Local and Systemic Glucocorticoid Metabolism in Inflammatory Arthritis

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ABSTRACT:

Context: We have shown that isolated, primary synovial fibroblasts generate active glucocorticoids via expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). This enzyme produces cortisol from inactive cortisone (and prednisolone from prednisone).

Objective: We have now examined glucocorticoid metabolism in patients with RA to determine how intact synovial tissue metabolizes glucocorticoids and to identify the local and systemic consequences of this activity.

Methods: Synovial tissue was taken from subjects with RA during joint replacement surgery. Glucocorticoid metabolism in explants was assessed using thin layer chromatography and specific enzyme inhibitors. RT-PCR and immunohistochemistry were used to determine expression and distribution of 11β-HSD enzymes. Systemic glucocorticoid metabolism was examined in RA subjects using gas chromatography/mass spectrometry.

Results: Synovial tissue synthesized cortisol from cortisone confirming functional 11β-HSD1 expression. In patients with RA, enzyme activity correlated with donor ESR. Synovial tissues were also able to convert cortisol back to cortisone. Inhibitor studies and immunohistochemistry suggested this was due to 11β-HSD2 expression in synovial macrophages, whereas 11β-HSD1 expression was primarily in fibroblasts. Synovial fluids exhibited lower cortisone levels compared to matched serum indicating net local steroid activation. Urinary analyses indicated high 11β-HSD1 activity in untreated RA patients compared to controls and a significant correlation between total body 11β-HSD1 activity and ESR.

Conclusions: Synovial tissue metabolizes glucocorticoids, the predominant effect being glucocorticoid activation, and this increases with inflammation. Endogenous glucocorticoid production in the joint is likely to impact on local inflammation and bone integrity.
INTRODUCTION
Since the discovery of cortisone and its first use in patients with rheumatoid arthritis (RA)[1] glucocorticoids have been extensively used to suppress synovial inflammation. However, in patients with established synovitis, glucocorticoids such as cortisol (hydrocortisone), prednisone and prednisolone do not cause permanent resolution of inflammation and long-term use has adverse effects on bone, skin and fat tissue.[2,3] Endogenous glucocorticoids also have a role in suppressing disease activity in RA. Early morning stiffness is attributed to the nocturnal decrease in circulating cortisol levels. Administration of metyrapone to reduce endogenous corticosteroid production increases disease activity in RA.[4] It is unclear however whether endogenous corticosteroid action contributes to susceptibility to, or severity of, RA. Subtle abnormalities of the HPA axis have been observed in glucocorticoid naive patients with RA[5-7] but their origin remains unclear.[8]

We have previously hypothesised that periarticular osteopenia in RA is partly due to excessive local glucocorticoid activation through the 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme.[9] This enzyme converts inactive steroids (cortisone and prednisone) to their active counterparts (cortisol and prednisolone).[10] Although 11β-HSD1 is bidirectional, its predominant action in vivo is conversion of inactive to active glucocorticoids. Hepatic 11β-HSD1 is essential for activation of oral cortisone/prednisone – patients who lack this enzyme are unresponsive to cortisone and prednisone but respond to hydrocortisone and prednisolone.[11] We have reported that synovial fibroblasts express 11β-HSD1 in vitro and in vivo.[12] In osteoblasts and synovial cells 11β-HSD1 activity is upregulated by pro-inflammatory cytokines.[9,12] This suggested that 11β-HSD1 might generate high levels of glucocorticoids within the joint and that this could contribute to periarticular osteopenia. By contrast, a related enzyme 11β-HSD2 solely inactivates steroids. This enzyme is expressed in mineralocorticoid target tissues, various developmental tissues and some tumors.[13-15] Recent studies have reported expression of 11β-HSD2 in peripheral blood mononuclear cells (PBMCs) and synovium of RA patients.[16-18] We have therefore examined glucocorticoid metabolism and function in synovial tissue from patients with RA using specific enzyme assays and inhibitors. In addition, we examined glucocorticoid concentrations in synovial fluid and compared the systemic metabolism of glucocorticoids in patients with RA and non-inflammatory joint conditions.

