Glucocorticoids inhibit induced and non-induced mRNA accumulation of genes encoding hyaluronan synthases (HAS): hydrocortisone inhibits HAS1 activation by blocking the p38 mitogen-activated protein kinase signalling pathway

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Objective. Glucocorticoids are still a mainstay in the treatment of rheumatoid arthritis (RA). Unfettered hyaluronan release is a hallmark of RA. The discovery of three genes encoding hyaluronan synthase (HAS) led us to investigate the effect of hydrocortisone and dexamethasone on the activation of these genes at the molecular level and, at least in part, the mode of action of these drugs.

Methods. Reverse transcription–polymerase chain reaction (RT-PCR) was used to monitor levels of HAS1, HAS2, and HAS3 mRNAs in cultured fibroblast-like synoviocytes (FS) and in leucocytes isolated from synovial fluid of RA patients. Western blot experiments were used to investigate the effect of hydrocortisone on transforming growth factor β (TGF-β)-induced activation of the p38 mitogen-activated protein kinase (MAPK) pathway.

Results. Hydrocortisone and dexamethasone suppressed HAS2 and HAS3 mRNAs accumulation concentration-dependently. Contrary to HAS2 and HAS3, HAS1 in FS was not constitutively activated. When cells were stimulated with TGF-β, a potent activator of HAS1 mRNA transcription, treating them with hydrocortisone suppressed induced activation of HAS1 in a concentration- and time-dependent manner. Similar suppressive effects of hydrocortisone were observed when leucocytes isolated from synovial fluid of inflamed joints were used instead of cultured FS. Furthermore, western blot experiments confirmed that hydrocortisone blocked TGF-β-induced phosphorylation of p38 MAPK, a kinase essential for TGF-β-induced HAS activation.

Conclusion. Our data demonstrate that glucocorticoids suppress all genes encoding hyaluronan. We speculate that inhibition of HAS genes might account for the beneficial effect of glucocorticoid treatment, and also for the detrimental effects of long-term use.

KEY WORDS: Hyaluronan, Hyaluron synthase, Glucocorticoid, TGF, p38 MAPK

For many decades, glucocorticoids have been successfully used to treat a series of ailments. Their many anti-inflammatory effects are thought to be attributable to the suppression of T-cell proliferation, the suppression of proinflammatory cytokines, the down-regulation of adhesion molecules and the induction of apoptosis, e.g. of monocytes and T cells. For these reasons glucocorticoids are among the most potent anti-inflammatory agents [1]. At the molecular level, it is generally believed that most, if not all of the beneficial effects of glucocorticoids are mediated via the glucocorticoid receptor [2]. The very first ailment that was treated with a glucocorticoid was rheumatoid arthritis (RA) [3], and more than 50 yr later glucocorticoids remain a mainstay of treatment for many patients with RA.

One of the characteristics of RA is unfettered hyaluronan (HA) production. Other ailments associated with increased HA levels are lung fibrosis, myocardial infection, transplant rejection and some forms of tumour [4]. HA is a molecule that can be found in nearly every tissue. HA is likewise a major constituent of the extracellular matrix and also plays a crucial role in wound healing, cell proliferation, cell migration and cell differentiation, and in angiogenesis and tumour biology [5].

A major step which will lead to a better understanding of the physiological and pathological functions of HA is the recent discovery of three hyaluronic acid synthase (HAS) genes. Although there is increasing evidence indicating different functions of these three genes, very little is known about the molecular mechanisms involved in the regulation of these genes [6]. Our working hypothesis is based on the assumption that unfettered HA synthesis may be involved in the progression of RA. The possible suppression of an HAS gene that is up-regulated in joints of RA patients by glucocorticoids may support such a working hypothesis. Here, we report our findings on the effect of glucocorticoids on levels of HAS1, HAS2 and HAS3 mRNAs in cultured fibroblast-like synoviocytes and in leucocytes isolated from synovial fluid of affected joints of RA patients.

