Intra-articular clodronate for the treatment of knee osteoarthritis: dose ranging study vs hyaluronic acid

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Objective. Bisphosphonates may have a chondroprotective effect in patients with knee OA (KOA), but the results of clinical trials with oral bisphosphonates have been contradictory. In this Phase 2 randomized, partially blind clinical trial, we tested the efficacy of IA clodronate vs HA in patients with primary KOA.

Methods. One hundred and fifty men or women aged 50–75 years suffering from KOA were randomized to one of five IA therapies: (i) clodronate 0.5 mg one IA injection/week for 4 weeks; (ii) clodronate 1 mg one IA injection/week for 4 weeks; (iii) clodronate 2 mg one IA injection/week for 4 weeks; (iv) clodronate 1 mg two IA injections/week for 2 weeks (clodronate 1 + 1 mg); and (v) HA 20 mg one IA injection/week for 4 weeks.

Results. Visual analogue scores (VASs) for different types of pain and the Lequesne index significantly improved in all treatment groups after the first injection and continued to improve even 2–4 weeks after the last injection without significant difference among the groups. A significant (P = 0.03) linear trend for a dose–response (0.5–2 mg clodronate) relationship was found for active movement VAS pain. Both joint extension and mobility scores improved significantly at all time points in all treatment groups without statistical differences among them.

Conclusions. This study indicates that IA clodronate provides symptomatic and functional improvements at least as good as those obtained with HA.

Trial Registration. Osservatorio Nazionale sulla Sperimentazione Clinica dei Medicinali - Agenzia Italiana del Farmaco. Comitato Etico Azienda Ospedaliera Universitaria Senese number CLIO 22/02 http://oss-sper-clin.agenziafarmaco.it

Key words: Osteoarthritis, intra-articular treatment, Knee, Clodronate, Hyaluronic acid, Bisphosphonate, Cartilage, Bone, Pain, Mobility.

Introduction

OA is a major cause of musculoskeletal pain, disability and handicap, and an important community health care burden. Knee OA (KOA) is particularly common, with radiographic OA of the tibiofemoral compartment occurring in 5–15% of people aged 35–74 years [1].

The ultimate objective of OA management is to limit the progression of structural changes in order to delay or avoid arthroplasty. However, the condition is often associated with pain and functional disability, for which analgesic agents and NSAIDs, including cyclo-oxygenase 2 inhibitors (COXIBs), are the most widely prescribed medications. Continuous long-term treatment is often required, but this may be contraindicated for the poor safety profile of both NSAIDs and COXIBs [2].

IA HA is generally considered as a valuable addition to the therapeutic armamentarium for the treatment of OA. The mechanism of action of HA is rather complex, although the most relevant benefits seem to come from viscosupplementation, restoring the normal viscoelastic properties of the pathologically altered SF [3].

Significant reductions in pain against placebo exceeding its half-life have been reported [3–7] associated with functional improvements on Lequesne’s index in one study [8] and with the delaying of disease progression >1 year [9].

Bisphosphonates have been demonstrated to have chondroprotective effects, to reduce the incidence and progression of osteophytes in animal models, and to modify osteoblast function in vitro [10–14].

Agents that suppress bone turnover, including bisphosphonates, have been associated with fewer subchondral bony lesions, as visualized by MRI, in patients with OA [15]. One uncontrolled study of etidronate demonstrated an improvement in subjective pain in individuals with spondylosis and KOA [16], and in a recent secondary analysis of data from a randomized controlled trial [fracture intervention trial (FIT)], alendronate was associated with less spinal osteophytes and disc-space narrowing progression than placebo [17].

In a pilot study in patients with KOA, daily treatment with 15 mg risedronate was associated with a trend of pain reduction, improvements in patient global assessment of disease scores and progress in delaying radiographic progression [18]. These results were not confirmed in a properly powered study, even though a significant reduction in the level of a marker of cartilage degradation was observed [19].

Although a direct chondroprotective effect of bisphosphonates is postulated, it is widely hypothesized that the potential positive effect of bisphosphonate therapy in OA may be related to their action on subchondral bone. The subchondral bone in OA is characterized by a decreased number and thinning of tibial cancellous trabeculae, localized osteoporosis [20–23], increased bone turnover and histological micro-fractures that are seen by MRI in many patients with OA [24–26].

