDF-1α expression was analysed by RT-PCR. cDNA was separated by gel electrophoresis on a 1% (w/v) agarose gel and visualized under a UV transilluminator. **Results:** There was detectable but low expression of both SDF-1α and Gro/KC in H4 hepatoma cells. Heat shock failed to induce expression of SDF-1α and Gro/KC in H4 hepatoma cells of rat. Hyperosmolarity also did not stimulate SDF-1α and Gro/KC expression. In this study we have also shown that oxidative stress did not induce expression of SDF-1α and Gro/KC. Overall, although detection is possible but regulatory responses were not observed in H4 hepatoma cells. **Conclusion:** Several known injurious conditions cause recruitment of macrophages, neutrophils and other immune cells to the liver. Immune cells are recruited to the hepatic vasculature following local liver injury and subsequent chemokine production. Our results demonstrated that failure to produce chemokines by hepatoma cells may be a way to escape from mechanism of immune surveillance.

Keywords: Hepatoma, SDF-1α, Gro/KC, Chemokine

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INTRODUCTION

It is now established that several environmental stress factors including UV radiation, DNA damage, pro-inflammatory cytokines, heat shock, oxidative, and osmolarity stress can cause the activation of p38 MAP kinase and JNK in various cell types (1). The effect of osmotic shock (e.g NaCl) on expression of IL-8 in human monocytes and bronchial epithelial cells has been shown to occur through a p38-dependent pathway (1, 2). The roles of p38, p42/44, and NF-κB in the expression of IL-8 and Gro/KC in response to hyperosmolarity in human intestinal epithelial cells have been identified (3). Oxidative stress is associated with several liver disorders such as bacterial and viral liver infections; toxin induced liver injury, ethanol consumption, and hepatocelullar carcinomas (4). These situations are associated with chronic inflammatory response and increased expression of Gro/KC, MIP-2, and IL-8 in rat and mouse hepatocytes and human Hep-G2 cells (5). H_2O_2 also activates NF-κB and regulates IL-8 expression in epithelial cells of lung (6). In this research, H4 hepatoma cell line was used as a model to detect CXC chemokines because it is a liver cell line which is reported to be reasonably differentiated and which has previously been well characterised. We investigated the expression of CXC chemokines Gro/KC as an ELR^+, and SDF-1α as an ELR^- CXC chemokines in rat's H4 hepatoma cells in response to heat shock, hyperosmolarity, and oxidative stress to explore the pattern of expression of these CXC chemokines by hepatoma cells.

MATERIALS AND METHODS

Hepatoma cells were maintained in MEM (minimal essential medium). Cells were subjected to different stress factors [(H_2O_2 0.15% (w/v), manitol and NaCl (160 mM) and heat shock (42°C for 20 minutes)]. Cells were harvested, RNA was extracted, purified, and expression of the CXC chemokines SDF-1α and Gro/KC was analysed by RT-PCR. cDNA was separated by gel electrophoresis on a 1% (w/v) agarose gel and visualized under a UV transilluminator. GAPDH mRNA was assessed in parallel as control.

H4 Hepatoma Cell Culture. Rat hepatoma H4-II-C3 cells were obtained from ECACC (European Collection of Animal Cell Culture, Porton Down. Wiltshire, UK). Cells were incubated at 37°C in atmospheric air: 5% CO_2. After 24 hours, medium and any unattached cells were removed and 20 ml of fresh growth medium was added. Cells were then subjected to routine subculture. For the maintenance of subculture, culture medium was removed and the cell sheet was washed with PBS. Cells were detached with gentle agitation and addition of 2 ml sterile trypsin (200mg/ml). Then fresh medium was added to stop trypsin action and the cell suspension was centrifuged at 400g for 5 min at room temperature. The supernatant was discarded, the pellet was suspended in fresh medium, and cells were diluted in medium to generate a density of 3.0-3.5 x 10^5 cells/ml in T75 ml flasks. Incubation was continued at 37°C and subculture was repeated until cells were 70% confluent (after about 3-5 days of culture).