Materials and methods

Reagents

Cytokines, phorbol 12-myristate 13-acetate lipopoly saccharide (serotype 055:B5), hydrocortisone, dexamethasone and cell culture
reagents were from Sigma (Vienna, Austria). The mitogen-activated protein kinase (MAPK) inhibitors JNK inhibitor II, SB 203580 and PD 98059 were from Calbiochem (Calbiochem, La Jolla, CA, USA). MAPK antibodies were from Cell Signaling Technology (Beverly, MA, USA).

Cell culture
Fibroblast-like synoviocytes, obtained from RA patients undergoing knee replacement surgery, were maintained in Dulbecco’s Modified Eagle Medium (DMEM; Sigma, Vienna, Austria) supplemented with 10% fetal calf serum (endotoxin, 0.05 ng/ml; Sigma), 50 U penicillin/0.1 mg streptomycin and 2 mM L-glutamine ( Gibco BRL Life Technologies, Paisley, UK). Cells were cultured in 75 cm² tissue culture flasks (Nalge Nunc International, Rochester, NY, USA) and passaged by the use of trypsin solution. Pooled cell populations were used for most experiments. FS were seeded into six-well plates (Iwaki, Funabashi, Chiba, Japan) and grown to high density. Cells at passages 5–13 (split ratio 1:3) were used.

Protein extraction and western blotting
Preparation of protein extracts and western blot experiments were carried out according to protocols provided with antibodies (Cell Signaling Technology).

RNA extraction and reverse transcription–polymerase chain reaction (RT-PCR)
Total RNA was extracted using TRI Reagent (Sigma), following the manufacturer’s protocol. The RNA pellet was dissolved in DNase/RNase-free water. One microgram of total RNA was reverse-transcribed using a First-Strand cDNA Synthesis Kit (Amer sham Biosciences, Freiburg, Germany). RT-PCR was performed in 50 μl reaction volume containing 5 μl of 10× buffer from BioTherm [160 mM (NH₄)₂SO₄, 670 mM Tris–HCl, pH 8.8, 15 mM MgCl₂, 0.1% Tween 20], 1 μl of 10 mM deoxynucleotide mix (dNTP; Sigma), 1 μl (100 pmol/μl) forward primer, 1 μl (100 pmol/μl) reverse primer, 2 U BioTherm (Biotaq) DNA polymerase (Qbioogene GmbH, Heidelberg, Germany) and 2.5 μl cDNA, and adjusted to the final volume with DNase/RNase-free water (Sigma). The following primers were used: HAS1, (sense) 5'-ACT CCG ACA CAA GGT TGG AC-3' and (anti-sense) 5'-AGC AGG GCC TCT CTG AGT AG-3'; HAS2, (sense) 5'-GTG ATG ACA GGC ATC TCA-3' and (anti-sense) 5'-GGG GGA AGT AAA CTC GA-3'; HAS3, (sense) 5'-CAC CCT GCA CCA TCG A-3' and (anti-sense) 5'-AGA GGT GGT GCT TAT GGA-3'; actin, (sense) 5'-CAC CTT CTA CAA TGA GCT GC-3' and (anti-sense) 5'-AGG CAC CTC GAT CTT CT-3'. The specificity of RT-PCR was confirmed by comparing the sizes of the amplified fragments with the calculated lengths and by sequencing the PCR products.

PCR products were separated by electrophoresis using 2% agarose gels. Gels were stained with 0.5 μg/ml ethidium bromide and analysed on a Fluorimag (Amer sham Biosciences). Values given on the y-axis of densitometry plots represent relative fluorescence units ×10⁶.