A major limitation in the studies assessing the efficacy of bisphosphonates in OA is the need of high doses, which are largely taken up by bone tissue and therefore, poorly concentrated on subchondral bone. An alternative approach might be represented by the administration of IA clodronate, a bisphosphonate with anti-inflammatory effects in experimental arthritis [27]. Clodronate has been also encapsulated in liposomes in order to increase its cellular uptake [28]. The clodronate-containing liposomes when administered IA lead to macrophage depletion and decreased expression of adhesion molecules in the synovial lining.
in patients with RA [29]. Unfortunately, the development of this pharmaceutical formulation was discontinued. Thus, the only efficient way to test the efficacy of clodronate in OA remains the direct IA administration of relatively high doses of clodronate.

In this study, we compare the symptomatic and functional changes occurring in patients with severe KOA of IA HA or clodronate at three different doses in a partially blind, randomized clinical trial.

Methods

This is a multicentre, randomized, partially double blind Phase 2 study, aimed at identifying the most appropriate dose of IA clodronate, for relieving pain and improving function of an osteoarthritic knee over 5 weeks. Men and non-pregnant women aged 50–75 years with KOA, according to the ACR criteria [30] radiographically confirmed with a Kellgren/Lawrence grades of 2 or 3, symptomatic for at least 3 months, were eligible. Additional inclusion criteria were starting spontaneous pain ≥40 on a visual analogue score (VAS) of 0–100 and functional disability ≥3 on a scale of 1–4. Patients were excluded if they had OA secondary to other joint diseases, if they were receiving anti-coagulant therapy or if they received corticosteroid therapy or chondroprotective agents during the 30 days prior to the study or viscosupplementation treatment within 3 months prior to the study. Patients were also excluded if they had undergone previous major surgery (joint replacement or re-alignment) in one of the knees, or arthroscopy within 6 months prior to the study, or with a history of allergy or intolerance to experimental preparations.

The trial was conducted in accordance with the ethics principles of the Declaration of Helsinki and was approved by the local research ethics committees. Written informed consent was obtained from each patient.

Interventions and randomizations

Patients were randomly allocated to receive one of the following treatments:

(i) clodronate 0.5 mg one IA injection/week for 4 weeks;
(ii) clodronate 1 mg one IA injection/week for 4 weeks;
(iii) clodronate 2 mg one IA injection/week for 4 weeks;
(iv) clodronate 1 mg two IA injections/week for 2 weeks (clodronate 1 + 1 mg); and
(v) HA 20 mg (Fidia, Padua, Italy) one IA injection/week for 4 weeks.

All clodronate formulations were dissolved in 1 ml saline solution. The vials were prepared at the Abiogen Pharma (S.p.A, Pisa, Italy) laboratories, where the stability and the final concentrations were periodically tested. Patients were blind to the treatment type and treatments (i–iii) were also blind to investigators. If both knees met the inclusion criteria, only the more symptomatic was treated. Six Italian centres participated in the trial. Patients were randomized in blocks of five by a computer program.

Study procedure and outcome measures

The recruited patients were asked to discontinue any NSAIDs at least 4 days before the first injection. Physical therapies and rehabilitation procedure were not permitted over the entire follow-up period. On the day of treatment and at weekly intervals for 5 weeks, the following data were collected:

(i) VAS (0–100 mm) to assess all types of pain: spontaneous, on passive and active movement (walking for >10 m), and at digital pressing of the two lateral articular spaces (tenderness). The area under the curve (AUC) was calculated for each VAS and for the average of the four VASs. The theoretical AUC obtained assuming no changes during treatment was used to calculate the percent changes in AUC for each treatment arm.
(ii) Extension score as assessed by a 1 (complete) to 4 (totally compromised) scale.
(iii) Joint mobility as assessed by the investigator on an arbitrary scale from 1 (complete) to 4 (totally compromised).
(iv) Lequesne algofunctional index [31].
(v) Paracetamol consumption (counting the returned pills that were provided weekly by the investigators).

Sample size and statistical analysis

A sample size of 25 patients per group was calculated to detect a 15-mm difference in VAS for spontaneous pain (>80% power, with α = 0.05), between the lowest to the highest clodronate dose, assuming a common s.d. of 18 mm.

Weekly paracetamol consumption was categorized by the proportion of patients who did not require its use. The continuous variables [VAS and Lequesne index changes, percent changes in the AUC (AUC, elapsed time × changes in score value) for the four VASs] were compared by analysis of covariance (ANCOVA) and then t-test after adjustment for paracetamol consumption (number of weekly tablets), using Bonferroni correction. Joint mobility scores were categorized as improved, unchanged or worsened, in comparison with the baseline. Analyses were conducted using an intent-to-treat (ITT) approach.

