Intra-articular corticosteroid preparations: different characteristics and their effect during inflammation induced by monosodium urate crystals in the rat subcutaneous air pouch

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Objective. To examine the effects of three commonly used intra-articular depot corticosteroid preparations tested in a rat air pouch model and their effect against monosodium urate (MSU) crystal-induced inflammation. Rheumatologists use intra-articular corticosteroid preparations to relieve pain and inflammation of acute monoarthritis without really knowing their effects on the synovial fluid and membrane or the differences between distinct preparations. This work compares the effect of three commonly used corticosteroid preparations in vivo, showing that they behave differently.

Methods. A subcutaneous air pouch was formed in male Sprague–Dawley rats. A first group of 6-day-old air pouches were injected with 10 ml of 6 mg/ml normal saline solution, 6 mg/ml betamethasone containing both depot betamethasone acetate and soluble betamethasone phosphate (Celestone) in 9 ml of normal saline solution, 20 mg/ml of prednisolone tebutate (Hydeltra) in 9 ml of normal saline solution or 20 mg/ml of triamcinolone hexacetonide (Aristospan) in 9 ml of normal saline solution. A second group (group 2) of air pouches were injected with 15 mg of synthetic MSU crystals and 24 h later they were reinjected with 1 ml of the same three corticosteroid suspensions. For each condition four rats were killed at 6, 24, 48 h and 7 days. Pouch fluid and tissue were analysed.

Results. In the first 6 h after normal saline solution or corticosteroid injection into the air pouch there were mildly increased leucocyte counts in the air pouch fluid. Betamethasone-injected pouches showed no cells in the fluid after 6 h and no crystals after 24 h, triamcinolone-injected pouches still showed rare cells at 7 days. Both triamcinolone and prednisolone crystals persisted in higher numbers and lasted longer in the fluid than did betamethasone (P < 0.05). In group 2 MSU crystal phagocytosis in the fluid was decreased in the betamethasone- (P < 0.01), prednisolone- (P < 0.003) and triamcinolone- (P < 0.006) injected pouches when compared with the MSU crystal-injected pouches alone. Pouches injected with MSU crystals alone showed the most intense tissue inflammation at all times. After MSU, betamethasone-injected pouches had a rapid but mild decrease in the number of lining cells and inflammation. In contrast, triamcinolone- and prednisolone-injected pouches showed a very thin tissue with few or no vessels and almost no inflammation at 7 days. The pouches injected with MSU crystals and any of the corticosteroid preparations had three times more tophus-like structures and persistent crystals identified than the ones injected with MSU crystals alone.

Conclusion. Each of the corticosteroid preparations by themselves produced very
mild transient inflammation. The betamethasone preparation with a soluble steroid component had a quicker but milder anti-inflammatory effect on MSU crystal-induced inflammation. In contrast to the doses used, prednisolone tebutate and triamcinolone hexacetonide preparations dramatically suppressed crystal-induced inflammation at 7 days, but both produced atrophy and necrosis of the membrane, yielding a very thin membrane with almost no vessels. When used for MSU crystal-induced inflammation these corticosteroid preparations suppressed some aspects of inflammation but may actually promote the persistence of MSU crystals and the formation of tophi.

**Key words:** Corticosteroids, Betamethasone, Prednisolone, Triamcinolone, Rat air pouch, Monosodium urate crystals, Inflammation, Tophi.

Intra-articular microcrystalline corticosteroid injections have been used for half a century as a simple, palliative and relatively safe therapeutic adjunct in non-septic local joint inflammation [1]. As intra-articular injection became widely used, anecdotes of post-injection steroid flares in the injected joints were reported. In 1964, a sterile inflammatory reaction was described with hydrocortisone, cortisone and dexamethasone crystals in normal joints [2]. In 1970, Hollander [3] reported his 20-yr experience with depot corticosteroids, noting post-injection flares in 1 to 2% of his patients. Since then new corticosteroid preparations have been developed containing methylprednisone, prednisolone, triamcinolone and betamethasone crystals. Increased white blood cell counts in the synovial fluid have still been observed after injections with triamcinolone acetate and prednisolone preparations, and their crystals have been demonstrated by polarized light and electron microscopy to be present intracellularly in synovial fluid 24 h and 1 week after injection [4]. Isolated reports of an immediate exacerbation or inflammation from intra-articular triamcinolone hexacetonide injection have been published from time to time [5]. Osteonecrosis, possibly secondary to repeated local corticosteroid injections with triamcinolone hexacetonide, has been reported in at least three cases involving the femoral head, the distal femur and proximal tibia [6, 7]. Also, regional epiphyseal osteonecrosis has been reported in children treated with intra-articular triamcinolone hexacetonide [8].