Data analysis
Data were analysed and quantitated using ImageQuant software (Amer sham Biosciences). mRNA for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or actin or both were used as controls for RT-PCR and scanner readings were used to recalculate PCR data. Films from western blot experiments were scanned on an Agfa Duoscan T 1200 or scanned and quantitated on a densitometer (Molecular Dynamics, Sunnyvale, CA, USA). Analysis of PCR data revealed that intra-assay variation (RT reaction plus PCR amplification) was ≤5% and inter-assay variation in the in vitro experiments was ≤12%. With the exception of the experiments using cells isolated from synovial fluid of untreated individuals (these experiments were done twice), experiments were repeated at least three times.

Results
Glucocorticoids inhibit HAS in FS
We demonstrated earlier (K. M. Stuhlmeier and C. Pollaschek, submitted for publication) that TGF-β, which is present in inflamed joints of RA patients, is a potent inducer of HAS1 mRNA in FS. We used TGF-β to induce HAS1 and to test the effectiveness of two glucocorticoids as inhibitors of HAS mRNA accumulation.

Figure 1 demonstrates the effects of hydrocortisone and dexamethasone on non-induced and TGF-β-induced activation of HAS1. In Fig. 1A, FS cultured in six-well plates were left untreated (lane labelled MEDIUM), pretreated with a high concentration of hydrocortisone (1 μg/ml) for 1 h [lane HC (1 μg/ml)], pretreated with a low concentration of hydrocortisone (0.2 μg/ml) for 1 h [lane HC (0.2 μg/ml)], treated with TGF-β (1 ng/ml) for 6 h [lane TGF-β (1 ng/ml)], pretreated with hydrocortisone (1 μg/ml) for 1 h followed by treatment with TGF-β (1 ng/ml) for an additional 6 h (lane HC (1 μg/ml) + TGF), or pretreated with hydrocortisone (0.2 μg/ml) for 1 h, after which TGF-β (1 ng/ml) was added and cells were incubated for a further 6 h (lane HC (0.2 μg/ml) + TGF). Isolation of mRNA and RT-PCR were performed as described in Materials and methods. Figure 1A shows a representative experiment in which the gel was scanned on a fluorimag and quantitated using ImageQuant software.

The lower panel shows a scan of the gel stained with ethidium bromide. The upper panel shows a quantitation of the HAS1 data normalized to actin. Such experiments demonstrated that hydrocortisone suppressed TGF-β-induced HAS mRNA accumulation in a concentration-dependent manner. Using 0.2 μg/ml consistently led to about 50% inhibition, while increasing the concentration of hydrocortisone to 1 μg/ml blocked TGF-β-induced HAS1 transcription almost entirely.

If dexamethasone was used instead of hydrocortisone, under otherwise identical conditions, comparable effects were noted. As shown in Fig. 1B, TGF-β activated HAS1 (lane TGF-β (1 ng/ml)) and preincubating FS (1 h) with a low concentration of dexamethasone (0.2 μg/ml) reduced HAS1 mRNA accumulation. Increasing the concentration of these drugs suppressed HAS1 mRNA accumulation in a concentration-dependent manner. Using 0.2 μg/ml consistently led to about 50% inhibition, while increasing the concentration of dexamethasone to 1 μg/ml reduced HAS1 mRNA to about 50% lower levels of HAS1 mRNA, treated with TGF-β for an additional 6 h (lane DEX (0.2 μg/ml) + TGF), or pretreated with hydrocortisone (0.2 μg/ml) for 1 h, after which TGF-β (1 ng/ml) was added and cells were incubated for a further 6 h (lane HC (0.2 μg/ml) + TGF). Isolation of mRNA and RT-PCR were performed as described in Materials and methods. Figure 1B shows a representative experiment in which the gel was scanned on a fluorimag and quantitated using ImageQuant software.

