



## Pre-clinical data



# INTESTINAL ABSORPTION OF MOBILEE®

## OBJECTIVE

To determine the intestinal absorption of Mobilee® using an everted gut sac model.

## INTRODUCTION

Male OFA-strain rats weighing approximately 200 g were used. Three different parts of the intestine were studied:

**Duodenum:** First part of the small intestine, located between the stomach and the jejunum. Food is combined with stomach acids and then enters the duodenum, where it is mixed with bile and digestive juices from the pancreas.

**Jejunum:** A portion of intestine that extends from the duodenum to the ileum to form the small intestine (although there is no morphological line of distinction between the jejunum and the ileum).

**Ileum:** The last part of the small intestine, located between the jejunum and the large intestine.

The amount of Mobilee® absorbed was analyzed following the technique described by Farndale et al. (1982) for glycosaminoglycan determination. This technique is based on measuring the absorbance of a glycosaminoglycan and dye complex at 535 nm.

It should be stressed that the absorption-assay model used measures intestinal absorption under conditions that are more similar to human physiological conditions and therefore closer to real conditions than in vitro cell models

## RESULTS

The following absolute absorption values were obtained for each portion of intestine.

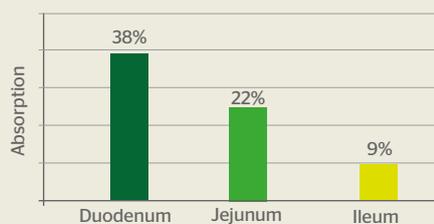
It may consequently be affirmed that Mobilee® is absorbed by the intestine, mainly in the duodenum, where the highest percentage of absorption was observed.

The other two parts of the intestine assayed (jejunum and ileum) also showed absorption activity.

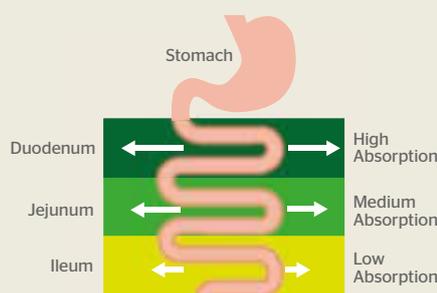
## CONCLUSIONS

From the results obtained, it can be affirmed that Mobilee® is absorbed through the intestinal mucous membrane.

Absorption takes place chiefly in the duodenum, and subsequently in the jejunum and ileum.



**Fig 6**  
**Absolute absorption values.**  
**These absolute values are approximate and should not be understood to be exact due to the variability of the method.**



**Centre where the study was performed:** Servicio de Análisis de Fármacos, Departamento de Farmacología, Terapéutica y Toxicología, Universitat Autònoma de Barcelona. España

**Bibliography:** Torrent A, Ruhi R, Martínez C, Castells G, Castellarnau-Castellà C. Anti-inflammatory activity and absorption of a natural rooster comb extract (Mobilee®). *Osteoarthritis and Cartilage* 2010;18(S2):S246-S247.

## OBJECTIVE

To study the effects of Mobilee® on the synthesis of cell-catabolism mediators prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and metalloproteinase 1 (MMP-1) in cases of inflammation (interleukin 1 $\beta$ ).

## INTRODUCTION

Increased synthesis of proinflammatory cytokines and proteases is produced in cases of rheumatic disorders (rheumatoid arthritis, osteoarthritis, etc.), with the resulting destruction of cartilage matrix.

Interleukin 1 $\beta$  is a proinflammatory cytokine that contributes to cartilage degradation. Among other things, it induces increased synthesis of prostaglandin E<sub>2</sub> and metalloproteinase.

Prostaglandin E<sub>2</sub> is directly involved in the mechanisms that trigger inflammation.

Metalloproteinase (MMP-1) is a collagenase that also causes cartilage degradation, given that it destroys collagen, a structural component of cartilage that gives strength and flexibility to the connective tissue.

A product capable of inhibiting prostaglandin synthesis or lowering MMP-1 levels could be an effective therapeutic aid for reducing inflammation, preventing the destruction of joint cartilage and curbing the evolution of arthritis.

## CONCLUSION

The study showed that Mobilee® can reduce inflammation because it significantly lowered PGE<sub>2</sub> levels in fibroblast cells cultured under conditions similar to those of inflammation.

