The Transdermal Profiles of Mediflex™ Glucosamine Cream in Mouse and Man

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ABSTRACT

There has been interest for many years in the use of glucosamine in degenerative joint disease (DJD). Glucosamine sulfate forms one half of the disaccharide subunit of keratan sulfate, which is decreased in osteoarthritis, and of hyaluronic acid. Hyaluronic acid forms the backbone of proteoglycan aggregates in articular cartilage, and is found in high concentration in the synovial fluid. However, the long-term effect of glucosamine on disease progression remains not well established, although numerous studies strongly suggested glucosamine was able to decrease osteoarthritis symptom severity. One of the major factors that contribute to the uncertainty in the efficacy of glucosamine (and many other drugs) was difficulty in providing a predictable pharmacokinetic profile and constant drug levels over extended periods of time without the extreme peak/trough fluctuations inherent in oral administration. Therefore, drug developers are turning to transdermal drug delivery technology, as a way to combine the advantages of IV infusion with the convenience of oral administration.

In this study, we had successfully created a highly stable lipovesicular system that allows us to encapsulate highly water soluble small molecules, such as glucosamine sulphate, at a salt concentration as high as 10% (w/w) in the cream. The pharmacokinetic profiles of oral and topical dosage of glucosamine sulphate salt (0.4 g/kg of body weight) were compared in mice. Results showed the topical application of glucosamine had the T_{max} value of 2 hrs with C_{max} value of 0.6 mg/ml of plasma. In contrast, little glucosamine was detected in the plasma of animals administered with glucosamine orally. In human study, 15 healthy volunteers were given a single dose of 10 g Mediflex™ Glucosamine Cream (10% w/w glucosamine sulphate salt and 5% MediLynk™ transdermal delivery vehicles) on both knees. The pharmacokinetic profile showed T_{max} of 4 hrs with C_{max} of 2.9 ± 0.5 µg/ml of plasma. At 8 hrs post-treatment, the plasma glucosamine levels remained at 1.4 ± 0.3 µg/ml of plasma. These studies demonstrated the superiority of MediLynk™ transdermal delivery vehicles in delivering clinical dosage of glucosamine across human skin.

INTRODUCTION

The most common cause of articular morbidity is degenerative joint disease (DJD), which results from the inability of articular structures to withstand applied stress. This process begins because the articular structures are abnormal, or because the stress is unusually high (Resnick & Niwayama, 1995). Eventually, both become pathologic, as the stress induces metabolic and structural changes in the articular cartilage and other tissues, and joint deterioration results in abnormal biomechanical loading. In synovial joints, articular cartilage experiences a loss of proteoglycans, disruption of the collagen matrix, and increased hydration (Resnick & Niwayama, 1995; Mankin & Brandt, 1992). DJD includes synovial joint osteoarthritis, such as in the knee and spinal apophyseal (facet) joints, and degenerative disease of cartilaginous joints, such as the intervertebral discs (Resnick & Niwayama, 1995).

Standard medical therapy includes nonsteroidal anti-inflammatory agents (NSAIDs), which provide analgesia and reduce secondary synovial membrane inflammation (Burkhardt & Ghosh, 1987). However, NSAIDs have well-known side effects. It is possible that some NSAIDs may accelerate disease progression through adverse effects on cartilage metabolism, or joint overuse associated with analgesia (Mankin & Brandt, 1992; Burkhardt & Ghosh, 1987; Brandt, 1997; Rashad et al., 1989). An alternative approach is to administer substances extracted from cartilage that are reputed to facilitate cartilage repair, with or without cofactors involved in tissue repair. The goal is to reduce immediate symptoms and long-term disease progression by stimulating proteoglycan production and cartilage healing.

There has been interest for many years in the use of glucosamine in DJD. Glucosamine sulfate forms one half of the disaccharide subunit of keratan sulfate, which is decreased in osteoarthritis (Mankin & Brandt, 1992), and of hyaluronic acid. Hyaluronic acid forms the backbone of proteoglycan aggregates in articular cartilage and is found in high concentration in the synovial fluid.