Reverse Transcription Polymerase Chain Reaction (RT-PCR). To make complementary DNA (cDNA), reverse transcription reactions were performed using the following protocol: 4μl 5x strand buffer [125mM tris-HCl pH 8.3, 188 mM KCl, 7.5

mM $MgCl_2$ 25 mM DTT]; 1 µl of each dNTP [dATP, dCTP, dGTP, dTTP (stock concentration of 10mM in DEPC-treated water)]; 4 µl oligo-dT (stock concentration of 125 µg/ml); 1 µl RNA (1µg/ µl); 4 µl DEPC–treated water; 1.5 µl M-MLV reverse transcriptase enzyme. After addition of M-MLV-reverse transcriptase and mixing, the tube was incubated for 1 hour at 37°C. The resultant product was stored at -20°C for further use in PCR reactions. To amplify DNA species, PCR reaction mixture was prepared by addition of the following reagents to a 0.5 ml micro-centrifuge tube in ice: 10 µl *Taq* polymerase buffer (10x); 3 µl $MgCl_2$ (stock concentration 1.5mM); 2 µl of each dNTP [(dATP, dCTP, dGTP, dTTP) stock concentration of 10mM]; 2 µl of each primer pair [(forward and reverse) stock concentration of 25ng/ µl]; 4 µl cDNA; and sterile double distilled water to a final volume of 99 µl. The above mixture was overlaid with 40-60 µl mineral oil. The PCR thermocycler was adjusted accordingly: 94 °C for 5 minutes, 94 °C for 1 minute (denaturation), 1 minute at appropriate annealing temperature for annealing of different targets (Table 1), and 72°C for 2 minutes (elongation). Denaturation, annealing and elongation procedures were repeated for 30 cycles and were followed by a final reaction at 72°C for 10 minutes (final elongation) and held at 4°C up to 24h. During the last 45 seconds of first stage 1µl *Taq* polymerase was added to the mixture. In addition to appropriate annealing and melting temperatures, table 1 indicates the size of products that should be achieved by PCR.

Table 1. Primers used to detect CXC chemokine mRNA

Gene Name	Primers (5` to 3`)	AT (°C)	MT(°C)	Fragment Size
GAPDH	F: aaggtcggtgtgaacggatt		62	835
	R: gagacaacctggtcctcagtgtag	57	62	
Gro/KC	F: ttcacttcaagaacatcca			680
	R: gagcattggttaaagaatataaa	51	56	
			56	
SDF-1α	F: agtgtgcattgacccgaaatta		65	1500
	R: ttacagcacgaaacagttggc	60	65	

Abbreviations: AT= annealing temperature; F= forward primer; GAPDH= glyceraldehyde-3-phosphate dehydrogenase; MT= melting temperature; R=reverse primer

RESULTS

Results obtained in this report revealed that there were detectable but low expression of both SDF-1α and Gro/KC in H4 hepatoma cells. Heat shock failed to induce expression of SDF-1α and Gro/KC in H4 hepatoma cells of rat. Hyperosmolarity also did not stimulate SDF-1α and Gro/KC expression. In this study we also noted that oxidative stress did not induce expression of SDF-1α and Gro/KC. Overall, although detection is possible but regulatory responses were not observed in H4 hepatoma cells. (Figures 1-3)