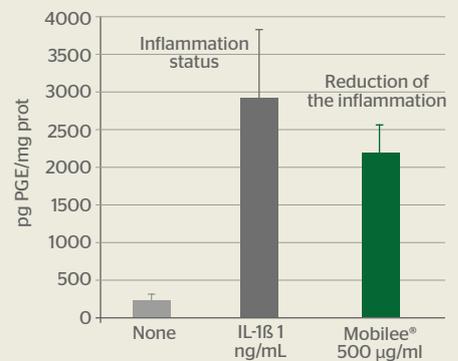
Furthermore, it showed a tendency to lower MMP-1 levels.

These results indicate that Mobilee® may have anti-inflammatory and chondroprotective effects.

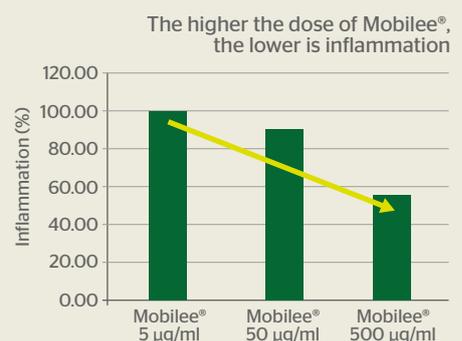
**Centre where the study was performed:** The study was performed by the company Advanced In Vitro Cell Technologies, S.L., which is located in the University of Barcelona Science Park. May 2003.

**Bibliography:** Torrent A, Ruhí R, Martínez C, Castells G, Castellarnau-Castellà C. Anti-inflammatory activity and absorption of a natural rooster comb extract (Mobilee®). *Osteoarthritis and Cartilage* 2010;18(S2):S246-S247Pages S246-S247.

## CHONDROPROTECTIVE EFFECTS OF MOBILEE®



**Fig 3**  
**Effects of Mobilee® on PGE<sub>2</sub> levels (pg/mg of total protein) induced by IL-1 $\beta$  in human dermal fibroblast cells.**



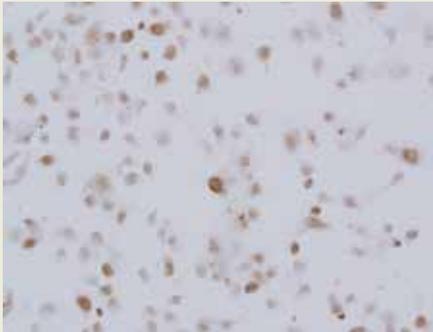
**Fig 4**  
**Mobilee® showed a statistically significant dose-dependent anti-inflammatory effect ( $p < 0.005$ )**

**NOTE:** With the objective to have a comparison between the different dosages, we have assumed the lowest effect (as reduction of inflammation) as 100, and then calculated the corresponding values for higher dosages

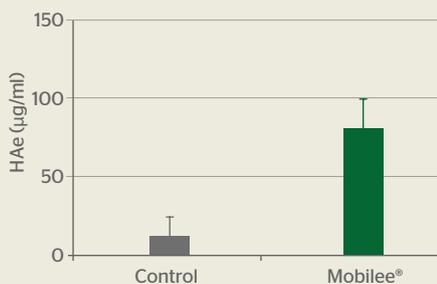
# EFFECTS OF MOBILEE® ON SYNOVIAL FLUID



**Fig 1a. (20x)**  
**Synoviocytes not stimulated with Mobilee® (control)**



**Fig 1b. (20x)**  
**Synoviocytes stimulated with Mobilee®**



**Fig 2.**  
**Effect of Mobilee® on endogenous synthesis of HA in human osteoarthritic synoviocytes**

## OBJECTIVE

To study the effectiveness of Mobilee® in stimulating the synthesis of endogenous hyaluronic acid (HA) in synoviocytes by evaluating levels of intracellular HA in human synoviocytes.

## INTRODUCTION

Hyaluronic acid (HA) is the main glycosaminoglycan in synovial fluid, where it is synthesized by synoviocytes.

HA provides viscosity to the synovial fluid, thereby improving joint function and reducing friction between articular-cartilage surfaces. In patients with osteoarthritis (OA), concentrations of endogenous HA in the synovial fluid are low, and the fluid's viscoelastic properties are limited.