A study of pain relief in the rheumatoid knee after injection of hydrocortisone succinate and triamcinolone hexacetonide or acetate did not report any flares and showed better results with triamcinolone hexacetonide [9]. An American College of Rheumatology survey carried out in 1994 showed little consensus among rheumatologists concerning the administration of intra-articular corticosteroids [10]. The reasons given for choosing a specific corticosteroid were primarily availability and habit.

Considering the sparse information on intra-articular corticosteroid preparations and their common use in the rheumatology clinic we have started studies to attempt to determine if there is one corticosteroid superior to others in various settings with regard to either efficacy and/or side-effects. We have studied three different corticosteroid preparations (betamethasone, prednisolone and triamcinolone hexacetonide) to compare their effects *in vivo* in two different situations. We observed their behaviour in the unaltered rat subcutaneous air pouch and in similar air pouches after induction of acute inflammation caused by monosodium urate (MSU) crystals.

**Materials and methods**

We used the same subcutaneous air pouch model with which our laboratory has worked to study inflammation caused by different crystals [11, 12]. A subcutaneous air pouch was formed in male Sprague-Dawley rats weighing 200–250 g, as described by Edwards *et al.* [13]. Rats were lightly anaesthetized intramuscularly with 40 mg/kg of ketamine and 5 mg/kg of xylazine. Their dorsal area was shaved and 10 ml of sterile air was injected subcutaneously. Air pouches were reinjected with sterile air every 2 or 3 days when needed to maintain inflation. On the 6th day after the air pouch was formed, two different sets of studies were performed. In group one, normal saline solution and three different microcrystalline corticosteroid preparations were injected in the ‘normal’ rat air pouches. In group two, synthetic MSU crystals were injected into the rat air pouch and 24 h later the same three corticosteroid preparations were injected. MSU crystals were synthesized in our laboratory by the method of Denko and Whitehouse [14] using endotoxin-free water and then autoclaved for 30 min and resuspended in sterile saline solution before use.

The corticosteroid preparations used in this study were betamethasone sodium phosphate combined with betamethasone acetate 6 mg/ml (Celestone soluspan, Schering, Kenilworth, NJ 07033, USA), prednisolone tebutate 20 mg/ml (Hydeltra, Merck and Co. Inc., Rahway, NJ) and triamcinolone hexacetonide 20 mg/ml (Aristospan, Fujisawa, Deerfield, IL 60015, USA). Doses used were approximately comparable with recommended doses in human joints. Sixteen rats were studied for each corticosteroid preparation in each group; four rats in each group were killed at 6, 24, 48 h and 7 days respectively. In group 1: 16 rats were injected with 10 ml of normal saline solution as a control; 16 rats with 9 ml of normal saline solution plus 1 ml of 6 mg/ml betamethasone acetate and soluble sodium phosphate betamethasone; 16 rats with 9 ml of normal saline solution plus 1 ml of 20 mg/ml prednisolone tebutate; and 16 rats with 9 ml of normal saline solution plus 1 ml of 20 mg/ml triamcinolone hexacetonide.
solution and 1 ml of 20 mg/ml triamcinolone hexacetonide. In group 2, 15 mg of MSU crystals were injected in all air pouches. After 24 h, 16 rats were studied without further injections; 16 rats were injected with 1 ml of 6 mg/ml of betamethasone acetate and soluble sodium phosphate betamethasone; 16 rats with 1 ml of 20 mg/ml prednisolone tebutate; and 16 rats with 1 ml of 20 mg/ml of triamcinolone hexacetonide.

