RELEASE OF LUBRICATING SYNOVIAL SURFACTANT BY INTRA-ARTICULAR STEROID

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SUMMARY

This study was undertaken to determine whether glucocorticosteroids promote the secretion of lubricating surfactant, i.e. surface-active phospholipid (SAPL), into the joint. A standard clinical dose (100 mg) of methylprednisolone acetate (MPA) in 2.5 ml of saline was injected into the load-bearing right radiocarpal joint of five horses and 2.5 ml of saline injected into each of the contralateral joints used as controls. Synovial fluid (SF) was aspirated from all 10 joints before injection and at intervals of 16 and 32 h after injection, and then analysed by standard methods. All test joints showed an elevated level of SAPL, the increases averaging 112% after 16 h and 76% after 32 h, which were highly significant relative to the control joints. A large increase at 16 h was also found in proteolipid as a possible further marker of surfactant release. Significant quantities of proteolipid were also found in human SF. Since intra-articular steroids can dramatically improve joint mobility in both humans and horses, it is proposed that part of the benefit may be derived from improved lubrication arising from the remarkable ability of SAPL to lubricate under high load. Other possible benefits of elevating surfactant levels in the joints include control of cartilage hydration, promotion of macrophage activity and the ability to scavenge oxygen free radicals.

KEY WORDS: Joint lubrication, Osteoarthritis, Steroids, Intra-articular, Synovial surfactant.

Since hydrocortisone was introduced in 1951 for local intra-articular administration, glucocorticosteroids have been widely prescribed for the treatment of rheumatoid arthritis and other inflammatory arthropathies [1]. Although controversial at first [2], these other arthropathies now include osteoarthritis, for which intra-synovial corticosteroids produce a beneficial response consisting of a significant reduction of pain and tenderness of the joint [3] which can lead to a dramatic short-term improvement in the mobility of the patient.

Much the same experience is reported in the veterinary literature where intra-articular injection of corticosteroids into the afflicted joint is a common therapy for degenerative joint disease in the horse [4]. Despite the controversial side-effects, corticosteroids, particularly methylprednisolone acetate (MPA), remain among the most effective drugs for the treatment of arthritic conditions in the equine joint [5].

The mechanism(s) of action of glucocorticosteroids remains open to debate in both humans [1] and horses [4], but would appear to extend beyond their potent anti-inflammatory properties [6], implying that they act at a fundamental level of physiological function. One of their basic properties in the lung [7] is their ability to promote the secretion of surfactant, which is predominantly surface-active phospholipid (SAPL), as employed clinically in preventing the respiratory distress syndrome (RDS) in the newborn [8].

There are now many studies [9–12] supporting the concept that SAPL is the vital load-bearing lubricant in the joint, where its adsorption to cartilage—necessary for boundary lubrication—can explain the extreme hydrophobicity of the normal articular surface [10, 13]. Moreover, SAPL has been shown to be deficient on these surfaces in human hips and knees afflicted with osteoarthritis [14].

The foregoing arguments imply that intra-articular steroids might also promote the secretion of surfactant in the synovial space, in which case it might lead to the administration of the mediator, i.e. SAPL, rather than steroids themselves, in attempting to gain the obvious clinical benefits without the side-effects. As a first step in this direction, this study is designed to determine whether a standard clinical dose of MPA, which is so effective in the equine joint, promotes the secretion of SAPL (synovial surfactant).

MATERIALS AND METHODS

Horses

Five healthy standardbred horses (mean body weight of 500 kg) with no overt symptoms of joint disease were selected for the trials. A standard dose for an equine joint [15] of 100 mg Depomedrol (MPA) in 2.5 ml of saline was administered by careful intra-articular injection into the right radiocarpal joint using aseptic technique. The same dose (2.5 ml) of saline was injected into the left radiocarpal joint as a control.

Methods

Samples of synovial fluid (SF) (2 ml) obtained by arthrocentesis were centrifuged to remove cellular debris and placed in vials containing 10 ml of chloroform, which were then sealed and shaken to extract lipids into the chloroform phase and suppress any biochemical degradation which could otherwise occur. Analysis for phospholipid followed the standard method of Rouser et al. [16], described in detail.
elsewhere. Essentially, the inorganic phosphate is left behind in the aqueous phase during the chloroform extraction. All elemental phosphorus in the chloroform extract is then oxidized to phosphate using perchloric acid and estimated colorimetrically in a spectrophotometer set at 660 nm, using ammonium molybdate as the indicator. Calibration was effected using dipalmitoyl phosphatidylcholine (DPPC; Sigma Chemicals #P6267). DPPC was used as the standard because phosphatidylcholine (PC) is the major phospholipid (PL) component of SF, while synovial PC is predominantly saturated [17]. Each test was repeated and the mean value recorded.

Proteolipid was determined according to the method of Bohlen et al. [18] as described in detail elsewhere. Essentially, the test uses the residue remaining after evaporation of chloroform in which the proteolipid is co-extracted with PL. A known weight of this residue is dispersed in sodium phosphate buffer at pH 8 with fluorescamine (Sigma # F-9015) in acetone as the indicator. This dispersion was analysed on a fluorescence spectrophotometer (Hitachi Perkin-Elmer Model 203) at an emission wavelength of 475 nm with excitation at 365 nm. Calibration was performed using bovine serum albumin (BSA) standards.

RESULTS

Phospholipid
Phospholipid was found in significant quantities in all samples of SF in both the test (right) joint and control (left) joint for all five horses. It was appreciably higher in the test joint administered MPA in all horses 16 h after injection; the results are summarized in Table I. It can be seen that the PL content of the test joints increased by 112% in comparison with 14% in the control joints. The difference is also significant 32 h after injection.