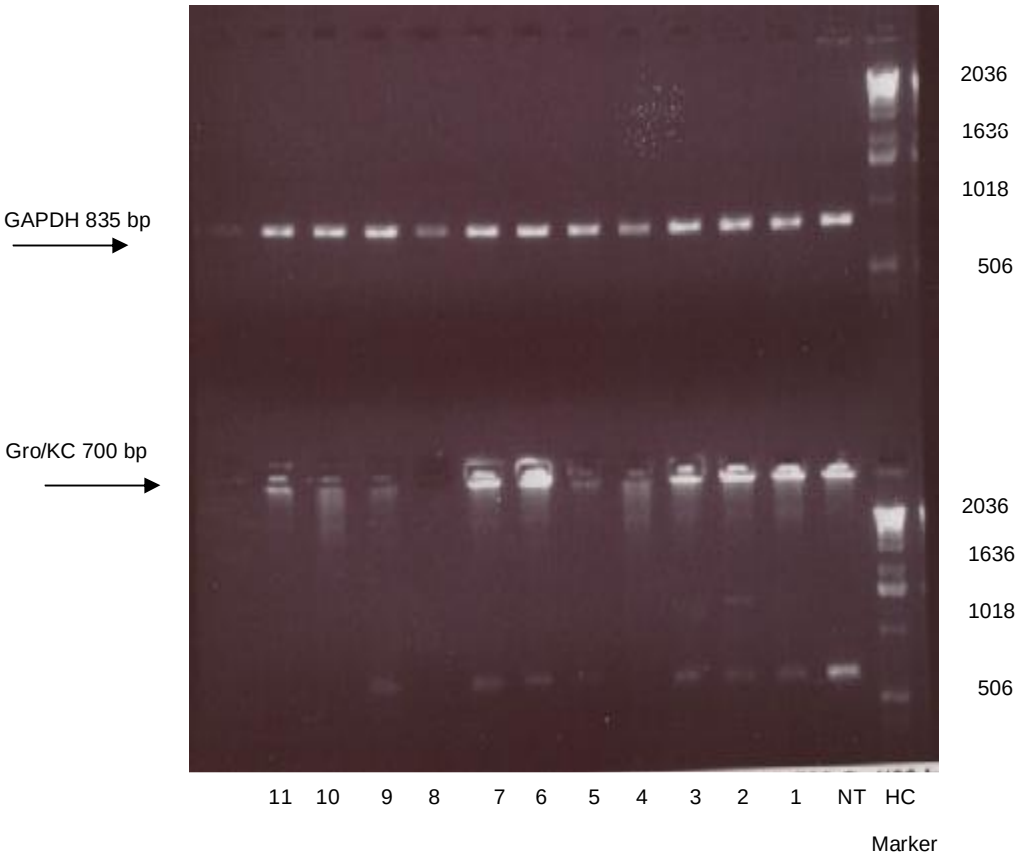

Figure 1. Expression of Gro/KC under heat shock, hyperosmolarity, and oxidative stresses in rat's H4 Hepatoma cells. Expression of CXC chemokine Gro/KC was analyzed by RT-PCR. GAPDH mRNA was assessed in parallel as control. Numbers on right represent the bands of the molecular marker. HC= hepatocytes control; NT= not treated; 1= heat shock treatment for 1h; 2= heat shock treatment for 3h; 3= heat shock treatment for 6h; 4= Manitol treatment for 1h; 5= Manitol treatment for 3h; 6= Manitol treatment for 6h; 7= H_2O_2 treatment for 1h; 8= H_2O_2 treatment for 3h; 9= H_2O_2 treatment for 6h; 10= NaCl treatment for 1h; 11= NaCl treatment for 3h.

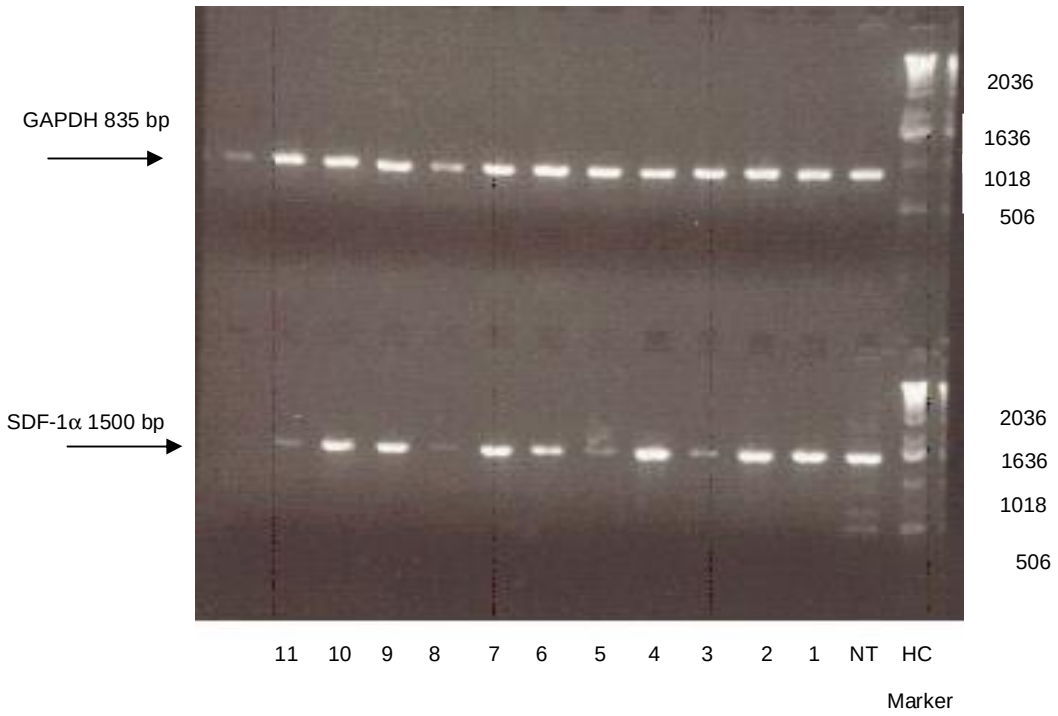

Figure 2. Expression of SDF-1α under heat shock, hyperosmolarity, and oxidative stresses in rat's H4 hepatoma cells. Expression of the CXC chemokine SDF-1α was analysed by RT-PCR. GAPDH mRNA was assessed in parallel as control. Numbers on right represent the bands of the molecular marker. HC=hepatocytes control; NT=not treated; 1= heat shock treatment for 1h; 2=heat shock treatment for 3h; 3=heat shock treatment for 6h; 4=Manitol treatment for 1h; 5=Manitol treatment for 3h; 6= Manitol treatment for 6h; 7=H_2O_2 treatment for 1h; 8= H_2O_2 treatment for 3h; 9= H_2O_2 treatment for 6h; 10= NaCl treatment for 1h; 11= NaCl treatment for 3h.