PATIENTS AND METHODS:
Patients:  
Biopsies of matched synovium and skin were obtained during hip, knee or elbow arthroplasty from consenting patients who fulfilled the American College of Rheumatology criteria for RA and OA. Clinical details are given in table 1.

Table 1: Clinical characteristics of subjects for synovial tissue corticosteroid metabolism studies.

<table>
<thead>
<tr>
<th>Age in years Mean±SD</th>
<th>F/M (number)</th>
<th>Site of operation (number)</th>
<th>Treatment (number)</th>
<th>ESR mm/hr</th>
<th>CRP mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>62±10</td>
<td>11/1</td>
<td>Hip (6) Knee (4) Elbow (2)</td>
<td>Methotrexate (3) Prednisolone (3) Anti-TNF (2) Sulphasalazine (1)</td>
<td>39±20</td>
<td>27±23</td>
</tr>
</tbody>
</table>
Hydroxychloroquine (1)
Azathioprine (1)

OA patients n=8:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>67±7</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Hip (7)</td>
</tr>
<tr>
<td>Knee (1)</td>
<td></td>
<td>14±11*</td>
</tr>
</tbody>
</table>

*P<0.05 compared to RA subjects

Synovial tissue was taken on ice and prepared within 2 hours by removing adherent non-synovial tissue. Tissue was divided into 100mg sections for enzyme assay or ELISA. Skin tissue was prepared by removing subcutaneous fat and dividing into 100mg pieces. Matched synovial fluid and serum samples were obtained from patients with active RA undergoing joint aspiration as part of routine care. Blood was drawn immediately prior to joint aspiration. Clinical details are given in supplementary table 2.

**Supplementary table 2: Clinical characteristics of subjects for serum and synovial fluid corticosteroid level measurement.**

<table>
<thead>
<tr>
<th>Age in years</th>
<th>F/M</th>
<th>RF+ (Y/N)</th>
<th>Erosions* (Y/N)</th>
<th>Treatment (number)</th>
<th>ESR mm/hr</th>
<th>CRP mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td></td>
<td></td>
<td></td>
<td>Gold (1) Methotrexate (1) Leflunomide (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65±20</td>
<td>4/1</td>
<td>4/1</td>
<td>4/1</td>
<td></td>
<td>51±41</td>
<td>45±38</td>
</tr>
</tbody>
</table>

* The presence of erosions was identified on hand and wrist radiographs

Urine samples for corticosteroid metabolite analysis were obtained from patients with newly presenting RA or non-inflammatory joint disease (localised OA n=5; trigger finger n=3; hypermobility n=1). Clinical details are given in supplementary table 3.

**Supplementary table 3: Clinical characteristics of subjects in urinary corticosteroid metabolite analyses:**

<table>
<thead>
<tr>
<th>Controls (n=9)</th>
<th>Rheumatoid arthritis (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>44±26</td>
</tr>
<tr>
<td>Sex F/M</td>
<td>5/4</td>
</tr>
<tr>
<td>ESR</td>
<td>10±8</td>
</tr>
<tr>
<td>CRP</td>
<td>3±3</td>
</tr>
<tr>
<td>RF+ (Y/N)</td>
<td>NA</td>
</tr>
<tr>
<td>Erosions (Y/N)</td>
<td>NA</td>
</tr>
</tbody>
</table>

All studies had ethical approval from the Local Ethics Committee and informed consent was obtained when samples were taken.