Proteolipid
In one horse, there was insufficient sample remaining after PL analysis for proteolipid determination. In the remaining four horses, proteolipid was present in all samples, as summarized in Table II. Proteolipid showed a 3.4-fold rise in the test joints after 16 h in comparison with a 23% fall in the control joints.

Statistical analysis
The paired t-test was used to compare PL in test and control joints. The difference was highly significant (P < 0.005) after 16 h and significant (P < 0.01) after 32 h. The difference in proteolipid between test and control joints was highly significant (P < 0.005) after 16 h, but not significant after 32 h.

DISCUSSION
The results leave no doubt that a standard clinical dose of MPA administered intra-articularly into the equine joint increases the quantity of SAPL in SF. This is clearly demonstrated by the 112% elevation of PL from pre-test levels (Table I), which are similar to those recorded in human SF [17]. It is also supported by the 239% rise in proteolipid. The apoproteins are present in synovial surfactant in essentially the same proportions as found in the lung, these including surfactant protein ‘A’ [19], and both SP-B and SP-C, which are proteolipids [11]. The role of proteolipid in the alveolus is essentially one of facilitating adsorption of the SAPL [20] and might also apply to the adsorption of SAPL in the joint as the oligolamellar lining, providing lubrication and, possibly, other desirable properties outlined above. Proteolipid is also essential in the deposition of SAPL as myelin [21] whose lamellated structure (and spacing) closely resembles the outermost layers described on the articular surface [11] and elsewhere in the joint [10, 11, 22]. The fact that proteolipid has also been identified in appreciable quantities in human SF in the ancillary experiment supports its relevance to the human joint.

The increase of 112% in synovial surfactant following steroid injection is particularly interesting because SAPL is such an effective load-bearing lubricant [9], which has been identified adsorbed to the articular surface [9, 10]. Most of the improvement in mobility of a patient administered steroids can be attributed to their anti-inflammatory action in reducing pain, but enhanced lubrication could contribute. SAPL is also an excellent release (anti-stick) agent [23] which could help in initiating motion in a joint and preventing articular gelling, just as crude surfactant of biological origin (lecithin) is used for that purpose in many industrial processes.

Another action of promoting surfactant secretion is that the enhanced lubricating lining, which imparts such hydrophobicity to the articular surface [10, 11], could become a better barrier to water, reducing the hydration characteristic of degenerating cartilage [24]. In fact, DPPC has been shown to be an effective

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Surfactant (PL) levels in SF following injection of a clinical dose of MPA</th>
</tr>
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<tbody>
<tr>
<td>Test joint (right)</td>
<td>Control joint (left)</td>
</tr>
<tr>
<td>Time (h)</td>
<td>Quantity* of PL</td>
</tr>
<tr>
<td>0</td>
<td>1.48 ± 0.13</td>
</tr>
<tr>
<td>16</td>
<td>3.25 ± 0.29</td>
</tr>
<tr>
<td>32</td>
<td>2.61 ± 0.10</td>
</tr>
</tbody>
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*Quantity in mg equiv. DPPC/ml SF. Mean values quoted ± s.e.m.
TABLE II

Proteolipid levels in SF following injection of a clinical dose of MPA

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Test joint (right) Quantity* of PL</th>
<th>Ratio to initial level</th>
<th>Control joint (left) Quantity* of PL</th>
<th>Ratio to initial level</th>
<th>Ratio (R/L)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.44 ± 0.95</td>
<td>1</td>
<td>3.47 ± 0.85</td>
<td>1</td>
<td>1.57</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>18.43 ± 5.43</td>
<td>3.39 ± 0.62</td>
<td>2.68 ± 0.76</td>
<td>0.77 ± 0.19</td>
<td>6.88</td>
<td>8</td>
</tr>
<tr>
<td>32</td>
<td>5.02 ± 1.03</td>
<td>0.92 ± 0.27</td>
<td>5.07 ± 1.02</td>
<td>1.46 ± 0.42</td>
<td>0.99</td>
<td>8</td>
</tr>
</tbody>
</table>

*Quantity in µg equiv. BSA/ml SF. Mean values quoted ± s.e.m.

'de-watering agent' [25], as surfactants are termed in the physical sciences when they can even remove water trapped in a matrix—with obvious implications to cartilage. Steroids even render all lung cells more hydrophobic [26] and this could be attributed to increased surfactant whose secretion they are known to promote [7, 8].

Other physiological advantages of surfactant secretion include a possible role in chondroprotection and enhancement of macrophage activity if findings in the lung [27] can be transposed to the joint. Many studies (e.g. [1, 28]) have implicated oxygen free radicals in the death of chondrocytes which have been implicated in the aetiology of osteoarthritis, so it is most interesting when a study of exogenous lung surfactant shows SAPL to be a scavernger for these free radicals [29].

In recent studies in this laboratory, it has been shown that the outermost surfactant lining of the articular surface is appreciably deficient in human hips and knees replaced by orthopaedic surgeons in patients with osteoarthritis [14]. As another indication of this deficiency, the same surfaces are also significantly less hydrophobic [14]. Hence, it is reasonable to speculate that one explanation for the dramatic improvement often observed in humans [3] and horses [4] for 1–3 months after the administration of corticosteroids is the replenishment of SAPL, i.e. lubricating surfactant, on the articular surfaces. In preliminary human trials administering 400 mg SAPL as one intra-articular dose in a carrier, similar improvements were recorded over a comparable time span [30]. Thus, in view of the undesirable side-effects of steroids, direct administration of exogenous SAPL would seem preferable if the underlying theory can be proven.

ACKNOWLEDGEMENT

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REFERENCES

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