Next, we tested the effects of hydrocortisone and dexamethasone on HAS3 mRNA. mRNA for HAS3 in unstimulated FS is readily detectable (K. M. Stuhlmeier and C. Pollaschek, submitted for publication). As shown in Fig. 1C, treating FS for 7 h with increasing concentrations of these drugs suppressed HAS3 mRNA levels. The comparison of dexamethasone and hydrocortisone shown in Fig. 1C demonstrates that the two reagents exerted comparable effects, as 0.2 μg/ml of both substances repeatedly led to a reduction of HAS3 mRNA by about 50%. As demonstrated by such experiments, the maximal inhibitory effects of dexamethasone and hydrocortisone on HAS3 activation were achieved using 0.2 μg/ml. Increasing the concentration of these inhibitors to 1 μg/ml did little with regard to a more pronounced inhibitory effect.

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Like HAS3 mRNA synthesis, the synthesis of HAS2 mRNA in cultured FS is activated constitutively. Shown in Fig. 1D are experiments demonstrating the suppressive effects of dexamethasone and hydrocortisone on HAS2 transcription. FS were incubated with the indicated concentrations of dexamethasone or hydrocortisone. After 8 and 22 h respectively, cells were harvested and HAS2 mRNA levels were monitored by RT-PCR and quantitated by densitometry.

The comparison of HAS2 mRNA levels in treated (lanes DEX 1.0 µg/ml, DEX 0.2 µg/ml, HC 1.0 µg/ml and HC 0.2 µg/ml) and untreated cells (lane MEDIUM) revealed that both drugs suppressed HAS2 mRNA accumulation. Furthermore, longer exposures of these cells to these drugs led to a more pronounced suppressive effect, as HAS2 mRNA levels were lower in cells that were treated for 22 h [lower section, labelled HAS2 (22)] instead of 8 h [upper section, labelled HAS2 (8)]. In the representative experiment shown in Fig. 1D, densitometry revealed that 8 h of exposure to 0.2 µg/ml hydrocortisone led to 35% inhibition while a similar concentration of dexamethasone led to 32% inhibition. Prolonging exposure to 22 h resulted in 60% (hydrocortisone) and 47% (dexamethasone) inhibition. This figure also shows that increasing the concentration of the drugs used to 1 µg/ml did not result in more effective suppression of HAS2 transcription. Figure 1E demonstrates properly adjusted RT-PCR cycling conditions. Such experiments were performed to ensure that RT-PCR experiments were terminated in the early log phase of product accumulation. This figure shows a representative experiment used to determine optimal cycle numbers for the amplification of the housekeeping gene actin. As demonstrated, using 23 cycles for the amplification of actin will ensure semiquantitative analysis of the PCR products.

Fig. 1. Suppression of TGF-β-induced HAS1 mRNA by hydrocortisone (A) and dexamethasone (B). Treating FS for 6 h with TGF-β leads to HAS1 mRNA accumulation. Preincubating FS with increasing concentrations of hydrocortisone or dexamethasone (0.2 and 1 µg/ml respectively) for 1 h prior to stimulation with TGF-β blocks TGF-β-induced HAS1 mRNA accumulation in a concentration-dependent manner. The upper panels show the quantitation of mRNA by densitometry. As in all other figures, actin levels were used for normalization. (B) Dexamethasone inhibits induced and non-induced HAS1 mRNA accumulation. Treating FS for 1 h with dexamethasone prior to stimulation with TGF-β for 6 h results in concentration-dependent inhibition of HAS1 mRNA accumulation. HAS1 mRNA is undetectable or present at very low concentration in unstimulated FS. As shown in this representative experiment, treating FS with dexamethasone also reduces non-induced HAS1 mRNA levels. (C) Dexamethasone and hydrocortisone inhibit HAS3 mRNA synthesis. As demonstrated by this RT-PCR experiment, HAS3 mRNA levels are high in unstimulated FS. Treating FS with hydrocortisone or dexamethasone for 7 h leads to a reduction of HAS3 mRNA levels detected by PCR. (D) Dexamethasone and hydrocortisone inhibit HAS2 mRNA accumulation. HAS2 mRNA was quantitated by densitometry. As indicated, FS were harvested 8 h [HAS2 (8h)] or 22 h [HAS2 (22)] after addition of dexamethasone (DEX) or hydrocortisone (HC). Increasing the concentration of glucocorticoid from 0.2 µg/ml (lanes DEX 0.2 µg/ml and HC 0.2 µg/ml) to 1 µg/ml (lanes DEX 1.0 µg/ml and HC 1 µg/ml) does not lead to a more pronounced suppressive effect. The label MEDIUM indicates unstimulated cells. The labels HC (1 µg/ml) and HC (0.2 µg/ml) indicate experiments in which cells were stimulated for 7 h with hydrocortisone and the labels DEX (1 µg/ml) and DEX (0.2 µg/ml) respectively indicate experiments in which cells were treated with dexamethasone. (E) Establishing semiquantitative RT-PCR conditions. Representative experiment involving amplification of the housekeeping gene actin. Such experiments demonstrate that special care was taken to adjust RT-PCR settings properly to ensure that the RT-PCR experiments were terminated in the early log phase of product accumulation. Similar optimization experiments were performed routinely for all genes prior to experiments. The cycle numbers are indicated on the x-axis.
Hydrocortisone inhibits HAS in leucocytes isolated from RA patients