**11β-hydroxysteroid dehydrogenase enzyme assays:**

Synovial or skin tissue (100mg per assay) was incubated in RPMI-1640 medium containing 1% non-essential amino acids, 1% penicillin/streptomycin, 1% sodium pyruvate, 2mM glutamine and 20% heat-inactivated FCS (Labtech International, Sussex, UK). 100nM
cortisol (to measure glucocorticoid inactivation) or cortisone (to measure activation) along with tracer amounts of $[\text{^3H}]$-cortisol (specific activity 78.4 Ci/mmol, NEN Life Science Products, Hounslow, UK) or $[\text{^3H}]$-cortisone (generated as previously described[19]) were added and tissue incubated at 37°C for 18h. Similar assays were performed using $[\text{^3H}]$-prednisolone or $[\text{^3H}]$-prednisone[20]. Steroids were extracted from medium/tissue using dichloromethane (5–7mls) and separated by thin-layer chromatography (TLC) using ethanol:chloroform as the mobile phase. TLC plates were analyzed using a Bioscan imager (Bioscan, Washington, DC, USA) and fractional steroid conversion calculated. Results were expressed as pmol product/mg tissue/hr. Experiments were carried out in duplicate or triplicate.

**RNA extraction and reverse transcription:**
RNA was extracted from synovium using a single-step method (TRI Reagent, Sigma, Poole, UK). Aliquots (1µg) of RNA were reverse transcribed using random hexamers in a 20µl volume as per the manufacturer’s protocol (Promega, Madison, USA).

**Real-time PCR:**
Expression of mRNA for 11β-HSD1/2 were assessed using real-time PCR in an ABI 7500 system (Applied Biosytems, Warrington, UK). Reactions were performed in 25µl aliquots on a 96 well plate (Sigma, UK). Primers for 18S were used as an internal reference. Reactions contained TaqMan PCR master mix (Applied Biosytems), 900nmol primers, 100-200nmol TaqMan probe and 25-50ng cDNA. Reactions occurred as follows: 50°C for 2 min, 95°C for 10min, 44 cycles of 95°C for 15s and 60°C for 1 min. Data were obtained as Ct values (cycle number at which logarithmic PCR plots cross a calculated threshold line) according to the manufacture’s guidelines, and used to determine ∆Ct values (Ct of target gene – Ct of housekeeping gene) as raw data for gene expression. Probe and primer sequences were: 11β-HSD1, forward AGGAAAGCTCATGGGAGGACTAG, reverse ATGGTGAATATCATCATGAAAAATTC, probe CATGCTCATCTCACAACCACATCACCAACA; 11β-HSD2, forward CAGGTGTCCTAGTGCACATTGAC, reverse GTAGCCCACTCTCTCGTCCAA, probe AAGGCACGCCCTCCCAGCG.

**Immunohistochemistry:**
Cryostat sections of synovial tissue were analysed with previously validated sheep polyclonal antibodies to 11β-HSD1 and 11β-HSD2 1/50 (The Binding Site, Birmingham, UK)[14,21] with anti sheep/goat biotin AB360 as secondary antibody 1/50. Immunohistochemistry was also carried out using polyclonal antisera to: the endothelial marker von Willebrand’s Factor (vWF) 1/1000 (Dako, Ely, UK); the fibroblast marker ASO2 (CD90) 1/100 (Invitrogen, Paisley, UK); and the monocyte/macrophage marker CD68 1/100 (BD Biosciences, NJ, USA). Immunohistochemical analyses were carried out as described previously.[12]

**Measurement of corticosteroid levels in serum and synovial fluids:**
Serum and synovial fluid cortisol and cortisone levels were measured using a specific cortisol ELISA (R&D systems, Abingdon, UK) and a previously reported radioimmunoassay for cortisone.[22] Since serum and synovial fluid samples contain cortisol binding proteins these techniques only give information about total cortisol concentration (rather than unbound ‘free’ levels).[23] Since there is much less protein binding of cortisone levels of cortisone ascertained by these methods are more likely to reflect ‘free’ levels.[22]

**Measurement of urinary corticosteroid metabolites:**
The GC/MS method was based on Palermo et al. [24] using a 5970 mass spectrometer (Hewlett-Packard, Houston, TX). Intra and interassay CV were <10% for cortisol and cortisone. 11β-HSD1 activity was calculated as the tetrahydrocortisol + allo-tetrahydrocortisol/tetrahydrocortisone [(THF+alloTHF)/THE] ratio. Renal 11β-HSD2 activity was calculated as the urinary-free cortisol/cortisone ratio (UFF/UFE). The sum of total corticosteroid metabolites (THF, alloTHF, THE, cortols, cortolones, UFF, UFE) was used as an index of total cortisol secretion.