Although the long-term effect on disease progression is not well established, glucosamine (Pujalte et al., 1980; Drovanti et al., 1980; Giacovelli & Rovati, 1993; Noack et al., 1994; Vajaradul, 1981; Reichelt et al., 1994; Lopes Vaz, 1982; Muller-Faßbender et al., 1994; Crolle & D'Este, 1980; D'Ambrosio et al., 1981) has been shown to decrease osteoarthritis symptom severity. *In vitro* studies (Noack et al., 1994; Lopes Vaz, 1982; Muller-Faßbender et al., 1994) reviewed evidence that: (1) glucosamine increases production of glycosaminoglycans and proteoglycans by fibroblasts and chondrocytes, and (2) in animal models, glucosamine has mild prostaglandin-independent anti-inflammatory effects, but no analgesic effects. Randomized, placebo-controlled trials demonstrated symptomatic relief by glucosamine given orally (Pujalte et al., 1980; Drovanti et al., 1980; Giacovelli & Rovati, 1993; Noack et al., 1994), intra-articularly (Vajaradul, 1981), or intramuscularly (Reichelt et al., 1994) for DJD of the knee (Pujalte et al., 1980; Noack et al., 1994; Vajaradul, 1981; Reichelt et al., 1994) and other joints (Drovanti et al., 1980; Giacovelli & Rovati, 1993). Compared with NSAIDs (Lopes Vaz, 1982; Muller-Faßbender et al., 1994), or NSAIDs followed by placebo (Crolle & D'Este, 1980; D'Ambrosio et al., 1981), glucosamine eventually produced similar (Muller-Faßbender et al., 1994) or superior (Lopes Vaz, 1982; Crolle & D'Este, 1980; D'Ambrosio et al., 1981) results for DJD of the knee (Lopes Vaz, 1982; Muller-Faßbender et al., 1994) and other joints (D'Ambrosio et al., 1981), although the relief with glucosamine occurred more slowly (Lopes Vaz, 1982; Muller-Faßbender et al., 1994).

Nevertheless, the controversy over the efficacy of glucosamine in preventing or improving the conditions patients suffering from DJD remains. One of the main contributors to the controversy is due to the liver first-pass phenomenon, which severely affected the pharmacokinetic profile of glucosamine delivered orally. Therefore, drug developers are

turning to transdermal drug delivery technology as a way to combine the advantages of IV infusion with the convenience of oral administration.

In this study, we had successfully deployed a highly stable proprietary lipovesicular system to encapsulate water soluble molecules such as glucosamine sulphate at a salt concentration as high as 10% (w/w) in the cream. The pharmacokinetic profiles of oral and topical dosage of glucosamine sulphate salt were compared in mice. Transdermal property of Mediflex^{TM} Glucosamine Cream (10% w/w glucosamine sulphate salt and 5% MediLynk^{TM} transdermal delviery vehicles) in man was also profiled.

MATERIALS & METHODS

Animal study

To evaluate the efficacy of Mediflex^{TM} Glucosamine Cream (containing 10% w/w of glucosamine sulphate salt and 5% w/w of MediLynk^{TM} transdermal delivery vehicles) in delivering glucosamine into the blood versus oral delivery of the same dosage in mature Balb/C male mice (National University of Singapore, Animal Holding Units).

The adult Balb/C mice were randomly divided into 2 groups containing 2-4 animals each. Animals were fast over night prior experimentation. Mice were shaven at the dorsal region, and topically applied with Mediflex^{TM} Glucosamine Cream formulation (dosage of 0.4 g of glucosamine sulphate salt per kg of body weight), over an area of approximately 4-5 cm^2. The cream was gently applied onto shaved area for 30 s. Thereafter, blood samples of 50 µl each were collected from the tail at time intervals of 0.5, 1, 2, 4, 6 and 7 hrs post-treatment, with EDTA added to each blood sample to inhibit coagulation. The plasma was separated via centrifugation, and derivatised prior to C18 reversed phase HPLC (AKTA^{TM}, Amersham BioScience) analysis for the level of freed glucosamine in the blood. For comparison, another group of mice were orally administered glucosamine sulphate salt (dissolved in distilled water) at the same dosage (0.4 g/kg of body weight) and similarly assayed for blood glucosamine level at similar time intervals. Blood samples collected from each animal prior glucosamine treatment (0 hr) were served as controls.