Just before the rats were killed, samples of each air pouch fluid were examined for leucocyte counts (cells/mm³), the presence of free MSU crystals per high power field and the percentage of cells with phagocytosed crystals. The air pouch tissue was dissected, examined unstained for crystals with compensated polarized light, fixed in formalin and embedded in paraffin for sectioning and staining with haematoxylin and eosin. Each pouch was scored semiquantitatively for the number and activity of lining cells, the number of vessels and the amount of inflammation. For all histological features we used a numerical scale beginning at 0 for the lowest and ending at 4 for the highest value seen. To analyse connective tissue elements of the air pouch membrane, a pentachrome stain was performed following the steps of the modified Russel–Movat pentachrome method [15, 16].

**Data analysis**

Data are presented as means. We analysed differences between the times and groups by Student’s *t*-test, considering *P* < 0.05 to be statistically significant.

**Results**

When corticosteroid preparations or normal saline solution were injected into ‘normal’ air pouches (group 1), fluid cell counts ranged from 0 to 500/mm³ with means for no groups higher than 162/mm³ (Fig. 1). Thus, only some very mild inflammation was common and was seen even with normal saline solution. Cell counts fell over time and rather more rapidly after the corticosteroids than normal saline solution, with all except the triamcinolone group showing rare cells by 7 days. Betamethasone-injected pouches showed no cells in the fluid after 6 h (Fig. 1). Triamcinolone and prednisolone crystals persisted in the pouch fluid in higher numbers and lasted longer than betamethasone crystals (*P*=0.005 and *P*=0.05, respectively) (Fig. 2). Betamethasone crystals disappeared 24 h after the injection. Prednisolone crystals were the only ones to last in the fluid for 7 days (Fig. 2).

When corticosteroid preparations were injected in MSU crystal-inflamed air pouches, fluid cell counts over the first 48 h were higher than with the steroids alone, but initially not lower than with the MSU alone (Table 1). At 7 days, fluid leucocyte counts were 0–50/mm³ in all steroid-injected pouches, but 666/mm³ with MSU alone (*P* < 0.05). The number of free MSU crystals was also not significantly different from the pouches injected with MSU crystals alone or between any groups at 6, 24 or 48 h. Crystal phagocytosis, however, was decreased in the betamethasone group (*P*=0.01), the prednisolone group (*P*=0.003) and the triamcinolone group (0.006) compared with the MSU crystal alone group in the first 24 h (Fig. 3). This difference had disappeared by 48 h.

Pouches injected with MSU crystals alone showed the most intense histological evidence of inflammation and had larger numbers and activity of pouch lining cells at all times (Table 2). Betamethasone-injected pouches were the only ones to have a significant decrease in the number of lining cells and interstitial inflammation as soon as 24 and 48 h (both *P* ≤ 0.05). At 7 days, betamethasone-injected pouches still showed a slight decrease in the lining cell numbers and activity as well as in interstitial vessels (Fig. 4). In contrast, triamcinolone- and prednisolone-injected pouches had areas with a very

![Fig. 1. Cell counts in air pouch fluids at 6, 24, 48 h and 7 days after being injected with normal saline solution (NSS), betamethasone (BTN), prednisolone (PDN) and triamcinolone (TMN). Betamethasone-injected pouches showed no cells in the fluid after 6 h.](image-url)
thin membrane with few or no vessels at 7 days, showing a significant decrease of all histopathological findings when compared with pouches injected with MSU crystals alone (\(P < 0.001\)) and with betamethasone-injected pouches (\(P < 0.005\)) (Fig. 4).