The intraarticular administration of exogenous HA is an alternative treatment currently used in cases of OA. The reason why intraarticular HA is so effective is not yet fully known, but the stimulation of endogenous HA synthesis has been shown to be one of its most important effects. A compound that stimulates synoviocyte production of HA would therefore be a valid alternative for improving the health of synovial fluid, and would be of particular interest if administered orally.

## RESULTS

A culture of human osteoarthritic synoviocytes was stimulated using Mobilee® (200 µg/mL). After incubating the samples for 48 hours, endogenous HA was detected using immunohistochemical techniques and microscope images.

The images showed an increase in intracellular HA when the synoviocytes were treated with Mobilee®.

The culture supernatants were then evaluated to quantify the increase in HA levels.

The Mobilee® values were 10 times higher than the control values, i.e. 10 times more HA was present when synoviocytes were stimulated with Mobilee®.

## CONCLUSION

Based on the results obtained, it can be affirmed that Mobilee® increases synthesis of HA. This increase would apparently be followed by HA secretion into the extracellular space.

Therefore, Mobilee® may stimulate HA secretion to synovial fluid and consequently improve joint function and patients' quality of life. This mechanism of action may explain part of the product's effectiveness.

**Centre where the study was performed:** Rheumatology Service Research Unit at Hospital Juan Canalejo, La Coruña, Spain. January 04.

**Bibliography:** Torrent A, Ruhí R, Theodosakis J, Blanco FJ. Comparative efficacy of IB0004, extracted hyaluronic acid (HA) and fermented HA on the synthesis of endogenous ha by human synoviocytes. *Osteoarthritis and Cartilage* 2009;17(S1):S278-S279

## OBJECTIVE

To determine the differences between Mobilee® and hyaluronic acid (HA) produced by bacterial fermentation in the stimulation of the synthesis of endogenous hyaluronic acid (eHA) by human synoviocytes.

## INTRODUCTION

Hyaluronic acid is the main glycosaminoglycan in synovial fluid, where it is synthesized by the synoviocytes.

HA provides viscosity to the synovial fluid, thereby improving joint function and reducing friction between articular-cartilage surfaces. In patients with osteoarthritis (OA), concentrations of endogenous HA in the synovial fluid are low, and the fluid's viscoelastic properties are limited.

Intraarticular administration of exogenous HA is an alternative treatment currently used in cases of OA. The reason intraarticular HA is so effective is not yet fully understood, but the stimulation of endogenous HA synthesis has been shown to be one of its most important effects.

A compound that stimulates synoviocyte production of HA would therefore be a valid alternative for improving synovial-fluid health, and would be of particular interest if administered orally.

## RESULTS

A culture of human osteoarthritic synoviocytes was stimulated using Mobilee® and HA from bacterial fermentation at different concentrations. After incubating the samples for 12 and 24 hours, the concentration of eHA in the cell cultures was measured.

Both molecules have a dose-dependent effect, with the most efficacious dosages being 100 and 200 µg/ml.

After 12 and 24 hours' incubation, Mobilee® presented higher values of eHA than HA from bacterial fermentation.

## CONCLUSION

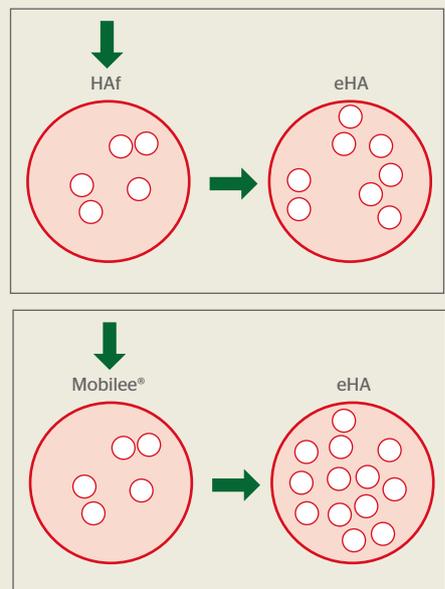
Based on the results obtained, it can be stated that, at the same concentration levels of each product, the highest levels of endogenous HA were measured in the cells stimulated with Mobilee®

According to this study, it can be concluded that there are differences between the action of Mobilee® and HA from bacterial fermentation in the synovial-fluid cells, with Mobilee® being more active in promoting synthesis of endogenous HA.