HPLC determination of glucosamine concentration in mouse plasma

Amersham BioScience AKTA Purifier 10 (equipped with P-903 pump, UV-900 detector, A-900 auto-sampler and UNICORN 5.01 data processing system) fitted with C18 reversed phase Inertsil ODS-3, 5 µ, 4.6 x 150 mm HPLC column (GL Sciences Inc. Japan) was used to determine the freed glucosamine concentration in mouse plasma. Plasma samples were run with isocratic mobile phase consisting of ACN : H_2O : AcOH : TEA (25 : 75 : 0.04 : 0.035) at a flow rate of 1 ml/min. A 10-µl aliquot of sample was injected and glucosamine was monitored at UV wavelength of 254 nm and the elution time was 15 min. Acetonitrile (60 % v/v) was used for column flushing In between runs and for column re-equilibration. Cysteic Acid was used as internal standard.

Human study

Fifteen subjects of healthy volunteers (men & women) were given a single dose (10 g) administration of Mediflex^{TM} Glucosamine Cream (equivalent to 1,000 mg of glucosamine sulphate salt). The cream was applied to both legs around the knee cap regions. These subjects had not taken any glucosamine product in the past 30 days of screening. Pharmacokinetic samples of the bioavailability of glucosamine were drawn from the arm at pre-dose, 0.5, 1, 2, 3, 4, 6, and 8 hours post-dose. A 24-hour telephone follow-up was conducted to assess any adverse effect.

All safety data (adverse events, laboratory toxicities, and vital signs) were recorded. The incidence of adverse events and laboratory toxicities were summarized by severity if any. Changes from baseline in vital signs and physical examination were summarized. Pharmacokinetic of transdermal glucosamine was evaluated at steady-state (pre-dose to 8 hrs post-dose). The trial was carried out by Clinical Trial Research Unit at Changi General Hospital, Singapore.

LC/MS/MS determination of glucosamine concentration in human plasma

LC-MS Thermo Finnigan LCQ series (consists of Surveyor auto-sampler, Surveyor MS pump, Finnigan LCQ Deca XP Max, ESI and Xcalibur Tune Plus data processing system) fitted with C18 reversed phase Inertsil ODS-3, 5 μ, 2.1x100 mm LC column (GL Sciences Inc. Japan) was used to analyse freed glucosamine concentration in human plasma. The samples were run with isocratic mobile phase consisting of MeOH : H_2O : AcOH : $NH_3.H_2O$ (12 : 88 : 0.09 : 0.10) with the flow rate of 150 μl/min. A 2-μl aliquot of plasma sample was injected and the retention time for glucosamine was 3.5 min. Methanol (90% v/v) was used for column flushing in between runs and for column re-equilibration. Capillary temperature was at 380°C. Detection parameters for glucosamine were at parent m/z 180.0, SRM, collision energy 24%, fragments m/z 144.0, 162.0, positive. Detection parameters for tyrosine (internal standard) were at parent m/z 182.0, SRM, collision energy 20%, fragments m/z 165.0, positive.

Chemicals and reagents

Glucosamine sulfate KCl, tyrosine, cysteic acid, 1-naphthyl isothiocyanate, acetonitrile (HPLC grade), glacial acetic acid (HPLC grade), methanol (AR grade or above), methylene chloride (AR grade or above) and triethylamine were obtained from Sigma-Aldrich. Mediflex^{TM} Glucosamine Cream (10% w/w glucosamine sulphate KCl, 5% MediLynk^{TM} transdermal delivery vehicles) was provided by PT Kalbe Farma Tbk, Kawasan Industri Delta Silicon, Jl. M.H. Thamrin Blok A3-1, Lippo Cikarang, Bekasi 17550, Indonesia.