MSU crystals were seen in non-stained tissue in all pouches injected with MSU crystals and were detected on the superficial surface of the lining cell membrane (Fig. 5). Although present in all non-stained tissues after MSU injection, crystals tended to be more prominent at 7 days in the groups later injected with triamcinolone and prednisolone. Microscopic tophi were found in stained tissue as empty round interstitial spaces surrounded by giant cells (Fig. 6). The pouches injected with MSU crystals and corticosteroid preparations had three times more of these apparent tophi than the pouches injected only with MSU crystals (\(P < 0.05\)) (Table 2). There was no significant difference in the number of tophus-like structures among the pouches injected with the different corticosteroid preparations. The pentachrome staining showed no evidence of collagen, proteoglycan or fibrin in the tophi. Immediately around the suspected tophi there

Table 1. Pouch fluid leucocyte counts (per mm\(^3\)) after MSU with/without depot corticosteroids

<table>
<thead>
<tr>
<th></th>
<th>MSU alone</th>
<th>MSU + BTN</th>
<th>MSU + PDN</th>
<th>MSU + TMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h</td>
<td>1462</td>
<td>1325</td>
<td>1200</td>
<td>1375</td>
</tr>
<tr>
<td>24 h</td>
<td>1200</td>
<td>610</td>
<td>1700</td>
<td>1655</td>
</tr>
<tr>
<td>48 h</td>
<td>1225</td>
<td>1012</td>
<td>1200</td>
<td>1100</td>
</tr>
<tr>
<td>7 days</td>
<td>666</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
</tbody>
</table>

BTN, betamethasone; PDN, prednisolone; TMN, triamcinolone. The only significant difference was at 7 days, \(P < 0.05\).

Fig. 2. Presence of corticosteroid crystals in air pouch fluids at 6, 24, 48 h and 7 days after injection of normal saline solution (NSS), betamethasone (BTN) and triamcinolone (TMN). Betamethasone crystals disappeared after 6 h.

Fig. 3. Percentage of cells with phagocytosed MSU crystals at 6, 24, 48 h and 7 days in all air pouches injected with MSU alone, MSU and prednisolone, MSU and triamcinolone, and MSU and betamethasone.
was fibrin but no increased collagen or proteoglycans. Both of the latter were seen in the remaining matrix with no trends for differences among treatments.

**Discussion**

The concept of a reversible inflammatory tissue response to microcrystals is well known. Crystals inducing inflammation are characterized by poor solubility in water, a size of approximately 0.5 to 20 μm and phagocytosis by polymorphonuclear and mononuclear cells during the inflammatory response [2]. Commercially prepared microcrystalline corticosteroid esters are sparingly soluble and of the required size range to cause transient inflammation, as described before but not seen to any greater degree than with normal saline solution. Any mild inflammation is generally not clinically detected although overt post-injection flares can occur [3]. Moreover, intraleucocytic steroid crystals can be observed by polarized light inspection of synovial fluid aspirated from joints which are involved in a post-injection flare.

**TABLE 2. Histopathological findings in the air pouch membranes injected with MSU crystals; MSU crystals and betamethasone preparation (BTN); MSU crystals and prednisolone preparation (PDN) and MSU crystals and triamcinolone preparation (TMN)**