The dose–response analysis relative to the AUC of the four VAS parameters was per protocol assessed for three clodronate groups, 0.5 mg/week, 1 mg/week and 2 mg/week. Whenever the linear trend analysis for a dose–response relationship was found statistically significant, this was again tested for the ‘step-down’ (exclusion of the less effective) and ‘fixed-sequence’ (from lowest to highest dose) of the Dunnett test [32].

Results

Study flow and patient characteristics

The 150 eligible patients were recruited over 18 months until September 2005. One hundred and forty-five patients received at least one IA injection and represent the study population included in the ITT analysis. Eight patients were lost to follow-up: one (clodronate 1 mg) after the first injection, three (clodronate 0.5, 1 and 2 mg) after the third injection and four (clodronate 0.5, 2, 1 mg and HA) did not attend the last evaluation (Fig. 1).

All reported results are by ITT analysis, whereby the missing values were all substituted throughout with the last available value. The results obtained with the per protocol analysis were virtually superimposable to those seen in the ITT analysis (results not shown) and not influenced by the Kellgren/Lawrence score. The five treatment groups had similar clinical characteristics at baseline (Table 1).

Treatment outcomes

The proportion of patients who did not require the use of paracetamol and the number of tablets taken during the observational period did not significantly differ [analysis of variance (ANOVA)] among treatment groups (Table 2), even though the individual values were occasionally considerably different.

A highly significant (P < 0.001) improvement of the four VAS scores and the Lequesne index was observed in all treatment groups after the first injection already at the first week of control. Pain continued to improve thereafter, 2 (clodronate 0.5, 1 and 2 mg or HA) and 4 (clodronate 1 + 1 mg) weeks after the last injection (Fig. 2).

No significant difference in any of the VAS scores was detected among the five treatment groups at any time point. The percent changes in AUC for each VAS and for the average of the four VASs are shown in Fig. 3. At the ANOVA test, none of the...
percent changes in AUC differed significantly among groups, after Bonferroni adjustment for multiple comparisons. When data were adjusted for paracetamol use, the spontaneous pain was significantly different among groups (ANCOVA, $P = 0.05$) with a highly significant difference ($P < 0.01$) between clodronate 1+1 mg and HA group.

The extension and mobility scores improved significantly at all time points in all treatment groups without statistical differences (chi square) among groups, even though a trend for better results was observed for clodronate 1+1 mg vs the other treatments ($P$-values ranging from 0.06 to 0.34; Fig. 4).

A significant ($P = 0.03$) linear trend for a dose–response (0.5–2 mg clodronate) relationship was found for active movement VAS pain.

The IA injection was considered ‘painful’ by only a few patients. However, local burning after the injection, lasting 1–2 min, was reported by 21% of the patients given 2 mg clodronate, and this proportion was statistically (chi square) higher than that reported in the patients given clodronate 0.5 mg and HA injections (Table 3).

### Discussion

We found that IA injection of either HA or different doses of clodronate in symptomatic KOA is associated with significant and clinically meaningful progressive improvement in pain and function extending for at least 2 weeks after the last injection. Non-significant differences were detected among the four clodronate treatment regimens, even though a significant dose–response relationship for the three doses of clodronate was found for active movement pain and a trend for extension and mobility scores and paracetamol consumption.

To what extent these results might be related to the well-recognized placebo response after aspiration of the knee [33, 34] remains unclear. Such a placebo effect is still advocated for HA [3], since in randomized placebo-controlled clinical trials ensuring adequate blinding during the study is considered impossible due to the high viscosity of the hyaluronan solutions, which makes this drug easily recognizable. This is the reason for which HA treatment was not blind to the investigator in this study. Recent reviews, guidelines and meta-analysis consistently attribute an appreciable therapeutic effect to HA [5, 6, 35, 36]. These position papers made and are still making a placebo arm for IA injections.

