High-dose hydrocortisone reduces expression of the pro-inflammatory chemokines CXCL8 and CXCL10 in SARS coronavirus-infected intestinal cells

JINDRICH CINATL Jr, MARTIN MICHAELIS, BIRGIT MORGENSTERN and HANS WILHELM DOERR

Institut für Medizinische Virologie, Klinikum der J.W. Goethe-Universität, Paul Ehrlich Str. 40, D-60596 Frankfurt am Main, Germany

Received August 30, 2004; Accepted October 22, 2004

Abstract. Clinical observations and our high-density oligonucleotide microarray results demonstrated increased expression of proinflammatory chemokines after SARS-CoV infection. Here, we investigated the influence of SARS-CoV infection on CXCL8 (interleukin 8) and CXCL10 (interferon-γ-inducible protein 10) in human intestinal epithelial (Caco2) cells. RT-PCR and ELISA showed time-dependent up-regulation of both chemokines after SARS-CoV infection. Electric mobility shift assay revealed increased DNA binding activity of the cellular transcription factors activator protein 1 (AP-1) and nuclear factor (NF-κB) in SARS-CoV infected cells. High hydrocortisone concentrations (>50 μg/ml) completely prevented increased DNA binding activity of AP-1 and NF-κB and inhibited up-regulation of CXCL8 and CXCL10, but did not reduce chemokine expression to basal levels. Ribavirin that does not inhibit SARS-CoV replication in Vero cells inhibited SARS-CoV replication in Caco2 cells at therapeutical concentrations. Hydrocortisone neither influenced SARS-CoV titres alone nor in combination with ribavirin. Our results show that corticosteroids may be of limited benefit in the suppression of chemokine production by SARS-CoV-infected cells.

Introduction

Severe acute respiratory syndrome (SARS) is caused by infection with the SARS coronavirus (SARS-CoV) (1,2). Approximately 25% of patients with SARS progress to severe respiratory failure with characteristics of acute respiratory distress syndrome (ARDS) (3,4). Pathologic findings demonstrated SARS to be a systemic disease that injures many organs (5,6) including the gastrointestinal tract, with diarrhoea being a common feature (7,8).

The clinical course of SARS can be divided in two phases. The first phase is characterised by viral replication (cytolytic activity) that results in tissue damage. In the second phase SARS progresses to respiratory failure in a notable proportion of patients despite lowering of virus load (9). These findings suggest an immunopathological nature of the second phase of SARS characterised by immune-mediated lung injury. Clinical investigations revealed an imbalance of inflammatory cytokines/chemokines in SARS patients (10,11).

Corticosteroids were used clinically with the aim of suppressing cytokine/chemokine storm and to stop disease progression (12). Their use often resulted in clinical improvement (9,12-17). Nevertheless, the value of corticosteroid treatment is still judged contradictory regarding efficacy, adverse immunosuppressive effects, and treatment outcome (18).

Corticosteroids were commonly co-administered with the antiviral drug ribavirin (8,9,12,13,15,19). The use of ribavirin was criticised because clinical findings did not reveal evidence for its anti-SARS efficacy and the severe adverse effects of the drug (20). First in vitro experiments that did not suggest anti-SARS-CoV activity of ribavirin had been performed in Vero cells (21,22), in which ribavirin is known to be of low antiviral efficacy (23,24). In other cells, ribavirin inhibited SARS-CoV replication (25).

We established a SARS-CoV infection in human intestinal epithelial cell line Caco2 allowing the investigation of SARS-CoV infection of cells from human origin (26,27). Herein, the influence of hydrocortisone on SARS-CoV replication with and without ribavirin was examined. Moreover, we examined the influence of SARS-CoV on the expression of the chemokines CXCL8 (IL-8 = interleukin 8) and CXCL10 (interferon γ inducible protein 10 = IP-10) on the mRNA and on the protein level, which were previously shown to be increased in the plasma of SARS patients. The influence of SARS-CoV infection on the DNA binding activity of the transcription factors nuclear factor κB (NF-κB) and activator protein 1 (AP-1), which are commonly involved in inflammatory processes, was investigated (28). Finally, the influence of hydrocortisone on SARS-CoV induced
chemokine expression and pro-inflammatory transcription factors were examined.

Materials and methods

Cell cultures. Colon carcinoma cell line Caco2 was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). The cells were grown at 37°C in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal bovine serum (FBS), 100 IU/ml of penicillin, and 100 µg/ml of streptomycin. All culture reagents were purchased from Biochrom (Berlin, Germany).

Virus preparation. SARS-CoV strain FFM-1 was isolated from respiratory specimens of a SARS patient admitted to the Infectious Diseases Department of Frankfurt am Main University Hospital, Germany, on Vero (African green monkey kidney; ATCC CCL81) cells (21). SARS-CoV stocks used in the experiments had undergone five passages on Vero cells and were stored at -80°C. Virus titres were determined as TCID\textsubscript{50}/ml in confluent cells in 96-well microtiter plates (21).

RT-PCR. CXCL8 and CXCL10 mRNA levels were examined by RT-PCR, according to standard protocols (29). PCR primer and amplification conditions were determined by software (Primer3, Whitehead Institute for Biomedical Research) (30).

Enzyme linked immuno sorbent assay (ELISA). ELISA was performed as described before (31). CXCL8 and CXCL10 protein levels were measured in cell culture supernatants by ELISA (R&D Systems, Wiesbaden, Germany) according to the manufacturer's instructions. Supernatants of cell cultures were stored at -80°C until measurement. Standards provided by the manufacturer were used as positive controls. Fresh culture medium was used as negative control. OD was determined using a microplate reader at 450 nm.