**Analysis of IL-6 levels by ELISA:**
Soluble IL-6 in tissue supernatants was measured using a sandwich ELISA (BD Biosciences Pharmingen, Torreyana, San Diego). The limit of detection was 2.2pg/ml and intra and interassay CVs were 5.2% and 9.3%. Data were expressed as pg IL-6/mg tissue.

**Statistics:**
Data were reported as the mean±standard deviation (SD) of replicate mean values for separate synovial explants unless otherwise stated. Regression analysis was performed using Microsoft Excel 2003. One-way ANOVA analysis was performed using SPSS Data Editor.

**RESULTS:**

**Enzyme activity studies:**
11β-HSD enzyme activity was present in all synovial samples from patients with RA (figure 1). Activity was also present in OA synovium. Activity was bidirectional in all cases although the relative amounts of activation (cortisone to cortisol conversion) to inactivation (cortisol to cortisone) varied between subjects. There was no significant difference between RA and OA synovium in overall levels of each activity or in the ratio of activation to inactivation. Compared to patient matched skin samples synovial samples had greater activating capacity (14.1±1.8 (mean±SE) pmol/mg protein/hr vs 8.9±1.3; p<0.001) and a higher ratio of activation to inactivation (ratio 1.7±0.4 vs 0.6±0.1; p<0.01) but there was no difference in inactivating capacity (16.1±3.5 vs 20.1±3.4; NS). For glucocorticoid activation in synovium there was a significant positive correlation with the preoperative ESR (figure 2A), but this relationship was not seen with CRP ($r^2$=0.04, NS). Inactivation did not correlate with either inflammatory marker. Synovial tissue also metabolised prednisone and prednisolone in a manner indistinguishable from cortisone/cortisol (for 100nM substrate activation assay: cortisone to cortisol 16.3±4.7pmol/mg protein/hr, prednisone to prednisolone 23.0±5.1; for inactivation assay; cortisol to cortisone 15.2±6.1, prednisolone to prednisone 12.5±4.7; n=4, NS).

**Characterisation of glucocorticoid inactivating capacity:**
The glucocorticoid activating (oxoreductase) capacity of synovial tissue appeared due to expression of 11β-HSD1 as this is the only enzyme known to convert cortisone to cortisol. Additionally, we have previously localized 11β-HSD1 in synovium by immunohistochemistry[12] and RT-PCR on synovial tissue demonstrated abundant 11β-HSD1 mRNA expression (CT value 32.0±0.7 relative to 12.9±1.9 for 18S).

The glucocorticoid inactivating (dehydrogenase) activity could be due to either 11β-HSD1, 11β-HSD2 or a combination of both. The lack of correlation between activation and inactivation (p=0.56) suggested the presence of more than one enzyme and a specific inhibitor of 11β-HSD1 (PF-877423; Pfizer Inc, New York, USA) was used to clarify this further. PF-877423 blocks both activating (oxoreductase) and inactivating (dehydrogenase) directions of 11β-HSD1 with no effect on 11β-HSD2 activity (Bujalska et al., submitted for publication).
Enzyme activity data indicated that the inhibitor reduced glucocorticoid activation in synovial tissue from RA and OA subjects but increased glucocorticoid inactivation in OA tissue (figure 2B). There was also a trend to higher inactivation in RA tissue treated with inhibitor (p=0.07). These observations suggested that glucocorticoid inactivation in synovial tissue was due to expression of 11β-HSD2 rather than 11β-HSD1. 11β-HSD2 was detectable by RT-PCR on RNA extracted from synovial tissue (CT 33.6±1.0 relative to 12.1±1.8 for 18S). Immunohistochemistry for 11β-HSD2 demonstrated expression of the enzyme in both RA and OA tissue, where it colocalised predominantly with CD68 positive macrophages (figure 3). This contrasted with 11β-HSD1 expression which was localised primarily to fibroblasts.