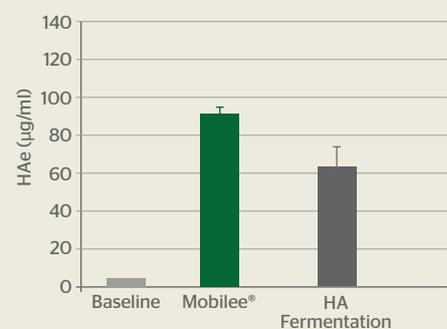
**Centre where the study was performed:** The study was carried out by a group of investigators under the direction of Dr. Blanco at the Rheumatology Service Research Unit at Hospital Juan Canalejo in La Coruña. October 05.

**Bibliography:** Torrent A, Ruhí R, Theodosakis J, Blanco FJ. Comparative efficacy of IBO004, extracted hyaluronic acid (HA) and fermented HA on the synthesis of endogenous ha by human synoviocytes. *Osteoarthritis and Cartilage* 2009;17(S1):S278-S279

## DIFFERENCES BETWEEN MOBILEE® AND FERMENTED HYALURONIC ACID ON SYNOVIAL FLUID



Cell culture of synoviocytes



**Fig 5**  
Effect of Mobilee® and fermented HA on endogenous synthesis of HA in human osteoarthritic synoviocytes. Results at a concentration of 200 µg/ml and 24 hours' incubation.

# MOBILEE® IN RATS DEVELOPING TYPE II COLLAGEN ARTHRITIS

## OBJECTIVE

To determine the effect of Mobilee® administered by twice daily oral dosing on inflammation, cartilage destruction and bone resorption that occurs in developing type II collagen arthritis in rats.

## INTRODUCTION

Twenty-four female Lewis rats weighing 136-154 g were used in this study. Arthritis was induced with subcutaneous collagen injections (300mL) on days 0 and 6. Nonimmunized rats (normal; n=4) were used as normal controls. Rats with developing type II collagen arthritis (n = 20) were treated orally twice daily on days 0-16 of the study with vehicle (H2O; disease control; n = 10) or Mobilee® (7.5 mg, BID; n = 10). Animals were terminated on study day 17. Livers, spleen and thymus were also collected, trimmed of extraneous tissue and weighed.

Efficacy evaluation was based on animal body weights, ankle diameter expressed as area under the curve (AUC), histopathologic evaluation of ankles and knees.

## RESULTS

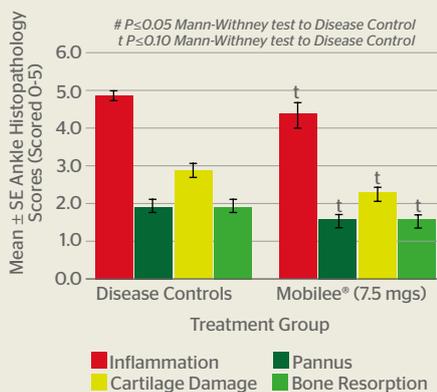
Body weight gain was significantly increased toward normal for rats treated with Mobilee® (24% increase) as compared to disease controls. Ankle diameter AUC was significantly reduced (14%; P = 0.15) for rats treated with Mobilee® as compared to disease controls. Relative liver, thymus and spleen weights for rats treated with Mobilee® were similar to those showed by normal controls, which confirmed Mobilee® safety. Individual ankle and knee histopathology parameters (inflammation, pannus, cartilage destruction, bone resorption) are shown in Figures 1 and 2, respectively. Summed histopathology scores were significantly reduced in ankle (17%, P < 0.05) and strongly reduced in knee (51%, P < 0.01) toward normal for Mobilee® treated rats as compared to disease controls. Collagen-induced arthritis led to a significant increase in knee synovial fluid volume in both vehicle and Mobilee® treated rats, being less pronounced in the second. HA concentration decreased 33% in disease controls, while maintained similar to normal values in Mobilee® group.

## CONCLUSION

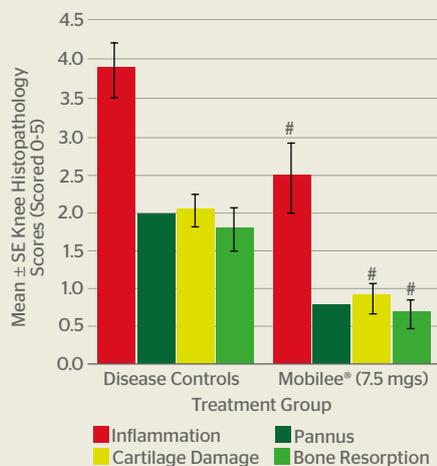
Our results suggest that the use of Mobilee® for the management of developing type II collagen arthritis in rats is safe and effective, having beneficial effects on the histopathology parameters in ankles and knees.