RESULTS

The results were expressed as Mean $\pm$ SEM. Where absent, the error bars are smaller than the symbols. Analysis of variance and subsequent multiple comparisons were used for statistical analysis. Data points marked with ** were considered as statistically significant at 0.01 level between treatment groups.

Development of transdermal delivery vehicles

A highly stable water-based emulsion matrix capable of holding unprecedented high concentration of water-soluble molecules was developed. This unique matrix is able to deliver highly hydrophilic molecules such as glucosamine sulphate across the skin into the blood stream effectively within minutes of exposure via topical application. The expansion of the stable region on the Water-Oil-Emulsifier phase diagram was achieved by controlled phase equilibra and formulation sequences and the use of natural biopolymers. The ability to incorporate the natural biopolymers into the formulation has minimized the potential adverse side effects (e.g. rashes, irritation, allergy etc), triggered by the synthetic polymers used by most transdermal technologies.

MediLynk's water-based emulsion matrix is highly stable and burn-in results show no breaking down of emulsion when matrix is subjected to high temperature (50°C for 60 min) or high centrifugation force (4,000 x g for 20 min). There is no hydrolysis of glucosamine under such conditions. The real time experiment shows shelf-life of greater than 18 months if the

emulsion is stored at a cool dry place. The texture of the emulsion remained at semi-solid state despite high concentration of glucosamine sulphate salts (up to 10% w/w), a feat not easily achievable with highly water-soluble drugs.

Determination of blood glucosamine concentration in mice

The results showed glucosamine sulphate salt administered orally to the mice at 0.4 g/kg of body weight gave a slight surge in blood level of freed glucosamine (70 µg/ml of plasma) after 1-2 hrs post-treatment.   No detectable amount of glucosamine in the blood was observed after 6 hrs of administration (Figure 1). In contrast, topical application of the same dosage using Mediflex™ Glucosamine Cream resulted in a huge surge of glucosamine (620 µg/ml of plasma, $p < 0.01$) in the blood within 2 hrs of post-treatment. Thereafter, it subsided to a level of approximately 200-280 µg/ml of plasma ($p < 0.01$) and sustained at this level for at least 7 hrs post-treatment.   The animals appeared and behaved normally throughout the period of the study.
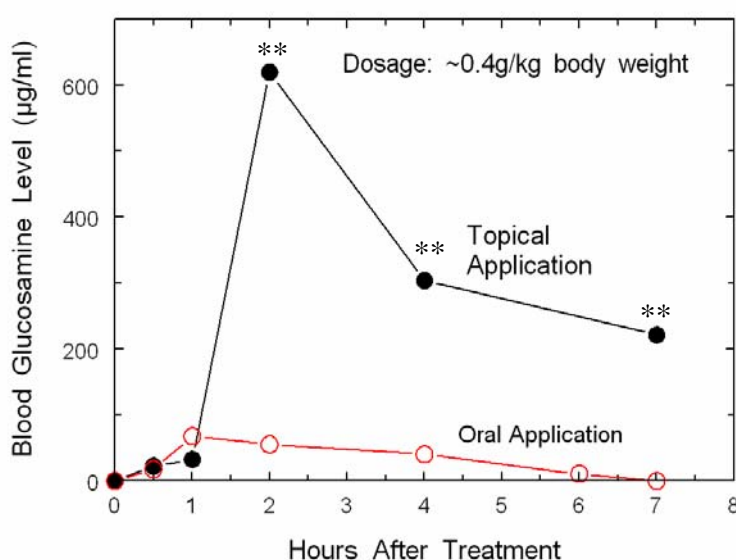

Figure 1. Comparing the efficacy of Mediflex™ Glucosamine Cream and oral delivery of glucosamine into blood of adult mice. Animals fasted and dorsal hair of each Balb/C mouse was shaved 24 hrs before use. Single dose of 0.4 g of glucosamine sulphate salt/kg of body weight was delivered into these animals via topical application of Mediflex™ Glucosamine Cream (●) or Oral application (O). Blood samples were collected and plasma glucosamine levels were measured by C18 reversed phase HPLC. ** $P < 0.01$.