Next, we tested the effectiveness of hydrocortisone as an inhibitor of HAS transcription in an experimental setting that more closely reflected its clinical use. Synovial fluid from affected joints of RA patients was obtained and processed immediately after collection. Mononuclear cells (MNC) and polymorphonuclear leucocytes (PMN) were separated by density gradient centrifugation. These cells were left untreated or treated with hydrocortisone (0.2 μg/ml) for 5 h, after which mRNA was isolated for RT-PCR experiments. A comparison of the HAS3 mRNA levels in hydrocortisone-treated cells vs untreated cells is shown in Fig. 2. Such experiments demonstrated that significant inhibitory effects on HAS3 mRNA accumulation could be noticed as early as 5 h after treatment of cells of the inflamed synovium with hydrocortisone.

Hydrocortisone inhibits TGF-β-induced phosphorylation of p38 MAPK

We have demonstrated (K. M. Stuhlmeier and C. Pollaschek, submitted for publication) that TGF-β uses the p38 MAPK pathway to activate HAS1 mRNA transcription. Because p38 MAPK is essential for the activation of TGF-β-induced HAS1 activation, we tested whether hydrocortisone suppresses HAS1 mRNA by blocking the p38 MAPK pathway. Phosphorylation of p38 MAPK is essential for the activation of this kinase, and we therefore used western blot experiments to detect possible glucocorticoid-induced changes in the TGF-β-mediated p38 phosphorylation pattern. FS were used for these experiments and, as shown in Fig. 3, the phosphorylated form of p38 MAPK was nearly undetectable in unstimulated cells. In contrast, phosphorylated p38 MAPK was abundant in cells treated with TGF-β (1 ng/ml, 45 min). More importantly, treating FS with increasing concentrations of hydrocortisone for 1 h prior to TGF-β treatment led to concentration-dependent inhibition of TGF-β-induced p38 MAPK phosphorylation. As shown in this figure, the use of 0.04 ng/ml hydrocortisone was not sufficient to prevent p38 phosphorylation completely. However, increasing hydrocortisone to 0.2 or 1 μg/ml resulted in complete inhibition of TGF-β-induced p38 MAPK phosphorylation. Equal loading of gels and uniform protein transfer to membranes was confirmed by staining blots with Ponceau red and/or staining blots with anti-tubulin antibodies (data not shown).
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Besides being essential for many physiological functions, HA
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effects. HA participates in many cellular reactions caused by
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Discussion

This study was motivated by our working hypothesis that
unfettered HA production may be involved in the progression of
RA. Levels of HA have been shown to be elevated not only in
affected joints but also in the circulation of RA patients [7].
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ism in human skin organ culture [12]. Another report demon-
strated that dexamethasone inhibited HAS2 in dermal fibroblasts
and osteoblasts [13]. The recent discovery of three genes encoding
HAS allows more thorough investigation into which of the HAS
genes are involved in RA and the study of signalling pathways
leading to the up-regulation of these genes. The effect of
glucocorticoids on the activation of these three genes has not yet
been studied in cells of the synovium.