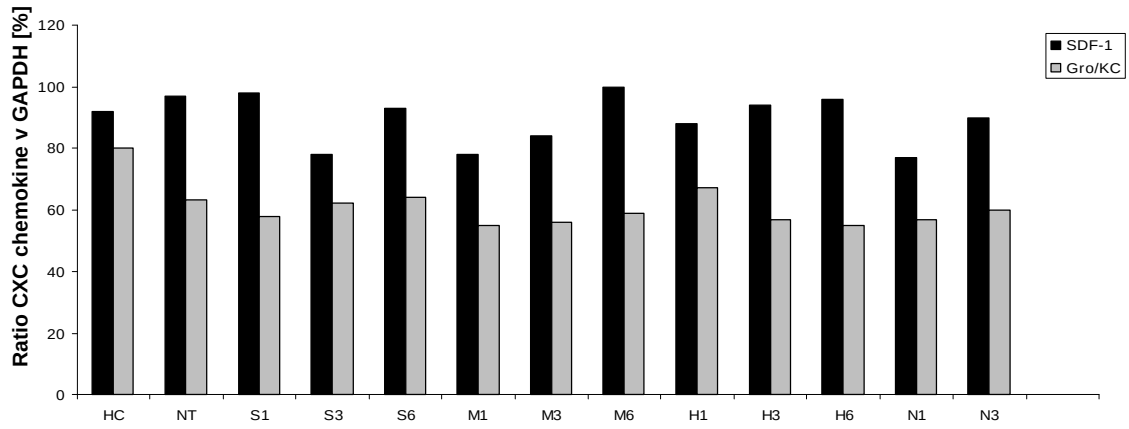

Figure 3. Alteration in expression of CXC chemokines SDF-1α and Gro/KC against GAPDH in rat's hepatoma H4 cells in different conditions and in the presence and absence of different stimuli. HC=hepatocytes control; NT=not treated; S1=heat shock treatment for 1h; S3=heat shock treatment for 3h; S6=heat shock treatment for 6h; M1=Manitol treatment for 1h; M3=Manitol treatment for 3h; M6=Manitol treatment for 6h; H1=H_2O_2 treatment for 1h; H3=H_2O_2 treatment for 3h; H6=H_2O_2 treatment for 6h; N1=NaCl treatment for 1h; N3= NaCl treatment for 3h.

DISCUSSION

Several known injurious conditions such as hepatitis, ischemia/reperfusion, sepsis/endotoxemia, and drugs cause recruitment of macrophages, neutrophils, and other immune cells to the liver parenchyma (7). Neutrophils are recruited to the hepatic vasculature following local liver injury and subsequent CXC chemokine production (8, 9) or following exposure to inflammatory cytokines (10, 7). Although neutrophils and Kupffer cells are widely considered to be the first defence line in liver, the liver immune response is not restricted to these cells, and other immune cells such as T lymphocytes and NK cells are also involved, mostly mediated by chemokines (11). Involvement of CC chemokines such as MCP-1, ELR^+ CXC chemokines (such as IL-8/MIP-2) and ELR^- chemokines (such as IP-10/Mob-1) has been reported (12, 13) in acetaminophen-induced liver injury and modulation of liver regeneration processes (14, 15). So far this report is the only one on expression of chemokines by H4 hepatoma cells. High constitutive levels of SDF-1α have been observed in the non-inflamed biliary epithelium of the liver (18) in association with CXCR4 expressing lymphocyte recruitment (19, 20). Immunohistochemical studies have revealed decreased levels of SDF-1α protein in hepatocellular carcinoma compared with other chronic liver diseases such as hepatitis C (21). Expression of Gro/KC in hepatectomized mouse has been shown and it may play an important role in hepatocyte proliferation and liver regeneration (22). Increased Gro/KC expression is associated with some hepatic injuries and diseases including: liver ischemia/reperfusion in mouse and rat (23), infections, paracetamol poisoning, sepsis and LPS injection in mice (24). Results obtained in this report revealed that there was detectable but low expression of both SDF-1α and Gro/KC in H4 hepatoma cells. In contrast to primary hepatocytes, heat shock did not induce expression of SDF-1α and Gro/KC in rat's H4 hepatoma cells (25). Hyperosmolarity also failed to stimulate SDF-1α and Gro/KC expression.

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