Transdermal profile of Mediflex™ Glucosamine Cream in healthy human volunteers

Distinctive levels of glucosamine were detected in the plasma of healthy human volunteers after being given a single dose of 10 g of Mediflex™ Glucosamine Cream (equivalent to 1,000 mg of glucosamine sulphate salt) on both of their legs around the knee cap areas. Rather high dose of glucosamine cream given in this study was due to low signal to noise ratio of the detection system (LC/MS/MS) for freed glucosamine in plasma. Figure 2 shows

a detectable level of glucosamine in the blood of volunteers within 30 min of topical application of Mediflex[TM] Glucosamine Cream. The T_{max} value was estimated to be 4 hrs post-dose with C_{max} value of 2.9 ± 0.5 µg/ml of plasma. The decline in plasma glucosamine level was gradual, at 8 hrs post-dose the plasma glucosamine was remained at 1.4 ± 0.3 µg/ml of plasma. There was no adverse reaction reported during the course of the study and 24-hr post study.
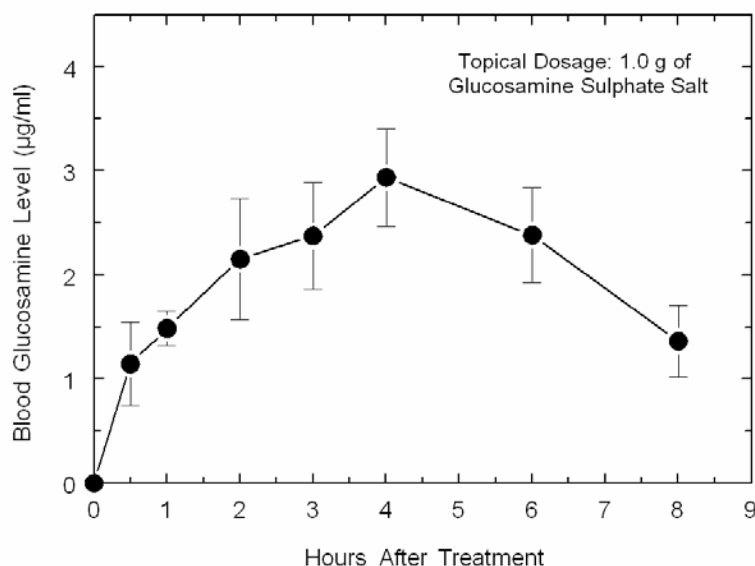

Figure 2. Transdermal profile of Mediflex[TM] Glucosamine Cream in healthy human volunteers. Human volunteers were given a single dose of 10 g of Mediflex[TM] Glucosamine Cream (equivalent to 1,000 mg glucosamine sulphate salt) on their legs around the knee cap areas. Blood samples were collected from forearm at pre-dose (0 hr), 0.5, 1, 2, 3, 4, 6 and 8 hrs post-dose. Plasma glucosamine levels were determined by C18 reversed phase LC/MS/MS.

DISCUSSION

The glucosamine component of glucosamine sulphate is quickly and almost completely absorbed from the gastrointestinal tract following an oral dose; however, it is unclear whether the entire glucosamine sulphate molecule is absorbed intact or to what extent it might be degraded prior to and after absorption. Based on the fecal excretions of radioactively labeled molecules, gastrointestinal absorption of glucosamine is about 87% in the dog. In humans, about 90% of glucosamine, administered as an oral dose of glucosamine sulphate, is absorbed (Setnikar et al., 1993).

After an oral dose, a substantial quantity of the absorbed glucosamine is concentrated in the liver, where it is either incorporated into plasma proteins, degraded into smaller molecules (e.g. H_2O, CO_2 and urea), or utilized for other biosynthetic processes, as it makes its "first-pass" through the liver. Hence, little or insufficient amount of freed glucosamine would be available to the cartilage for the rehabilitation and regeneration processes. As a result, the bioavailability of glucosamine is an issue that constantly clouded the general acceptance or recognition of glucosamine for treatment of osteoarthritis and joint injuries.