**Functional consequences of enzyme activity:**
High levels of IL-6 were produced by synovial tissue. As expected levels were higher in patients with RA than OA. Incubation of synovial tissue with cortisol significantly reduced IL-6 production indicating tissue sensitivity to glucocorticoids. Incubation with cortisone also caused a significant reduction in IL-6 expression, suggesting that synovial 11β-HSD1 was functionally active converting cortisone to cortisol in an autocrine fashion (figure 4A). This was confirmed by studies using PF-877423, which blocked the suppressive effect of cortisone on IL-6 (figure 4B).

**Measurement of tissue glucocorticoid levels in vivo:**
To evaluate net synovial tissue glucocorticoid metabolism within the joint we measured corticosteroid levels in paired serum and synovial fluid samples. To minimize the confounding effect of cortisol-binding proteins we measured serum-to-synovial fluid concentration gradients in cortisone since cortisone has limited binding to these proteins. Cortisone levels in synovial fluid obtained from RA patients were significantly different from plasma levels (serum 56.7±11.8nmol/L, synovial fluid 20.9±8.7nmol/L; mean fall from serum to synovial fluid 63%, p<0.001). The low cortisone levels in synovial fluid suggested local net conversion of cortisone to cortisol.

**Measures of systemic glucocorticoid metabolism in inflammatory arthritis:**
Systemic measures of 11β-HSD1 activity were assessed in subjects with untreated RA compared to patients with non-inflammatory joint disease. There was a significantly higher ratio of cortisol to cortisone metabolites in the urine of RA patients suggesting higher 11β-HSD1 activity (figure 5A,B). No change was seen in the ratio of urinary cortisol to cortisone indicating that renal 11β-HSD2 was unchanged. There was a positive correlation between 11β-HSD1 activity and ESR (but not CRP) in RA patients (figure 5C,D). The urinary cortisol/cortisone ratio also correlated with ESR in patients with RA (r=0.42, p<0.05) probably reflecting the contribution 11β-HSD1 makes to this ratio. There was no correlation between the urinary cortisol/cortisone ratio and CRP (r=0.11, NS). There was no correlation between total urinary corticosteroid metabolites and ESR or CRP (r=0.29 and 0.08, both NS).

**DISCUSSION:**
Using a variety of measures, substantial glucocorticoid metabolism was identified in the joint. The net consequence was local glucocorticoid excess. In patients with RA, glucocorticoid generation within synovium, and systemic levels of active steroid, correlated with disease activity and had functional effects on the inflammatory response. Although this may form part of an endogenous mechanism for immunoregulation, a subset of synovial cells were able to inactivate glucocorticoids through expression of 11β-HSD2 and thus may be glucocorticoid resistant.
Soon after the initial use of cortisone and hydrocortisone for RA it was realised that these steroids were interconverted \textit{in vivo} and that 11\(\beta\)-HSD activity was essential for conversion of cortisone to cortisol.[25] This suggested that synovial tissue itself might metabolize corticosteroids. Initially it was thought that RA patients would have increased capacity to inactivate hydrocortisone.[26] Although the understanding of tissue steroid metabolism was rudimentary bidirectional 11\(\beta\)-HSD activity was noted in rheumatoid synovial tissue[27] and significant amounts of cortisol were generated from cortisone injected into inflamed joints.[28] Recently interest has been renewed with reports implicating 11\(\beta\)-HSD2 expression in RA. 11\(\beta\)-HSD2 was the most upregulated of >4,300 genes in a study examining PBMCs from patients with recent onset compared to long-standing RA.[18] 11\(\beta\)-HSD2 was one of 3 (out of 20,000) significantly upregulated genes in PBMCs from identical twins discordant for RA.[17] Analysis of rheumatoid synovium indicated that 11\(\beta\)-HSD2 was expressed predominantly in synovial macrophages. A separate study examined the capacity of synovial explants to inactivate steroids and described how this activity was higher in patients with RA.[16] That study did not involve direct measurement of glucocorticoid activation and relied on 11\(\beta\)-HSD inhibitors with limited specificity. Furthermore, the association between local and systemic glucocorticoid metabolism was not assessed. To address this, we used specific enzyme assays and selective inhibitors to define the nature of glucocorticoid metabolising activity. We were additionally able to examine possible consequences of corticosteroid metabolism on local and systemic levels of glucocorticoids.