**Centre where the study was performed:** BolderPATH Inc., Boulder, CO; KineMed Inc., Emeryville, CA

**Bibliography:** Castillo V, Bendele AM, Li K, Martinez-Puig D, Turner SM, Chetrit C. Effects of oral administration of Mobilee® in 17 day rat developing type II collagen arthritis. *Osteoarthritis and Cartilage* 2010;18(S2):S244-S245



**Fig 1.**  
**Individual ankle histopathology scores of rats treated with Mobilee® as compared to disease control.**



**Fig 2.**  
**Individual knee histopathology scores of rats treated with Mobilee® as compared to disease control**

## OBJECTIVE

The objective of this study was to determine the effect of the oral administration of an HA concentrate, (Mobilee®) on synovial fluid quality and on the clinical condition of horses with osteochondrosis (OCD).

## INTRODUCTION

The horse was used as an animal model because it allows the extraction of high amounts of synovial fluid at different time points. Twelve horses with a radiographic diagnose of OCD were randomly divided in two groups and assigned to receive orally 250mg of Mobilee® or placebo during 60 days in this blinded randomized controlled clinical pilot trial.

At the end of the treatment (d60) and 30 days after finalization (d90) a sample of synovial fluid was extracted from each animal to analyse HA concentration. The degree of synovial effusion measured with ultrasonographic evaluation and the degree of lameness according to AAEP scale were also evaluated.

## RESULTS

On day 0 no differences on intra-articular HA concentration were detected among groups.

However during the experimental period intra-articular HA concentration increased numerically in the Mobilee® group but decreased in the placebo group, resulting in differences among groups on day 60 (384 vs. 208 µg/L;  $P=0.07$ ) and on day 90 (424 vs 209 µg/L;  $P=0.05$ ) which tended to reach statistical significance. Increases of the intra-articular HA concentration in Mobilee® treated horses were associated on d90 with numerical improvements on the synovial effusion scale (1.25 vs 2.00 points for treated and control groups respectively; and on the degree of lameness (0 vs. 1.5 degrees for treated and control groups respectively), although differences among groups failed to reach statistical significance due to the reduced number of animals.

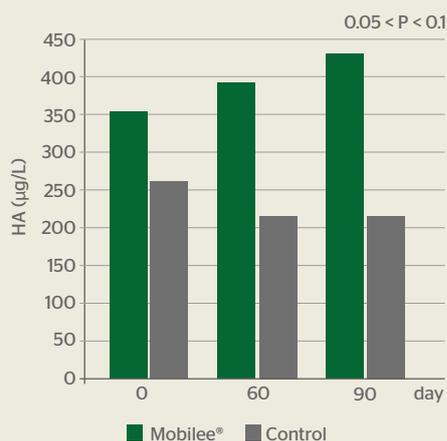
## CONCLUSION

The overall results suggest that oral Mobilee® administration could increase HA concentration, which could also be related to improvements in the clinical condition of the affected joint.

**Centre where the study was performed:** Dept. Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona, Barcelona, Spain; Servei de Bioquímica i Genètica Molecular, Hospital Clínic Universitari de Barcelona, Barcelona, Spain

**Bibliography:** Martínez-Puig D, Carmona JU, Arguelles D, Deulofeu R, Ubia A, Prades M. Oral hyaluronic acid administration improves osteochondrosis clinical symptoms and slightly increases intra-articular concentration of hyaluronic acid in a horse model: a pilot survey. *Osteoarthritis and Cartilage* 2007;15(S3):C62-C63

## MOBILEE® INCREASES INTRA-ARTICULAR CONCENTRATION OF HYALURONIC ACID IN A HORSE MODEL



**Fig 1.**  
**Intraarticular concentration of hyaluronic acid of OCD horses treated with Mobilee® or with placebo (control).**



MOBILEE® IS A TRADE MARK OF BIOIBERICA S.A.

Y405502



**BIOIBERICA**

© Bioiberica 2013

Plaza Francesc Macià, 7  
08029 Barcelona, Spain  
[www.bioiberica.com](http://www.bioiberica.com)