Electrophoretic mobility shift assay (EMSA). EMSA was performed as described before (32). Nuclear extracts (5 µg total protein) were assayed for DNA-binding activity of NF-κB or AP-1 using a gel shift assay kit (Promega, Mannheim, Germany) following the manufacturer's manual.

Results

Influence of hydrocortisone and ribavirin on SARS-CoV replication Caco2 cells. SARS patients were commonly treated with a combination of corticosteroids and antiviral drugs (9,12-17). Ribavirin was initially found to be ineffective in the inhibition of SARS-CoV replication in Vero cells (21). Newer results revealed that ribavirin inhibits SARS-CoV replication in foetal Rhesus kidney-4 cells (25). Our results show SARS-CoV replication inhibition by ribavirin in human Caco2 cells in therapeutic concentrations of 12.5-50 µg/ml in a dose-dependent fashion (Fig. 1). Hydrocortisone in concentrations up to 250 µg/ml (the maximum dose tested) neither influenced SARS-CoV titres alone nor in combination with ribavirin (Fig. 1).

Influence of SARS-CoV infection on expression of CXCL8 and CXCL10 in Caco2 cells. High-density oligonucleotide array studies had demonstrated up-regulation of different
pro-inflammatory cytokines and chemokines in SARS-CoV-infected Caco2 cells (27). The chemokines CXCL8 and CXCL10 had been shown to play a role in the clinical course of SARS (11,33,34). Therefore, we investigated the time-dependent influence of SARS-CoV infection on mRNA levels of CXCL8 and CXCL10 in Caco2 cells by RT-PCR.

Influence of hydrocortisone on the SARS-CoV-induced up-regulation of CXCL8 and CXCL10. Corticosteroids are a common part of treatment strategies for SARS patients (9,12-17). Therefore, we investigated the influence of hydrocortisone on SARS-CoV-induced expression of CXCL8 and CXCL10 in Caco2 cells. As shown in Fig. 3, hydrocortisone concentrations >50 μg/ml inhibited increased CXCL8 and CXCL10 expression on the mRNA level 24 h after SARS-CoV infection.

Hydrocortisone 250 μg/ml also inhibited up-regulation of CXCL8 (Fig. 4A) and CXCL10 (Fig 4B) on the protein level. However, the expression of both proteins was not reduced to basal levels.

Influence of hydrocortisone on the SARS-CoV infection on the DNA binding activity of the transcription factors AP-1 and NF-κB. The transcription factor AP-1 had already been found to be activated by the SARS-CoV nucleocapsid (35). AP-1 and NF-κB binding sites have been described for the CXCL8 promoter (36,37) and a NF-κB binding site has been described for the CXCL10 promoter (38). Therefore, the influence of SARS-CoV infection on the DNA binding activity of AP-1 and NF-κB was investigated. Electrophoretic mobility shift assay demonstrated increased DNA binding activity of AP-1 and NF-κB after SARS-CoV infection (Fig. 5).

Treatment of SARS-CoV infected cells with hydrocortisone 250 μg/ml prevented the increase in DNA-binding activity of both transcription factors (Fig. 5).
Discussion

Inflammatory cytokines/chemokines play both beneficial and harmful roles in infectious diseases caused by different viruses including human coronaviruses (39). Recent investigations found dynamic changes in blood cytokine levels of patients with SARS which may be responsible for injury of lung and other organs (10,11). By the use of high-density oligonucleotide arrays, we previously demonstrated that infection of cultured intestinal epithelial cell line Caco2 with SARS-CoV alters mRNA levels of several cytokines/chemokines (27). In this study, the chemokines CXCL8 and CXCL10, that play a role in the clinical course of SARS pathology (10,11), were found to be up-regulated on mRNA and protein levels after SARS-CoV infection of Caco2 cells.

The transcription factors NF-κB and AP-1 are commonly involved in inflammatory processes (28). The promoter of CXCL8 has NF-κB and AP-1 binding sites and for the CXCL10 promoter a NF-κB binding site has been described (36-38). The induction of CXCL8 by rotavirus in intestinal epithelial cells required binding of AP-1 and NF-κB transcription factors to the CXCL8 promoter (40). The porcine transmissible gastroenteritis virus (TGEV), an animal coronavirus, was shown to activate NF-κB in different cell lines (41). Recently, the SARS-CoV nucleocapsid was shown to activate AP-1 but not NF-κB pathways (35). Our results reveal that SARS-CoV infection increases the DNA binding activity of both transcription factors indicating a possible involvement in SARS-CoV induced inflammatory processes. While the present results are in concert with AP-1 activation by SARS-CoV nucleocapsid (35), they for the first time demonstrate that SARS-CoV utilizes signal pathways activating NF-κB which are not induced by the SARS-CoV nucleocapsid.

Corticosteroids, that inhibit NF-κB and AP-1 activation (28,42), are a common part of SARS treatment regimens to control immunopathological processes during the course of SARS (9,12-17). Clinical findings show reduction of CXCL8 and CXCL10 expression in infected patients (10,11), were found to be up-regulated on mRNA and protein levels after SARS-CoV infection of Caco2 cells. In spite of general improved outcome of SARS patients treated with corticosteroids, severe side-effects have been reported. The 30-day mortality was reported to be increased and a disseminated fungal disease was described (43). Moreover, avascular necrosis of bone was detected in >30% of SARS cases (44). Other clinical observations reported corticosteroid therapy to be of low efficacy or ineffective (19,45). The different treatment outcomes may be due to different dosing, timing, and/or duration of corticosteroid treatment. Therefore, exact criteria for the use of corticosteroids for SARS patients have been defined (15-18). Our results, showing that corticosteroids may be of limited benefit in the suppression of chemokine production by SARS-CoV-infected cells, strongly support that corticosteroids should be carefully used for SARS patients.

References


