Purpose: TissueGene-C (TG-C) is a mixture of normal human chondrocytes (hChonJb) and genetically modified chondrocytes to express TGF-β1 (hChonJb#7), and currently undergoing phase 2 clinical trials for osteoarthritis. Phase 2a clinical trials have shown that TG-C treatment significantly reduced pain when administered to the osteoarthritic patients. In this study, we investigated the effect on pain reduction by the chondrocytes expressing TGF-β1 (hChonJb#7) which is a component of TG-C.

Methods: Monosodium iodoacetate (MIA)-induced osteoarthritis rat model was used in the study. Three milligram of MIA was intra-articularly injected into the left knee of SD rat (280-300g) to induce degradation of cartilage (Ponemis et al, Pain; 2005: 339). The rats showing pain-related behaviors measured by von Frey filament tests were selected for the evaluation of hChonJb#7. At two weeks post MIA administration, various doses of hChonJb#7 were injected into the same knee. The effect on the mechanical allodynia by hChonJb#7 treatment was evaluated by von Frey filament test. After completing the filament tests, the knees were harvested for the histological analysis in the cartilage.

Results: The improvement of pain related behavior was dose-dependently observed earliest from 1 week post hChonJb#7 injections. The minimum effective dose was 3 x 10^4 cells of hChonJb#7. The effect was maintained upto 4 week post hChonJb#7 injections. The histological analysis showed that the integrity of cartilage was severely affected by MIA treatment but hChonJb#7 treatment protected the cartilage from MIA-induced damage when compared with the untreated group.

Conclusions: The current study indicated that the chondrocytes expressing TGF-β1 (hChonJb#7) reduced pain in a rat model which might explain pain reduction in osteoarthritis patients demonstrated during the clinical trials.

522

A SELECTIVE CCR2 ANTAGONIST SHOWED MONOCYTES MIGRATION-INDEPENDENT ANALGESIA IN RAT MODELS OF INFLAMMATORY PAIN

L. Corradini1, S. Hbbie1, S. Hobbie1, B. Stierstorfer1, H. Doods1, Boehringer-Ingelheim Pharma GmbH & Co. KG, Dept. CNS Diseases Res., Biberach an der Riss, Germany; 1Boehringer-Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany; 2Boehringer-Ingelheim Pharma GmbH & Co. KG, Milan, Italy

Purpose: The aim of this study is to demonstrate whether the Chemokine C-C motif (CCR2) receptor is involved in inflammatory pain and which mechanisms could describe its physiological contribution in pain pathways.

Methods: Compound A was tested for in vitro properties in native cells (rat whole blood assay) and recombinant cells lines (CHO cells expressing rat CCR2). Binding assay was performed in rat CCR2 receptor membrane (RMB) while functional assays were rat whole blood monocytes shape change and calcium mobilization assay in CHO cell line. Selectivity over CCR5 was investigated by binding assay on CHO cells expressing rat CCR5. To assess the in vivo profile of compound A, adult (200-275 g) male Wistar-Han (Charles River, Germany) rats underwent intraplantar injection of complete Freund’s adjuvant (CFA, 25ug/ul by Sigma Aldrich) or intraarticular injection with 1 mg of monosodium iodoacetate (MIA, 50 ul by Sigma Aldrich) for induction of paw inflammatory pain or osteoarthritis (OA), respectively.

Analgesic properties of compound A were assessed by using paw pressure test (Randall Selitto test, UgoBasile, Italy) at 24 hrs post CFA injection and by Incapacitance tester (manufactured by Boehringer-Ingelheim) the weight bearing (WB) deficit was assessed at 3 days post MIA injection. In addition anti-edema properties were investigated in the CFA model measuring changes in the paw volume as assessed by plethysmography (UgoBasile, Italy).

Tissues were collected from injured paws or joints, for further histological examination. All experimental procedures were approved by the ethics committee and the Regierungspräsidium Tübingen (VHV 06-007).

Results: In rat compound A is a selective CCR2 antagonist with Ki in rat RMB of 3.6 nM, with no binding properties to the closest homologous CCR5 (Ki=2920 nM). In addition, it reversed monocytes shape changes (IC50 4.3 nM) and inhibited Calcium mobilization in CHO cells (IC50=14 nM) proving to be a full antagonist devoid of any agonistic activity.

In the CFA model, Compound A showed a dose dependent anti-hyperalgesic effect after acute treatment. The highest dose of 10 mg/kg, orally (PO) achieved full effect comparable to celecoxib (30 mg/kg, PO) while the minimal effective dose was measured at 1 mg/kg, PO. Moreover, unlike celecoxib repeated doses of compound A did not prevent edema formation in the CFA model.

In the MIA model compound A, like celecoxib, showed no effect in the WB deficit when dosed acutely, however it significantly reversed pain-like behaviour when given by subchronic treatment. Furthermore histological findings revealed that compound A has no effect in reducing monocytes migration in the CFA paw or MIA joint.

Conclusions: Overall our data support a CCR2 antagonist to have the potential to provide analgesia in nociceptive pain condition. In addition preclinical data suggest that analgesic properties of CCR2 antagonists in pain inflammatory models can not be attributed to blockage of monocytes migration.

## Table 2

<table>
<thead>
<tr>
<th>Test day</th>
<th>Day -8</th>
<th>Day 0</th>
<th>Post lidocaine</th>
<th>Day 14</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>Mean</td>
<td>SE</td>
</tr>
</tbody>
</table>
<br>Computer-controlled algometer, kg<br>Corpus Hofa | 2.4    | 0.1   | 2.4            | 2.8    | 0.1        |<.0001|
| Vastus lateralis | 2.4    | 0.1   | 2.5            | 2.9    | 0.1        |<.0001|
| Tibialis anterior | 2.8    | 0.1   | 2.6            | 2.8    | 0.1        | 0.0006|
<br>Cuff algometer, kPa<br>Lower leg | 15.6   | 0.4   | 15.4           | 16.0   | 0.4        | 0.0214|

Purpose: Measurement of pain in an acute murine arthritis pain model using a dynamic weight bearing device and evoked pain responses: effect of intra-articular capsaicin pretreatment

H.E. Krug1, C. Dorman1, S. Funkenbusch1, S. Frizelle1, M. Mathiowalk2, 3Univ. of Minnesota, Minneapolis, MN, USA; 2Minneapolis VA Hlth.Care System, Minneapolis, MN, USA

Purpose: Murine models are important to study arthritis pain and new analgesics, but the measurement of pain in mice is challenging. The Dynamic Weight Bearing (DWB) device measures individual limb forces and time spent bearing weight on each limb during spontaneous activity. Evoked pain behaviors in mice are sensitive to change due to arthritis pain and analgesia. We hypothesized that mice with acute arthritis would have measurable changes in DWB due to joint pain that would correlate with evoked pain behaviors and that this could be prevented by pre-treating with intra-articular (IA) capsicain. We measured DWB and Evoked Pain Scores (EPS) in acute arthritis to determine if DWB correlates with EPS and whether it is a reliable measure of spontaneous pain behavior in animals with arthritis. To test the reliability of DWB in differentiating arthritic from nonarthritic animals, some mice were pretreated with capsicain to prevent development of arthritis. We also measured substance P in the dorsal root ganglia of animals with and without arthritis and with morphine (MOR) and capsicain (CAP) treatment.

Methods: C57Bl6 mice were used for all experiments. Acute inflammatory arthritis was produced by IA injection of 10ul of 2.5% carrageenan into the left knee 2-4 hours prior to pain behavior testing. Analgesic controls were injected with 2.5% carrageenan diluted in MOR solution.