<table>
<thead>
<tr>
<th></th>
<th>Number of lining cells</th>
<th>Number of vessels (0–4)</th>
<th>Inflammation score (0–4)</th>
<th>Number of tophi per pouch</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSU/6 h</td>
<td>3.5 ± 0.57</td>
<td>2.25 ± 0.95</td>
<td>1.25 ± 0.95</td>
<td></td>
</tr>
<tr>
<td>MSU/24 h</td>
<td>3 ± 1.41</td>
<td>2.5 ± 0.57</td>
<td>2.75 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>MSU/48 h</td>
<td>3.6 ± 0.57</td>
<td>2.3 ± 1.15</td>
<td>2 ± 1</td>
<td></td>
</tr>
<tr>
<td>MSU/7 days</td>
<td>3.2 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>3 ± 0.8</td>
<td>1.25</td>
</tr>
<tr>
<td>MSU + BTN/6 h</td>
<td>3.75 ± 0.5</td>
<td>2.5 ± 1</td>
<td>2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>MSU + BTN/24 h</td>
<td>2 ± 0</td>
<td>2 ± 0</td>
<td>0.57 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>MSU + BTN/48 h</td>
<td>1.75 ± 0.5</td>
<td>2 ± 0</td>
<td>1.75 ± 0.5</td>
<td>3</td>
</tr>
<tr>
<td>MSU + BTN/7 days</td>
<td>2.5 ± 0.5</td>
<td>1.5 ± 1</td>
<td>2.5 ± 1.29</td>
<td></td>
</tr>
<tr>
<td>MSU + PDN/6 h</td>
<td>3.75 ± 0.5</td>
<td>1.5 ± 1</td>
<td>1.5 ± 1.29</td>
<td></td>
</tr>
<tr>
<td>MSU + PDN/24 h</td>
<td>3 ± 0.8</td>
<td>1.25 ± 0.95</td>
<td>1.25 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>MSU + PDN/48 h</td>
<td>3 ± 0.8</td>
<td>1.4 ± 1</td>
<td>1.25 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>MSU + PDN/7 days</td>
<td>2 ± 0.5</td>
<td>0.5 ± 0.5</td>
<td>0.5 ± 0.47</td>
<td>3.5</td>
</tr>
<tr>
<td>MSU + TMN/6 h</td>
<td>3.5 ± 0.57</td>
<td>2.75 ± 1.5</td>
<td>3.25 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>MSU + TMN/24 h</td>
<td>2.75 ± 0.95</td>
<td>0.75 ± 0</td>
<td>1.25 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>MSU + TMN/48 h</td>
<td>2.5 ± 0.57</td>
<td>0.75 ± 0.5</td>
<td>1 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>MSU + TMN/7 days</td>
<td>2 ± 0.81</td>
<td>0.25 ± 0.5</td>
<td>0.5 ± 0.47</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**FIG. 4. Photomicrographs of air pouch membranes at 7 days post-injection, haematoxylin and eosin stain (magnification ×400).**

(A) Air pouch injected with MSU crystals alone. There are prominent numbers of lining cells (LC), infiltrating neutrophil and mononuclear cells and vessels (V). (B) Air pouch injected with MSU crystals and betamethasone. The number and activity of lining (LC) and inflammatory cells is clearly less and there are almost no vessels. (C) Air pouch injected with MSU and triamcinolone. There is only one layer of interrupted lining cells, almost no cellular infiltration and no patent vessels.
There is little information on the effects of intra-articular corticosteroids on the synovial membrane. In the 1950s Selye [18], using the ‘granuloma pouch’ technique, studied the effects of hydrocortisone administration during different inflammatory conditions. He found that the hydrocortisone-treated animal’s pouch was thin, consisting of rather atrophic connective tissue and small fibroblasts with narrow capillaries. He concluded that, depending on the nature of the irritant agent causing inflammation, antiphlogistic corticosteroid may be useful by suppressing inflammation or may be harmful by induction of extensive local necrosis.

The different morphological characteristics of the crystals of the three corticosteroid preparations that we used have been described before [19]. Prednisolone tebutate crystals are small, rather pleomorphic with a branched and irregular configuration and positive birefringence. Triamcinolone hexacetonide crystals are 15 to 60 μm, rod and rhomboid shaped with a negative birefringence. Both prednisolone and triamcinolone crystals have a strong tendency to agglutinate. Betamethasone acetate crystals are 10 to 20 μm, rod shaped with blunted edges, negative birefringence and a weak tendency to agglutinate.

Betamethasone is four to six times more potent on a weight basis than triamcinolone and prednisolone steroids. We used an approximately equivalent strength dose estimated for clinical use by the manufacturers for each preparation. An injection of 6 mg of betamethasone or 20 mg of triamcinolone hexacetonide or prednisolone per pouch had only mild anti-inflammatory effects on MSU crystal-induced inflammation as measured by the pouch fluid. We do not know if a lower corticosteroid dose into the pouch would still have anti-inflammatory effects without showing the atrophic and necrotic changes that we noted in the pouch membrane.

Betamethasone preparation crystals disappeared from the fluid as soon as 24 h. Early anti-inflammatory effects were present on the MSU-inflamed membrane, but were only very slightly progressive at 48 h and 7 days. On the other hand, triamcinolone and prednisolone preparations behaved similarly. Their crystals remained longer in the fluid, most dramatically suppressed the tissue inflammation at 7 days and both produced a striking atrophy and necrosis of the membrane. The effects of the corticosteroids on the pouch membrane were the same whether the pouches were previously injected with normal saline solution or MSU crystals. The clinical and longer term effects of synovial-like tissue damage seen in these studies remain to be evaluated. Whether the antiphlogistic characteristic of betamethasone is adequate for a given inflammation to provide a long enough lasting relief of inflammation and pain for the patient has to be analysed with a well-designed clinical study.