The generation of substantial amounts of active glucocorticoids within the joint could account for why therapeutic glucocorticoids are so effective at dampening flares of synovial inflammation. In the inflamed joint, cells will be exposed to both circulating glucocorticoid and glucocorticoids generated from circulating inactive precursors by cells expressing 11\(\beta\)-HSD1. Locally produced glucocorticoids will be free to diffuse into surrounding tissues. This could affect the integrity of adjacent connective tissue. The periarticular osteoporosis seen in RA is of multifactorial origin but high local glucocorticoid levels would suppress bone forming capacity and thus contribute to uncoupling of bone resorption from formation.[29] High glucocorticoid levels would also be expected to have an immunosuppressive effect. This could contribute to the increased risk of septic arthritis in RA.[30]

The finding of 11\(\beta\)-HSD2 expression in synovium raises the possibility that some cells within synovium are resistant to steroids through expression of this enzyme. Further assessment of the functional implications of 11\(\beta\)-HSD2 expression is beyond the scope of this study but there is a need to further define the phenotype of 11\(\beta\)-HSD2 expressing synovial cells and to clarify the relationship these cells have with 11\(\beta\)-HSD2 positive PBMCs. An important and unexpected finding in this study is the presence of considerable glucocorticoid metabolising capacity in synovium from OA patients. This tissue was initially used as a control for patients with RA with the expectation that glucocorticoid metabolism would be less prominent. However net activity appeared similar to that of RA. Additionally the relative decrease in IL-6 production in response to cortisone treatment was similar. The inability of the inhibitor to block the capacity of OA synovium to convert cortisol to cortisone also suggests that 11\(\beta\)-HSD2 is expressed. 11\(\beta\)-HSD2 expression in OA has been identified in a previous study[16] which suggested that its expression is lower in OA than RA. The roles of 11\(\beta\)-HSD1/2 in OA remain to be defined.

Glucocorticoid activation was associated with ESR but not CRP measurements. A potential explanation for this is the confounding effect glucocorticoid activation had on synovial IL-6 production. CRP synthesis is primarily an IL-6 driven process thus the relationship between joint inflammation and CRP will be complicated by the inhibitory effect of glucocorticoids on
IL-6. The ESR is less dependent on IL-6 and so less likely to be directly influenced by local glucocorticoid metabolism.

The data presented here have several limitations. Firstly, synovial tissue was obtained from patients with RA treated with various disease modifying drugs. The small number of patients studied makes it difficult to comment about potential effects of anti-rheumatic therapy on steroid metabolising enzyme activity. Secondly, synovial tissue was examined in patients that had established RA. It will be of interest to study temporal changes in glucocorticoid metabolism in patients with RA at different disease stages and to assess whether findings are specific to RA or a general feature of persistent synovial inflammation. Although urinary measures of systemic steroid metabolism correlated well with measures in synovial tissue and fluid an intrinsic limitation is that these could reflect altered steroid metabolism in tissues other than synovium. We have previously reported that inflammatory cytokines increase 11\(\beta\)-HSD1 expression in fibroblasts from several tissues[12] and 11\(\beta\)-HSD1 expression has been reported in subpopulations of peritoneal macrophages[31] and T-cells.[32] Regardless of the extent of altered glucocorticoid metabolism in RA the functional consequences of this deserves further study.

Acknowledgements and affiliations

We would like to thank Mr Andrew Thomas, Dr Shinner and the operating theatre staff of the Royal Orthopaedic Hospital in Birmingham for their help in sample collection. Sue Hughes and Beverley Hughes carried out enzyme assays and biochemical measurements. Debbie Hardie performed immunohistochemical analysis on synovial tissue.