It is obvious that delivery is an important issue in the development of any drug product, and the choice of a delivery route is contingent upon optimizing drug delivery; while maintaining convenience and ease of administration. Drug developers are now turning to transdermal drug delivery as a way to combine the advantages of IV infusion with the convenience of oral administration. Transdermal drug delivery, if achievable, presents several advantages over existing drug delivery systems. For oral and parenteral drugs with very short biological half-lives and high body clearance rates, a transdermal delivery system has the potential to provide a steady maintenance of therapeutic levels of the drug in the blood over a predictable period of time. Transdermal drug delivery can also help improve patient compliance, reduce the side effects of the drug due to fluctuations in drug concentration in the blood, the elimination of first-pass effect and the prompt interruption or termination of treatment when necessary, in addition to other benefits.

Emerging technologies within transdermal drug delivery are expanding the number of deliverable drugs (predominantly with hydrophobic drugs). At MediLynk, a high-loading yet highly stable lipovesicular system was developed to deliver highly hydrophilic molecules such as glucosamine sulphate across mammalian skin.

Pharmacokinetic studies with transdermal glucosamine were conducted in mice in accordance with GLP guidelines. After administration of transdermal glucosamine, maximum plasma concentrations and exposures were higher than those after oral administration. Likewise, bioavailability of transdermal glucosamine was rapidly and well absorbed, with peak plasma concentration occurring within 2 hours; the half-life of elimination of transdermal glucosamine was about 4 hours. Mediflex^{TM} Glucosamine Cream (containing 10% w/w of glucosamine sulphate salt and 5% w/w MediLynk^{TM} transdermal delivery vehicles) is able to increase blood glucosamine concentration significantly ($p < 0.01$) by greater than 15 folds compared to oral dose of same amount (0.4 g/kg of body weight) in mice. Furthermore, blood glucosamine level could be sustained at a very significant level for up to 7 hrs post-treatment.

Similar transdermal glucosamine profiles were observed with human volunteers (given a single dose of 1.0 g glucosamine sulphate salt). Observed T_{max} and C_{max} values were 4 hrs and 2.9 ± 0.5 µg/ml of plasma, respectively. More importantly, the plasma glucosamine concentration remained relatively high (1.4 ± 0.3 µg/ml of plasma) for up to 8 hours post-treatment. This provides a good and constant source of glucosamine that could be beneficial to patients suffering from osteoarthritis (Bucci, 1994), as glucosamine is needed in the synthesis of chondroitin sulphate and hyaluronic acid, and these two compounds are depleted in osteoarthritis condition.

Articular cartilage has no blood, nerve, or lymphatic supply (Curtiss, 1964). As a result, it depends heavily on synovial fluid as medium to transport nutritional substances, such as glucose and glucosamine, from periarticular vasculature to the cartilage. It had been shown in fasted human that synovial fluid glucose concentration is much higher than plasma glucose concentration 2 to 3 hrs after a meal (Cohen et al., 1975). Hence it is very likely that, the synovial fluid glucosamine concentration may also be significantly higher than plasma glucosamine concentration under current study. It is also possible that transdermal delivery is much more effective than oral delivery in delivering glucosamine to the synovial fluid as glucosamine is applied closely to the joints. All these issues will be addressed in our future studies.

CONCLUSION

MediLynk Pte Ltd has formulated the highest concentration of glucosamine sulphate salt topical cream in the market with their proprietary transdermal delivery technology. This is the first study to show the superiority of transdermal delivery versus oral delivery of glucosamine sulphate in mice and to demonstrate the pharmacokinetic of 10% (w/w) glucosamine sulphate salt topical cream in human. Our findings strongly suggested that the high and sustainable level of glucosamine in the blood achieved via topical application of Mediflex^{TM} Glucosamine Cream, is able to provide enzymes with a sufficient amount of substrate for their cartilage regeneration and rehabilitation processes.

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