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The technology behind KNOSIS

Strategic Science & Technologies, LCC (SST) brings a transformational technology to the field of localized transdermal active pharmaceutical ingredient (API) delivery. Called KNOSIS, SST’s proprietary platform technology offers the ability to deliver APIs transdermally. Target therapeutic opportunities include the delivery of new or current systemic therapeutics that treat localized physiological derangements. APIs which failed due to systemic toxicity are also candidates for delivery via KNOSIS. Two completed formulations, a transdermal non-steroidal anti-inflammatory (NSAID) for muscle and joint pain and inflammation, and transdermal L-Arginine for treatment of poor peripheral circulation in patients with neuropathy and cold feet (both diabetic and non-diabetic) will be the first marketed products employing KNOSIS.

A simple idea with profound implications

SST achieves KNOSIS utilizing ingredients that are included on the Food and Drug Administration’s generally regarded as safe (GRAS) list. A wide range of APIs can benefit from KNOSIS as their underlying delivery platform. They include APIs that act locally, APIs withdrawn from market for safety reasons, and APIs with beneficial activity, but that possess toxic effects which have prevented them from ever reaching market. KNOSIS ensures improved, safer API delivery vs. oral medications by permitting more effective local, and much lower whole-body doses. It also makes APIs faster-acting than if they are administered orally, and can help to create a new proprietary position for APIs with patent protection as the key attribute.

The many inherent advantages of delivering APIs transdermally have long been recognized. They include reduction or elimination of many of the side effects, substantial reduction in total body dose, and a potential for higher localized dosing. These are achieved because the API is delivered where it is needed, with the highest exposure at the delivery site and other tissue in the body exposed to much lower concentrations as the API diffuses from the application site. With oral API delivery, APIs are distributed to tissue through the blood, placing the API in the blood first. With transdermal API delivery, APIs are distributed to the tissue first, and then to the blood, but at a lower level.
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**KNOSIS breaks through previous barriers**

Despite the general recognition of the advantages of transdermal delivery, only a limited number of APIs have been successfully developed in the transdermal format. KNOSIS greatly expands the range and classes of APIs that can be delivered transdermally by overcoming two major obstacles.

The outer layer of the skin, the *stratum corneum*, though only a few microns in thickness, constitutes a highly effective barrier to skin penetration. SST’s technology, aptly named KNOSIS, embodies a kenotic ability of the vehicle to cause the API to cross this barrier. Kenosis is a Greek word meaning or describing an act of self-emptying. SST’s KNOSIS employs two complementary features addressing the two major barriers to transferring the API from the vehicle into the tissue through the *stratum corneum*. First, KNOSIS raises the free energy of the API in the vehicle with respect to the free energy of the API in tissue, creating a positive chemical potential, which drives the API from the vehicle into the tissue. Second, a major feature of the *stratum corneum* barrier is the ability of the membranes of its cellular constituents to form hydrogen bonds with the API, preventing the API from traversing the *stratum corneum*.

KNOSIS prevents the formation of hydrogen bonds between the API and the components of the *stratum corneum*, allowing the API to pass through, driven by the chemical potential of the API in the vehicle. It does so by employing two components in the vehicle – Hostile Biophysical Environment (HBE), which raises the thermodynamic activity coefficient of the API and the Anti-Hydrogen bonding feature. Manipulating the chemical properties causes a mismatch between the chemical properties of the API and the vehicle. This creates the HBE, which raises the chemical potential of the API (Gibbs Free Energy), which, in turn, produces the kenotic effect.

As stated previously, KNOSIS will expand substantially the range of APIs that can be delivered transdermally. Until now the only clinically available transdermal API preparations were those where the API formed few or no hydrogen bonds with the *stratum corneum*. These included nicotine, nitroglycerine, scopolamine and steroids such as estrogen and testosterone. KNOSIS is changing that.
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SST's transformational transdermal technology has already been employed in the development and testing of transdermal preparations of certain NSAIDs and the nitric oxide precursor, L-Arginine. The KNOSIS platform delivers these APIs locally, helping NSAIDs to more effectively reduce local pain and inflammation, and L-Arginine to more effectively increase localized blood flow in the feet of patients with neuropathy and cold feet (both diabetic and non-diabetic).

The background on TransDermal L-Arginine

In the early 1990s, the role of nitric oxide, the simple diatomic molecule NO, in controlling local blood flow was being described in scientific literature. In 1998, it was the subject of the Nobel Prize in Medicine and Physiology. In the body, NO is generated from the amino acid L-Arginine by the enzyme nitric oxide synthase (NOS), which exists in at least three isoforms. The form of NOS localized in the endothelial cells is referred to as eNOS and is the form responsible for regulation of local blood flow. A variety of localized derangements of the eNOS/L-Arginine system result in impaired blood flow. This is particularly severe in patients with neuropathy and diabetes. Dr. Fossel realized that the ability to deliver L-Arginine transdermally to effect local blood flow would be an important medical contribution. From March of 1995, when he left Harvard Medical School, until the summer of 1997, he studied and perfected a system for such a transdermal delivery of L-Arginine.