Competing interests: The Authors have no competing interests to disclose

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Reference List


**Figure Legends:**

**Figure 1:** Glucocorticoid metabolism in synovial explants taken from patients with RA and OA:
The capacity of synovial tissue to interconvert cortisone and cortisol was examined using tissue freshly isolated following joint replacement surgery. Data is steroid generation per sample adjusted for protein content and assay time and is expressed as mean±SD of samples from each patient.

**Figure 2:** Relationship between glucocorticoid activating capacity of synovial explants and the systemic inflammatory response and the effect of a specific inhibitor of 11β-HSD1 on glucocorticoid activation and inactivation:
A. A significant correlation was seen between glucocorticoid activation (oxoreductase activity) and the ESR measured prior to surgery in patients with RA. B. The effect of PF-877423, a specific bidirectional inhibitor of 11β-HSD1 enzyme activity, on glucocorticoid activation (oxoreductase activity, cortisone to cortisol conversion) and inactivation (dehydrogenase activity, cortisol to cortisone conversion) in synovium from patients with RA or OA. The inhibitor reduced the capacity of synovium to activate glucocorticoids but was unable to block the inactivating capacity. In OA tissue inactivation increased with 11β-HSD1 inhibition. These results indicate that the inactivating capacity is not due to 11β-HSD1.

**Figure 3:** Immunohistological localization of 11β-HSD enzymes in synovium:
The expression of 11β-HSD1 and 11β-HSD2 within rheumatoid synovium were examined using specific antibodies. 11β-HSD1 expression colocalised predominantly with a fibroblast marker. Expression of 11β-HSD2 colocalised with expression of CD68.

**Figure 4:** The functional effect of glucocorticoid activation on IL-6 synthesis in patients with RA and OA:
A. Synovial tissue was incubated for 24 hours in the presence or absence of 100 nM cortisone. In both RA and OA synovium cortisone had a significant suppressive effect on IL-6 production. B. A specific 11β-HSD1 inhibitor (PF-877423) was able to block the decrease in IL-6 production caused by cortisone treatment.

**Figure 5:** Systemic measures of local glucocorticoid metabolism in patients with RA and non-inflammatory joint disease:
A,B. Measurement of the balance of glucocorticoid activation/inactivation in non-inflammatory controls and patients with untreated RA. There was a significant increase in the (THF+alloTHF)/THE ratio in patients with inflammatory arthritis whilst the urinary free cortisol/cortisone ratio was unchanged. This indicated that 11β-HSD1 mediated glucocorticoid activation was enhanced in patients with RA. C,D. In patients with RA the relationship between the (THF+alloTHF)/THE ratio, a systemic measure of
11\( \beta \)-HSD1 activity, and inflammatory markers was examined. The ESR, but not CRP, demonstrated a significant correlation with this measure suggesting increasing 11\( \beta \)-HSD1 mediated glucocorticoid activation with increasing degrees of inflammation.
Figure 1.

Cortisone to cortisol
Cortisol to cortisone

Conversion pmol / mg protein / hr

Cortisone to cortisol / cortisol to cortisone ratio (mean±SE)

RA

OA

1.3±0.3
2.2±0.7

NS
Figure 2.

A

B

RA SYNOVION

11β-HSD1 inhibitor

activation

inactivation

* p<0.05 compared to control

OA SYNOVION

11β-HSD1 inhibitor

activation

inactivation

* p<0.05 compared to control

$\text{r}^2=0.40; \ p<0.05$
Figure 3.
Figure 4.

A. RA SYNOVrium

mean fall 31±15%
p<0.05

OA SYNOVium

mean fall 36±9%
p<0.05

B.

* p<0.05 compared to control
Figure 5.

A. 11β-HSD1

B. 11β-HSD2

C. (THF+alloTHF)/THE (11β-HSD1)

D. (THF+alloTHF)/THE (11β-HSD1)

Controls Cases

Controls Cases

r=0.40; p<0.05

r=0.34; p=0.08