### Table 1. Baseline characteristics of the study population

<table>
<thead>
<tr>
<th>Assessment</th>
<th>HA</th>
<th>Clodronate 0.5 mg</th>
<th>Clodronate 1 mg</th>
<th>Clodronate 2 mg</th>
<th>Clodronate 1+1 mg</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>28</td>
<td>30</td>
<td>30</td>
<td>29</td>
<td>28</td>
<td>145</td>
</tr>
<tr>
<td>Age, mean ± s.d., years</td>
<td>65.2 ± 6.9</td>
<td>65.7 ± 6.4</td>
<td>66.2 ± 6.6</td>
<td>64.7 ± 7.4</td>
<td>63.6 ± 6.7</td>
<td>65.1 ± 8.8</td>
</tr>
<tr>
<td>Female/male, n</td>
<td>26/2</td>
<td>25/5</td>
<td>24/6</td>
<td>24/5</td>
<td>25/3</td>
<td>25/3</td>
</tr>
<tr>
<td>Kellgren/Lawrence score 2/3, n</td>
<td>10/18</td>
<td>10/20</td>
<td>12/18</td>
<td>11/18</td>
<td>11/17</td>
<td>54/91</td>
</tr>
<tr>
<td>Right/left knee</td>
<td>11/17</td>
<td>18/12</td>
<td>13/17</td>
<td>16/13</td>
<td>16/12</td>
<td>74/71</td>
</tr>
<tr>
<td>Lequesne score, mean ± s.d.</td>
<td>13.1 ± 3.4</td>
<td>13.0 ± 3.0</td>
<td>12.2 ± 2.9</td>
<td>12.3 ± 3.4</td>
<td>12.8 ± 3.6</td>
<td>12.7 ± 3.2</td>
</tr>
<tr>
<td>Pain active movement (VAS), mean ± s.d.</td>
<td>71.3 ± 15.5</td>
<td>61.7 ± 15.4</td>
<td>63.1 ± 11.7</td>
<td>67.4 ± 14.9</td>
<td>63.8 ± 15.2</td>
<td>65.4 ± 14.8</td>
</tr>
<tr>
<td>Pain passive movement (VAS), mean ± s.d.</td>
<td>60.1 ± 19.7</td>
<td>58.4 ± 11.8</td>
<td>55.7 ± 13.8</td>
<td>57.7 ± 18.6</td>
<td>56.6 ± 17.8</td>
<td>57.7 ± 16.3</td>
</tr>
<tr>
<td>Tenderness (VAS), mean ± s.d.</td>
<td>61.5 ± 17.3</td>
<td>51.6 ± 14.6</td>
<td>51.7 ± 18.5</td>
<td>53.8 ± 20.7</td>
<td>53.1 ± 16.8</td>
<td>54.3 ± 17.8</td>
</tr>
<tr>
<td>Spontaneous pain (VAS), mean ± s.d.</td>
<td>63.0 ± 14.5</td>
<td>61.4 ± 12.9</td>
<td>58.8 ± 11.5</td>
<td>62.7 ± 13.7</td>
<td>62.2 ± 12.9</td>
<td>61.8 ± 13.0</td>
</tr>
<tr>
<td>Extension score 1/2/3/4, n</td>
<td>3/6/19/0</td>
<td>2/6/22/0</td>
<td>3/9/18/0</td>
<td>5/7/17/0</td>
<td>3/5/20/0</td>
<td>16/33/96/0</td>
</tr>
<tr>
<td>Mobility score 1/2/3/4, n</td>
<td>0/0/28/0</td>
<td>0/0/30/0</td>
<td>0/0/30/0</td>
<td>0/1/28/0</td>
<td>0/0/28/0</td>
<td>0/1/144/0</td>
</tr>
</tbody>
</table>

There were no significant differences among treatment groups (ANOVA or chi-square test).

### Table 2. Number and proportion of patients who did not take paracetamol week-by-week in each treatment group

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Cumulative mean ± s.d. of tablets</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA, n (%)</td>
<td>10 (35.7)</td>
<td>9 (32.1)</td>
<td>10 (35.7)</td>
<td>12 (42.9)</td>
<td>11 (39.3)</td>
<td>16 (57.1)</td>
</tr>
<tr>
<td>Clodronate 0.5 mg, n (%)</td>
<td>14 (46.7)</td>
<td>13 (43.3)</td>
<td>12 (40.0)</td>
<td>14 (46.7)</td>
<td>19 (63.3)</td>
<td>22 (73.3)</td>
</tr>
<tr>
<td>Clodronate 1 mg, n (%)</td>
<td>15 (50.0)</td>
<td>13 (43.3)</td>
<td>19 (63.3)</td>
<td>19 (63.3)</td>
<td>22 (73.3)</td>
<td>22 (73.3)</td>
</tr>
<tr>
<td>Clodronate 2 mg, n (%)</td>
<td>15 (51.7)</td>
<td>17 (58.6)</td>
<td>19 (65.5)</td>
<td>21 (72.4)</td>
<td>19 (65.5)</td>
<td>21 (72.4)</td>
</tr>
<tr>
<td>Clodronate 1+1 mg, n (%)</td>
<td>10 (35.7)</td>
<td>12 (42.9)</td>
<td>11 (39.3)</td>
<td>13 (46.7)</td>
<td>16 (57.1)</td>
<td>21.0 ± 23.7</td>
</tr>
</tbody>
</table>