This was no small feat, because the transdermal delivery of L-Arginine is difficult for two main reasons. First, at physiological pH it is charged. Charged molecules are notably difficult to deliver transdermally. Second, L-Arginine is potentially capable of forming six hydrogen bonds. As described above, passive diffusion across the stratum corneum as described by Fick's first law of diffusion becomes virtually non-existent if two or more hydrogen bonds can form between the API being delivered and the membranes of the cells of the stratum corneum.

The solution for delivering L-Arginine, and potentially many other APIs transdermally as well, is two-fold. Taking advantage of the charged nature of L-Arginine, a vehicle was designed that would raise its chemical potential relative to that in tissue. By creating a high ionic strength vehicle, the chemical potential of L-Arginine (see above) was raised. In addition, this same high ionic-strength vehicle prevents formation of hydrogen bonds between L-Arginine or another API and the membranes of the cells of the stratum corneum.

The resulting transdermal L-Arginine system was first tested and demonstrated to be effective on people with cold hands. Cold hands or feet are the result of an insufficient supply of warm blood reaching the extremity. Warm blood from the core of the body is, among other things, the heating system of the body. Many tests were conducted. The warming effect of the transdermal L-Arginine preparation on cold hands was achieved by using the first product created by SST Inc., Warm Cream.
The upper table above summarizes the results of tests done by an independent commercial testing laboratory. The lab selected a group of subjects with index finger temperatures between 22 and 26°C. After equilibrating in the test room for a half an hour, index finger temperatures were measured by an infrared thermometer. Ten minutes later the subjects rubbed the cream into their hands for five minutes and the index finger temperatures were recorded every ten minutes for an hour. As can be seen in the figure, a temperature increase of approximately 10°C was recorded at the end of the hour.

Three different placebo creams also were tested. In the lower table the results are shown for a placebo, which contains all components, including L-Arginine, but which omits the ionic components that constitute the delivery system. With this placebo, no temperature increase is seen. Two other placebo creams also were tested (but not shown in this paper). In one, the L-Arginine, but not the ionic components was omitted. In the second, the optical isomer, D-Arginine, was substituted for L-Arginine. The enzyme eNOS is able only to convert the natural L isomer to nitric oxide. Neither of these creams resulted in a temperature increase.
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A Pilot Study of Transdermal L-Arginine Cream in patients with diabetes who have impaired circulation in their feet

The transdermal L-Arginine cream was tested in a pilot study for its ability to improve temperature and flow in the feet of patients with diabetes, exhibiting symptoms of impaired circulation in their feet. This study, which was published in the January 2004 issue of the journal Diabetes Care, is described below.

Poor blood circulation in the legs and feet is a major source of complications in patients with diabetes. These complications begin as coldness and progress first to pain (neuropathy), and then to open ulcers. Ulcers can progress to a point where the only treatment available is amputation. In the US approximately 90,000 amputations are performed each year on the toes, feet or limbs of patients with diabetes. These are directly caused by impaired blood flow.

The eighteen million people with diabetes in the US either have compromised peripheral circulation or are at high risk of developing it. In order to test the hypothesis that SST’s transdermal L-Arginine cream would be beneficial to people with diabetes, SST conducted a pilot study in a patient population with type II (non-insulin dependent) diabetes who had some degree of neuropathy, but had not yet developed ulcers.

As background to this study, it has been shown that in diabetes the functionality of the endothelial nitric oxide (NO)/nitric oxide synthase (eNOS) system is impaired. NO is generated in the endothelium through the oxidation of the amino acid, L-Arginine by the enzyme eNOS. NO causes vascular smooth muscle to relax resulting in increased blood flow. In addition to being a substrate of eNOS, L-Arginine facilitates the dimerization of two identical subunits forming a homodimer. The enzyme is only active in the dimeric form. Under proper conditions, dimerization occurs rapidly, on a timescale of minutes. Once formed, the dimer is stable.

Subjects with diabetes have abnormally low levels of L-Arginine and elevated levels of the eNOS inhibitor, asymmetric dimethylarginine (ADME) in their plasma. Though the value of increasing L-Arginine levels in cases of impaired circulation is now recognized, practical schemes for therapeutic use of L-Arginine have been elusive. In this pilot study SST sought to determine whether supplying L-Arginine transdermally would improve vascular function of the feet in patients with diabetes as indicated by flow and temperature.