Fig. 5. Air pouch membrane at 7 days after injection with MSU crystals observed under polarized light. A linear surface deposit of negatively birefringent needles (arrow) is present (magnification ×400).

Fig. 6. A suspected tophus in the air pouch membrane 7 days after MSU injection represented by a cluster of clear clefts (arrows) surrounded by giant cells and collagen (C) (pale yellow). Crystals are proposed to have been dissolved by formalin fixation. Pentachrome stain (magnification ×400). Monosodium urate injection followed by triamcinolone hexacetonide. No such tophus-like areas were seen in any pouch without MSU injection.
Our model of depot steroid treatment of MSU-induced inflammation may provide some clues to the possible clinical implications of depot steroid treatment in crystal-associated disease. Possibly the most important finding was the presence of increased MSU aggregates on the synovial surface and tophus-like structures after using the corticosteroid preparations. The formation of tophi might be related to alteration or loss of matrix molecules favouring solubilization of crystals, and/or the loss or necrosis of the lining membrane phagocytic cells allowing the crystals to accumulate in neighbouring tissue, or to the fact that phagocytosis was delayed in the pouch fluid. It is known that the connective tissue sites where urate crystals deposit are normally rich in proteoglycans. Some of these polysaccharides can enhance the solubility of urate in vitro. It appears that the integrity of the proteoglycan molecule is essential. Chondroitin sulphate or proteoglycans digested by trypsin do not augment urate solubility. Katz and Schubert [20] suggested that when proteoglycan is enzymatically destroyed as a result of normal and accelerated connective tissue turnover, its binding capacity for urate is reduced. With the pentachrome staining we observed that the tophi seen after the administration of depot corticosteroids were surrounded by fibrin but no stainable proteoglycans.

Sedwick et al. [21] worked with a rat air pouch model to study the effects of local administration of hydrocortisone on cartilage degradation. Their results also suggested that steroid injections enhanced proteoglycan loss only if insoluble preparations were injected and then led to cartilage damage. Our findings of corticosteroid-associated increases in suspected local tophi are interesting in light of the clinical observation that gout patients who take corticosteroids have more tophi. Vazquez-Mellado et al. [22] did a case-control study to determine which factors associated with the presence of intradermal tophi in a group of patients with gout and cutaneous tophi. All patients had similar age, duration of gout and associated diseases such as hypertension, diabetes mellitus, hyperlipidaemia, chronic renal failure, urate lithiasis and obesity. Only corticosteroid use was strongly associated with the presence of the dermal tophi.

Corticosteroid preparations continue to be one of the easiest and relatively safe ways of giving relief to the patient for non-septic local joint inflammation. Knowing how they act and the properties of the different preparations is important. The use of intra-articular corticosteroids in the clinic has to be more wisely based on objective findings not ruled only by availability and habit. Acute reversible problems like bursitis might well respond best and most safely to an agent like betamethasone. However, symptomatic relief in chronic rheumatoid arthritis may not be adequate with betamethasone. Some published clinical experience suggests better symptomatic effect with triamcinolone hexacetonide preparations, but results have been inconsistent [23, 24]. Results may vary in part owing to differences in injection techniques and post-injection rest of the joint [23, 24].

This study reminds us that each corticosteroid preparation has different crystals with different very short term antiphlogistic characteristics and different longer term effects; their use is not always free of side-effects. The necrotizing process that we have shown in these injected pouches merits further study. Might it actually be beneficial in rheumatoid arthritis, by diminishing numbers of protease- or cytokine-producing cells although possibly favouring local crystal persistence and tophi in gout? Does something similar to the pouch wall atrophy occur in cartilage that should be a concern? Will lower dosage patterns change these results? As for other therapies, more controlled clinical and basic studies need to be done on corticosteroid preparations.

References