None of the differences was statistically different by chi-square test.
ethically unacceptable, despite the clinically minimal effect of HA as compared to placebo. The issue of the potential placebo effect is also obviously critical to attribute a therapeutic effect to clodronate. Nonetheless, if we agree that HA is an effective treatment for KOA, then this study provides strong evidence that IA clodronate should be considered as a new agent with an extraordinary potential for the management of KOA. This positive view is also somewhat supported by the trend of better results observed with clodronate 2 mg and 1 + 1 mg vs HA treatment in percent AUC changes for VAS for spontaneous and active movement pain.

A clear dose–response relationship could not be identified, even though in some occasions it appears that a trend for better results should be expected with 2 mg/week.

The lack of a clear dose–response relationship might be due to the shadowing placebo effect, but it is also possible that the lowest dose we adopted is already close to the upper part of the dose–response curve. The decision regarding the dose to be selected for a Phase 3 study, should also take into account the significantly greater proportion of patients complaining of a mild on site sensation of burning with the highest clodronate concentration.

Symptoms and functions with all treatments were still improving at the last time point and this might suggest a therapeutic effect extending over several weeks after the treatment course.

The use of bisphosphonate for the management of OA was hypothesized not only on the basis of a number of experimental findings, but also on some preliminary clinical observations concerning the effect of oral alendronate [17] or risedronate [18].
in spinal and KOA, which are also of interest. However, in a large ad hoc randomized clinical trial, doses of oral risedronate 2- to 3-fold higher than those commonly used for the treatment of post-menopausal osteoporosis failed to prevent the progression of KOA [19]. These conflicting results may be explained by the pharmaco-dynamic properties of bisphosphonates. When given orally, 50–70% of bioavailable bisphosphonates are avidly taken up by the bone tissue and only a tiny amount is bound to knee subchondral bone and even less cartilage [37]. An interesting alternative approach tested in the past was represented by the use of clodronate-containing liposomes, which gives very high concentrations of the drug in the cells, with enhanced anti-inflammatory activity, particularly when given intra-articularly [28, 29]. Unfortunately, the development of this formulation was discontinued, but with the IA doses we used, the amount of clodronate absorbed by knee articular cartilage in our patients is likely to be considerably higher than that possibly achieved with extremely large doses of oral clodronate. When the IA concentration of clodronate is adequately high it was reported that the typical target cells are the activated macrophages, but an action on proliferating glial cells secondary to trauma has been also reported [38], which might explain the symptomatic effect that we observed in this study.

In conclusion, our study demonstrates that IA clodronate provides symptomatic and functional improvements at least as good as those obtained with HA. The precise dose regimen and the persistence of the effect remain to be established. The uncertainties still persisting on the real therapeutic value of HA also leave the interpretation of the results reported here with IA clodronate open. This problem might find a solution from a more conservative position by both scientific and regulatory authorities, whereby placebo-controlled clinical trials for IA treatments should be considered still ethically acceptable.

| Table 3. Number of patients who complained for any type of pain from the injection and burning after |
|---------------------------------|-------|-------|-------|-------|-------|
| Side effect                      | HA    | Clodronate 0.5 mg | Clodronate 1.0 mg | Clodronate 2.0 mg | Clodronate 1–1 mg |
| Total number                     | 28    | 30    | 30    | 29    | 28    |
| Injection site pain, n           | 1     | 2     | 2     | 1     | 1     |
| Injection site burning, n        | 0     | 1     | 4     | 6     | 5     |

The burning was statistically more frequent in the clodronate 2 mg group as compared with the other groups given < 2 mg clodronate/week (chi-square test, P < 0.05).

it with representatives of the sponsors, Abiogen S.p.A. prior to finalization of the protocol. The manuscript was written by the authors and edited by a medical writer selected by the study sponsors. The sponsor and each individual author agreed with the contents of the manuscript and gave their agreement to submit the manuscript for publication.

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