Here we report our findings on the effects of dexamethasone
and hydrocortisone on the accumulation of HAS1, HAS2 and
HAS3 mRNAs in cultured FS. Our data demonstrate that both
glucocorticoids repress the accumulation of all three HAS genes in
a concentration- and time-dependent manner without affecting the
viability of FS. We also demonstrate that hydrocortisone also
suppresses HAS in leucocytes isolated from inflamed synovium of
RA patients. Furthermore, we show that hydrocortisone blocks
TGF-β-induced HAS1 accumulation by blocking the p38 MAPK
pathway. The effect was demonstrated with drug concentrations
that are clinically relevant, since patients are adjusted to blood
levels of 0.2 µg/ml. Clearly, concentrations significantly higher
than this are achieved when glucocorticoids are administered
locally for shorter periods. Interestingly, while work on this
manuscript was under way, two groups also demonstrated the
ability of glucocorticoids to interfere with the p38 MAPK
signalling cascade [14, 15]. We found earlier (submitted for
publication) that blocking the p38 MAPK pathway with a specific
inhibitor completely blocked TGF-β-induced HAS1 activation but
had no effect on levels of HAS2 and HAS3 mRNAs. Therefore,
blockage of p38 MAPK by hydrocortisone cannot account for all
the effects observed. As demonstrated in Fig. 1, hydrocortisone
blocked mRNA of all three HAS genes; therefore, other mechan-
isms must also be at work. Although it seems unlikely that the three HAS genes produce
molecules with identical biological functions, there are few reports
investigating potential functional differences of the HAS gene
products [16–21].

What is clearer is that degradation products of HA are potent
inducers of a series of undesirable effects. HA has been shown to
undergo rapid degradation at sites of inflammation [22, 23].
Low-molecular-weight degradation products of HA have been found to
elicit various proinflammatory responses, such as the stimulation
and invasion of macrophages in affected joints [24, 25], as well as
the functional activation of dendritic cells [26]. Noble et al. [24],
for example, reported that degradation products of HA activate
NF-κB, a transcription factor that has been shown to be involved in
the activation of most, if not all, proinflammatory molecules
[27–29]. Plasminogen activator inhibitor 1, a cytokine with many
undesirable effects, is up-regulated by HA fragments, as is metallo-
elastase, a molecule that is implicated in lung disorders such as
emphysema and pulmonary fibrosis [30, 31].

Whether the beneficial effect of steroid treatment is due to
inhibition of one or more of the HAS genes that might play a
role in disease progression or whether glucocorticoids are
beneficial because inhibition of the accumulation of HAS
mRNA ultimately also prevents the appearance of proinflamma-	ory HA degradation products will have to be clarified by further
experiments. The observation that glucocorticoids suppress all three genes
encoding HAS may also explain some of the side-effects of these
drugs that are observed after long-term use. Blocking HA, with its
undoubtedly many important physiological functions, will
eventually lead to pathological manifestations. Taking the results
together, we believe that HA is involved in many ways in the
progression of RA. The experiments described here support such a
concept indirectly, as it might well be that the down-regulation of
all HAS genes is the underlying mechanism that accounts (at least
in part) for the beneficial effects of glucocorticoids in RA. Just
as blocking CD44 is a proven way of impeding inflammation
by blocking cell trafficking, down-regulating HAS might have
a similar beneficial effect.
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Conflict of interest

The authors have declared no conflicts of interest.

References