The study was designed as a double-blind vehicle-controlled two-period crossover protocol, with washout periods of one week. Sixteen subjects were enrolled and thirteen completed the study (age 56 +/- 8 yrs.). After analyzing the data it was clear that the effect of L-Arginine persisted throughout the washout periods. Because of this, except for the initial exposure of L-Arginine to virgin feet, the analysis was altered to determine the effect from cumulative exposure to L-Arginine throughout the protocol. Flow was measured at the metatarsal and Achilles area.
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using a Doppler flow meter\(^7\) and temperature was measured at the metatarsal and big toe areas using an infrared thermometer.

The active cream was a water-based moisturizing vehicle containing 12.5% L-Arginine hydrochloride in a hostile biophysical environment comprised of high concentrations of choline chloride, sodium chloride and magnesium chloride. The vehicle control was identical except that the L-Arginine was omitted.

At the first visit, after baseline measurements were made, each subject rubbed active cream (4 mg L-Arginine/cm\(^2\)) into one foot and vehicle into the other. After thirty minutes, measurements were made again. A one-week washout period followed. Patients returned after the washout period at which time, flow and temperature measurements were made. They were then randomly given either an active or placebo cream, and told to rub it into their feet in the morning and evening every day for two weeks.

At the end of two weeks, subjects returned, and again measurements were made. A second one-week washout period followed that third visit. At the end of that period, subjects returned and measurements were made. They were given the crossover product and told again to rub it into their feet morning and evening for two weeks. The subjects returned for final flow and temperature measurements at the end of that period.

At the first visit, flow was increased at the Achilles in the foot with active cream from 8.1 +/- 3.3 Absolute Units (AU) to 11.5 +/- 5.5 (p=0.05) thirty minutes after application. In the foot that received placebo cream, flow failed to increase (8.1 +/- 1.4 vs. 8.3 +/- 2.2). Further, at the last visit, the temperature at the metatarsal area had risen from the initial value of 82.0o F. +/- 2.3 to 86.9 +/- 2.4 (p<0.0001), and the temperature of the big toe had risen from the initial visit value of 74.4 +/- 4.2 to 82.4 +/- 4.8 (p<0.0001). And at the last visit, the flow at the metatarsal area had risen from 8.7 +/- 4.3 to 11.6 +/- 5.5 (p<0.0001), and flow at the Achilles area had risen from 8.4 +/- 2.5 to 11.4 +/- 5.5 (p=0.02). While the failure of the L-Arginine effect to wash out removed the opportunity for placebo control, the improvement in temperature and flow were substantial and highly statistically significant. Though puzzling, one explanation of the persistence of the L-Arginine effect is that the local tissue concentration of L-Arginine becomes high enough to cause inactive monomers of eNOS to form active dimers.

From these data it is clear that, in the patients SST studied with diabetes, treatment of their feet with a transdermal preparation of L-Arginine improved both flow and temperature, and that this effect was surprisingly long lasting. Such improvement of compromised local blood flow should be beneficial and could reduce the complications of the disease.
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Flow-Assisted TransDermal Drug Delivery has arrived

One of the classical limitations of transdermal drug delivery is imposed by Fick’s first law of diffusion.

\[ J_f = -D_i \left( \frac{\partial C_i}{\partial x} \right) \]

Simply put, the amount of API transferred across the skin \( J \), is governed by a constant \( D \) and is directly proportional to the concentration gradient \( C \), and inversely proportional to the thickness of the barrier \( x \) posed by the stratum corneum. If the gradient \( C \) collapses (becomes 0), drug delivery comes to a halt. The gradient becomes 0 when the amount inside the skin and outside the skin becomes equal.

In order to prevent the collapse of the gradient and maintain the flux of API across the stratum corneum, KNOSIS, SST’s flow-assisted transdermal delivery system, incorporates the transdermal L-Arginine technology described above.

Blood flow is enhanced in the target tissue by providing L-Arginine for the production of nitric oxide. The enhanced blood flow prevents the gradient from collapsing by not allowing build-up of API under the skin, but rather washing it deeper into the tissue.
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In principle, this is a generally applicable technique for a wide range of APIs. Moreover, it is illustrated clearly above in diagrams, which show how increased blood flow removes the API from the viable epidermis, preventing collapse of the gradient.

In conclusion

Based on the information and findings described in this paper, it is clear that the intellectual property, and underlying science and technology of KNOSIS, which has been developed, perfected and patented to facilitate the delivery of new or current systemic therapeutics, is effective, unique and transformational for the field of transdermal treatment of localized physiological derangements and their resulting medical conditions.

Footnotes:


