Expert Opinion

Water-in-oil microemulsions for effective transdermal delivery of proteins

Gregory Russell-Jones[†] & Roy Himes
[†]*Mentor Pharmaceutical Consulting Pty Ltd, 23 Greenfield Ave, Middle Cove, Sydney, New South Wales, Australia*

Introduction: A water-in-oil microemulsion is a thermodynamically stable emulsion that has the capacity to 'hide' water-soluble molecules within a continuous oil phase. The very small size of the water droplets within the microemulsion means that these types of formulation can be applied topically to the skin, with the result that peptides and proteins can be delivered effectively into the dermal layer.

Areas covered: This review discusses the general problems of peptide and protein delivery following topical application, and compares the possible routes of peptide and protein clearance and distribution within the body following topical administration. Several examples of successful peptide and protein delivery using microemulsions are discussed, in addition to the possible alterations in biological profiles following administration via this route.

Expert opinion: Water-in-oil microemulsions present themselves as an effective means of topical delivery of peptides and proteins of all sizes, and in high doses. These formulations are a cheap, stable, pain-free means of delivery of peptides and proteins to the skin. An exciting area of potential development is the area of weight control management. The results using insulin, IGF-I and GHRP-6 given topically are particularly intriguing. Whether these results can be replicated in humans and whether the use of these drugs for potential treatment of obesity will be commercially viable will be particularly interesting.

Keywords: GHRP-6, IGF-I, insulin, microemulsion, transdermal, vaccine delivery, weight loss

Expert Opin. Drug Deliv. (2011) 8(4):537-546

1. Introduction

The skin is the largest organ in the body, and presents itself as a readily accessible site for administration of pharmaceutical products, particularly since there are a plethora of skin conditions, which could potentially benefit from local delivery. Transdermal drug delivery has been a commercial reality for over 40 years. The molecules delivered, however, have been restricted to small lipid-soluble molecules applied in a variety of devices, including patches, ointments, creams and gels. Molecules delivered include testosterone, oestradiol, scopolamine, fentanyl, diclofenac, nitroglycerin, clonidine, apomorphine, nicotine and various non-steroidal anti-inflammatory molecules, among others. A review of methods of delivery can be found in the article by Subedi *et al.* [1].

Many different methods of delivery have been developed for topical applications, including patches (Climira™, Minitran™ (3M, MN, USA)), thermoporation (Passport™ Apomorphine (Altea Therapeutics Corp., GA, USA)), reservoir patches (Duragesic®, (Alza Corp., MN, USA)), diffusive patches (Elestrin™, ATD™

informa
healthcare

Article highlights.

- Water-in-oil microemulsions are a cheap, easy to formulate, device-free, and highly effective vehicles for transdermal delivery of peptides and proteins.
- High bioavailabilities can be achieved for proteins from 1000 to 150,000 Da molecular mass.
- The biological response to various peptides can be altered following transdermal delivery in water-in-oil microemulsions.
- Effective needle-free vaccination can be achieved through the use of topically applied vaccines formulated in water-in-oil microemulsions.
- Topically applied IGF-I, GHRP-6 and insulin, each formulated in water-in-oil microemulsions, are effective at increasing muscle mass in obese mice.
- Topically applied IGF-I, GHRP-6 and insulin, each formulated in water-in-oil microemulsions, are effective at decreasing total body fat in obese mice.
- Topically applied anti-TNF molecules, each formulated in water-in-oil microemulsions, are effective at decreasing inflammation in the foot of carrageenan-treated mice.

This box summarizes key points contained in the article.

(Antares Pharma, NJ, USA)), intrafolicular delivery (Dermatec, Nevada, USA), electroporation, ultrasonic and iontophoretic devices (Medpulser® (Genetronics, CA, USA), PM2000™, Iogel™, Optima™ (Iomed, UT, USA), LidoSite® (Vyteris, NJ, USA), Sonoprep® (Sontra Medical, MA, USA)), biphasic vesicles (Biophasix™Helix Pharma, Pakistan), ultradeformable carriers (Diraclin® (IDEA AG, Munchen, Germany)), absorption and permeation enhancers (SEPA® (Macrochem), NexAct® Alprox-TD and Femprox creams Nexmed; Solaraze® (Skye Pharma, London, UK), Androderm® (Watson Labs, NJ, USA)) [2,3], among others [4], and microneedles (Georgia Tech; Macroflux™ (Alza, MN, USA)).

Similar results for testosterone delivery have been found for sprays [5], ethosomes [6], patches [7] and solid lipid microparticles [8]. Delayed transport of estradiol across excised rat skin has also been reported by Fang and co-workers [9] using proniosome formulations. Studies by Ghafourian and Fooladi [10] have shown a high correlation between skin permeablility and octanol:water partition coefficients for a variety of sterol derivatives.

A review by Cevc and co-workers [11] describes the utility of the skin as a pathway for systemic delivery of drugs using lipid-based vehicles. Their studies concentrated on the use of transferosomes for delivery, whose ultimate fate appeared to be the liver. Other lipid-based delivery systems have been developed, including liposomes, niosomes and transfersomes [12-16]. An alternative, lipid-based system, which has also been used for delivery of water-soluble molecules, is a water-in-oil microemulsion (W/O ME) formulation. Most of the studies with these formulations have dealt with the delivery of small water-soluble molecules, and are discussed later in the review.

Transdermal delivery systems, such as reservoir and matrix-type patches, have been used in the development of several products for the delivery of low-molecular-mass and/or hydrophobic drugs such as clonidine, fentanyl, estradiol, testosterone, nitroglycerin, nicotine, scopolamine, oxybutinin and lidocaine [17]. Although the transdermal delivery of these molecules has commercial and pharmaceutical validity in its own right, there are many disease applications that are treated with peptide or protein preparations, usually by means of injection, as they cannot be delivered via topical application at present.

Despite the barrier function of skin, small lipophilic molecules are easily administered through the skin, but it is difficult to administer water-soluble protein and peptide drugs that are on the market or under development.

When disruptive mechanisms such as electroporation have been used for protein delivery, they have shown an inverse relationship between size and transdermal transport [18]. These problems are exacerbated further in iontophoresis, which has been shown to be highly variable depending on conditions of solvent ions, cargo charge, pH and amperage applied [19].

Recently, however, a new delivery formulation has been described that overcomes most of the problems associated with transdermal peptide and protein delivery. This technology utilizes the ability of water-in-oil microemulsions effectively to 'hide' water-soluble molecules within an oil layer, which can then be used to 'smuggle' water-soluble molecules such as proteins and peptides though the epidermis into the dermal layer, in a similar fashion to the way in which 'vanishing creams' vanish when applied topically. Topical delivery of peptides and proteins using water-in-oil microemulsions is the topic of this review.

2. Advantages of topical delivery

Topical delivery has several obvious advantages when compared with either oral delivery or subcutaneous injection, including improved patient comfort, lack of needle-stick injuries, reduced pain, improved safety, possible site-directed delivery, controlled and continuous drug delivery, with the avoidance of sharp peaks in delivery profile, improved efficacy and fewer side effects [12,20], all of which should lead to an improvement of patient compliance, and ultimate drug efficacy. Also, it has been found that the biological/pharmaceutical activity of the applied pharmaceutical can be altered, particularly when peptides and proteins are applied topically. This new activity is discussed later.

2.1 Circulation in the skin

The skin is amply supplied with a rich vascular network of arteries and veins, which are involved in temperature regulation, nutrient supply to the skin and the supply of many immunocompetent cells. Topically applied peptides and proteins cannot enter the circulation in the skin as there is no basal-to-apical transport of such molecules through the vascular endothelium, and as such they must travel in the lymphatics in order ultimately to reach the circulation [21].

2.2 Lymphatic clearance

Several workers have shown that clearance of large molecules such as proteins and large sugars, when injected intradermally, is almost exclusively by means of lymph vessels [22-27]. Transit of material within the lymph vessels is relatively rapid and varies from ~ 1.5 to 10.2 cm/min [28]; however, movement of molecules that have not reached the lymph vessels appears to be quite a bit slower, and is more akin to radial diffusion. The radial diffusion pattern observed is very similar to that observed following intradermal injection of sensitizing antigen in hypersensitive mice [29] and also following topical application of various water-soluble agents to human skin [30,31]. Thus, the applied material appears to diffuse symmetrically by means of simple radial diffusion, in which the applied material persists for many hours. Thus, when diffusion is visualized using the water-soluble fluorescent molecule umbelliferone, formulated in a water-in-oil microemulsion and applied topically, there are obvious signs of lateral diffusion in the dermal layer, reaching ~ 5 cm after 6 h (**Figure 1**). In this instance there is no obvious sign of concentration of material within a draining lymph vessel, rather, diffusion appears lateral. Such diffusion would be dependent on the absence of specific receptors for the solute molecule. Thus, for allergens applied by intradermal injection, there is a concentration-dependent diffusion of allergen and subsequent mast cell activation, which is the basis of the standard skin test for allergen hypersensitivity (see **Figure 2**). These observations are also consistent with those seen in humans, where the majority of intradermally injected doses persists at the site of application for several hours [28], and ultimately drains from distal or proximal lymph nodes many centimeters from the site of injection [28,32-39].

2.3 Choice of models

Many skin permeation studies have been performed *in vitro* on sections of excised skin from various sources, including rat, pigs and humans. In many instances the skin could be regarded as rather less than live, having been prepared and frozen before defrosting and use [40,41]. One must question the relevance of these studies in light of the many observations of lateral diffusion of applied drug through the dermal layer of the skin, the lack of muscular movement in the chambers, plus the degree of pressure that is applied to the skin samples during their 'mounting' in the chambers. Even small amounts of pressure, such as that exerted by a sock on a human leg, can be sufficient to inhibit vascular drainage. As such it is questionable as to whether these 'clamped skin' models are representative of reality [28]. For the reasons outlined above, the studies reported here have been on live, conscious animals, or humans.

3. Transport across the dermal layer using microemulsions

There appears to be little evidence for topical delivery of peptides and proteins in water-based formulations. By contrast, there is now a growing body of evidence that oil-based formulations, more particularly water-in-oil microemulsions, may have the potential for the delivery of milligram quantities of peptides and even large proteins through the epidermal layer and into the dermis [30,31,42,43]. Microemulsions have enormous advantages in delivery because of their high solubilization capacity, ease of preparation, transparency, thermodynamic stability, and high diffusion and absorption rates [44,45]. The microemulsions are easily formed, and an excellent discussion on the theory and practice of microemulsions can be found in the publication edited by Prince [46]. Although it is not the object of this review to describe the methods for formation of microemulsions, the essential conditions for their formation have been described by authors such as Schulman *et al.* [47], Kumar and Mittal [48] and Constantinedes *et al.* [44].

Water-in-oil microemulsions have been shown to have a highly favorable drug delivery potential, which may be attributed to both their excellent solubility properties as well as the possible permeation enhancement through easy dispersion through the dermal lipids [49-51]. Most previous work, both in small animals and humans, has utilized microemulsions containing small hydrophobic molecules [52-63], or small 'model' hydrophilic molecules [64-66]. Often this work has been performed in chambers, rather than on live conscious animals [59,64,67]. The validity of these models in measuring lateral movement of topically applied material is rather questionable, and so data from these studies are not included in this review.

3.1 Peptide and protein delivery with microemulsions

There are only a few studies of transdermal peptide delivery using microemulsions. These studies describe delivery of small peptides such as desmopressin [68], cyclosporine [69] and the folate analogue methotrexate [49]. Although these studies do point to the potential utility of water-in-oil microemulsions for transdermal delivery of small water-soluble molecules, it has recently become evident that these microemulsions may have a much more generic role in the delivery of a wide range of water-soluble molecules, extending from small peptides (described above) to large proteins of 100 – 150,000 Da molecular mass [31,42,43]. In their studies, Russell-Jones and co-workers topically radiolabeled anti-TNF receptor Fc-fusion protein formulated in W/O ME to the shaved skin of conscious mice, and found that the label rapidly entered the dermal layer of the mouse skin, and subsequently moved laterally through the skin to distal areas of the skin, and muscle. The systemic bioavailability following topical administration was found to be > 50%, most of which (~ 70%) remained in the skin for > 4 h. During the time of the experiment there was no discernable evidence of breakdown of the topically applied molecules as evidenced by the lack of ^{125}I counts detectable in the thyroid of the mice. By contrast, there was evidence of breakdown of orally administered antibodies, which could be measured by the presence of ^{125}I counts in the thyroid and urine. The

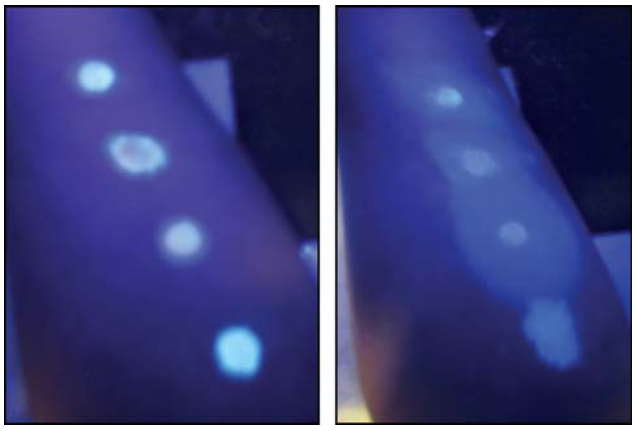

Figure 1. Penetration of umbelliferone in various microemulsion formulations into the forearm of a human volunteer. Time 0 (left panel) time 6 h (right panel).

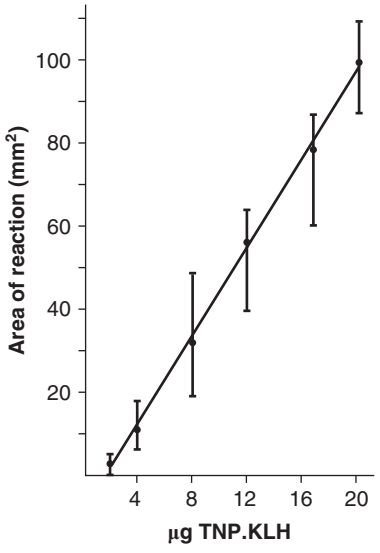

Figure 2. Area of active cutaneous anaphylactic reaction following increasing doses of the allergen TNP.KLH injected intradermally in mice. (Russell-Jones, 1983, PhD thesis.).
TNP.KLH: Trinitrophenylated keyhole limpet haemocyanin.

bioactivity of the formulated proteins was found to be substantially maintained during formulation within the microemulsion and during transit through the skin, as judged by the ability of anti-TNF molecules such as Enbrel™ (Amgen, USA), Remicade™ (Centocor, PA, USA), Humira™ (Abbott Labs, IL, USA); and the human cell-expressed TNF-RII molecule to reduce carrageenan-induced inflammation of the foot following topical administration in the W/O ME.

3.2 Use of microemulsions for topical vaccination

Delivery of protein antigens into the skin is perhaps one of the most desirable routes for vaccination. This is owing to the large number of dendritic cells resident in the dermal layer of the skin. These cells have previously been shown to have a major function in antigen presentation to the immune system [70]. Preliminary work has shown the effectiveness of transferosomes in stimulating an immune response following topical application of tetanus toxoid to rats [71]. More recently, the authors have shown that it is possible to formulate antigens such as tetanus toxoid within W/O ME and generate comparable antibody responses to that observed following subcutaneous injection (**Table 1**, **Figure 3**). The topical route of delivery in W/O ME was particularly good at generating good cellular immunity, which was found to be equal to or higher than intramuscular injection of antigen administered in adjuvant (**Figure 3**).

Interestingly for both the antibody response and the cellular response, complexation of tetanus toxoid with the adjuvant alum greatly reduced the subsequent immune response. This was presumably owing to complexation with the antigen, with resultant precipitation. It would be expected that this would prevent the oil-penetrating ability of the subsequent W/O ME.

3.3 Use of microemulsions for modification of obesity in mice

The studies outlined above have shown the utility of the water-in-oil microemulsions for transdermal delivery of peptides and proteins; however, they did not address whether there is any difference in the biological response seen when topically administered peptides were compared with the more traditional subcutaneous administration. It was therefore decided to compare the biological response with topical or subcutaneously administered peptide hormones. The first of these, insulin-like growth factor 1 (IGF-I), is an insulin-like molecule, which is an important factor in childhood growth and also in maintaining muscle mass in adults [72]. Also, it has been shown to have an effect in controlling obesity [73]. IGF-I is one of the main hormones released though the action of growth hormone [73], which in turn can be stimulated by the administration of several small peptide agonists, including growth hormone releasing hormone 6 (GHRP-6), which was also included as a comparator [74]. Insulin has also been shown to have an anabolic effect on muscle [75-78], but has been implicated in weight gain following subcutaneous administration to diabetic individuals, which is a result in part of its ability to stimulate differentiation of preadipocytes, and to increase the uptake of glucose into adipocytes and inhibit triglyceride breakdown [79-82]. It was therefore of interest to compare the effect of topical and subcutaneous administration of these three peptides in obese animals.

Outbred Swiss mice when kept on an ad lib diet of mouse chow have a tendency to become obese and may reach weights > 45 g (normal weight 25 g). Dissection of mice following euthanasia reveals obvious deposits of visceral fat (omental and mesenteric), subscapular fat and paired inguinal

Table 1. Immune response to tetanus toxoid given by various routes.

Vaccine	Route	Adjuvant	Antibody response
Saline	Intramuscular	–	ND
TT in ME	Topical	–	140,000 ± 30,000
TT in ME	Topical	Alum	25,000 ± 10,000
TT in saline	Intramuscular	–	60,000 ± 25,000
TT in saline	Intramuscular	Alum	170,000 ± 60,000

ME: Microemulsion; TT: Tetanus toxoid.

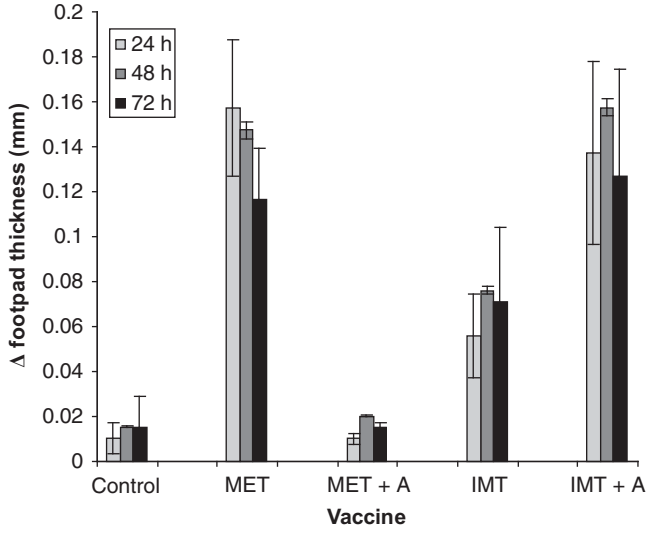

Figure 3. Topical vaccination with tetanus toxoid. Tetanus toxoid (T, 1.8 LF or 20 μg) alone or precipitated with alum (T + A, 1:5) was administered topically to abdominal skin (ME) or by injection into the quadriceps muscle (IM) in C57 black mice (n = 5). Tetanus-specific serum IgG titers were measured two weeks after three rounds of vaccination (**Table 1**). Delayed type hypersensitivity was measured by injection of 20 μg of T into the right footpad and saline into the left footpad of mice and measurement of footpad swelling relative to the saline control at 24, 48 and 72 h post-injection using electronic calipers. Columns represent the average and error bars the standard error for 10 mice [31].
LF:Limit of flocculation.

deposits. Also, the obese mice show a considerable increase in subcutaneous fat, as evidenced by an increase in the weight of the skin from 3.65 ± 0.46 g to 4.37 ± 0.75 g.

Studies have been carried out on obese mice of varying weight to compare the effect of subcutaneously and topically administered insulin, IGF-I and GHRP-6 on body weight, muscle mass and weight of skin and fat. In paired studies comparing subcutaneous injection with topical application in a W/O ME, several interesting findings were noted regarding the anabolic effects of insulin, IGF-I and GHRP-6. Topically applied insulin, IGF-I and GHRP-6 all had an anabolic effect on the percentage muscle mass measured in treated animals when compared with control animals. By comparison, there was no increase in muscle mass in animals receiving these doses by means of subcutaneous injection. By contrast, all treatments (insulin, IGF-I and GHRP-6) had an effect on reducing the weight of adipose tissue, regardless of route. The greatest effect, however, was observed with topically applied insulin in a W/O ME (**Figure 4A, B**).

In a separate study, the effect of increasing the dose of topically administered insulin formulated in a water-in-oil microemulsion was compared with subcutaneously administered insulin. It was possible to increase the dose of topically administered insulin from 10 to 100 μg as there was no reduction in serum glucose seen at this dose (results not shown). By contrast, it was not possible to increase the dose of subcutaneously administered insulin owing to the potential of death through induction of hypoglycemia. As is normal for this strain of mice, the control animals gained weight during the experiment. By contrast, topical or subcutaneously administered insulin had a differing effect, which depended on the initial weight of the mice. Thus, those mice whose starting weight was > 30.0 g lost weight, with the largest weight loss observed with the mice that were heaviest at the start of the experiment and who received the highest dose of topical insulin (100 μg). By contrast, the mice whose starting weight was < 30 g gained weight, with the highest weight gain on the smallest mice given the highest dose of topical insulin (100 μg) (**Figure 5**). These data are consistent with the known anabolic effect of insulin [75-78] and possibly suggest receptor downregulation on adipocytes, with resultant breakdown of stored fats (lipolysis) [79-82]. Presumably the greater effect seen with the topical insulin is due to the depot-like effect of this route of administration, leading to a longer stimulation of both adipocytes and muscle cells.

4. Conclusion

Effective peptide and protein delivery to the skin has received much attention in the pharmaceutical industry, with many companies developing a variety of delivery devices to force peptides and proteins into and across the epithelium of the skin. Despite these many attempts, effective delivery of high-molecular-mass compounds has at best been poor. The water-in-oil microemulsion system as described in this review effectively overcomes the water-impermeable barrier of the epidermis and allows for effective delivery of highly water-soluble molecules such as peptides and proteins following topical application. Using this delivery formulation, high bioavailability of topically administered peptides and proteins, ranging from 900 Da molecular mass to > 150,000 Da molecular mass, has been achieved. Effective needle-free vaccine delivery has been achieved, as well as a new regime for weight loss, and the stimulation of anabolic activity in muscles has been achieved using topically delivered insulin, GHRP-6 and IGF-I. The studies presented add to earlier findings, which have shown effective delivery of monoclonal

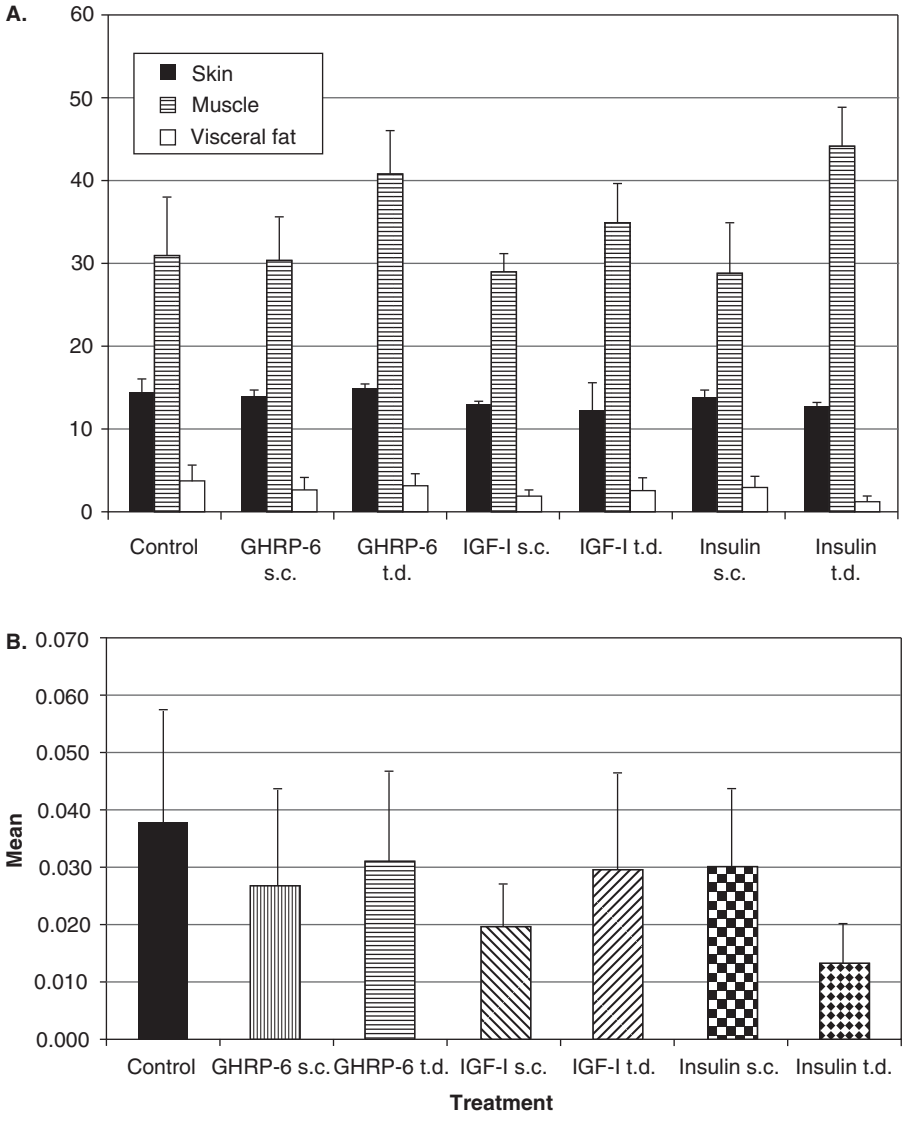

Figure 4. A. Variation in skin, muscle and visceral fat following subcutaneous (in saline) or transdermal (in water-in-oil microemulsion) administration of GHRP-6 (10 μg/dose), IGF-I (10 μg/dose) and insulin (10 μg/dose) (n = 5). **B.** Variation in visceral fat following subcutaneous (in saline) or transdermal (in water-in-oil microemulsion) administration of GHRP-6 (10 μg/dose), IGF-I (10 μg/dose) and insulin (10 μg/dose) (n = 5). Daily doses for 14 days.
s.c.: Subcutaneous; t.d.: Topical.

antibodies and Fc-fusion proteins using topically applied water-in-oil microemulsions.

5. Expert opinion

The examples outlined in the preceding text do much to overcome many of the weaknesses in the field of topical delivery of peptides and proteins. In the past the general 'feeling' about delivery of peptides and proteins transdermally was that it would require some method of permeation enhancement or skin damage to achieve successful delivery. Even then the quantity of material delivered was small, and the molecular mass of the material delivered was generally low (< 6000 Da). Furthermore, the molecules delivered were generally hydrophobic, and the area of application was small. Transdermal delivery using water-in-oil microemulsions overcomes many of these problems. Thus, it is now possible to deliver large quantities of high-molecular-mass hydrophilic peptides or proteins using a vehicle that is well tolerated, does not cause mechanical disruption of the skin, and which can deliver material locally, regionally, or systemically.

This mode of delivery has enormous potential in many different areas of medicine, including vaccine delivery, topical delivery of therapeutic proteins, particularly molecules such

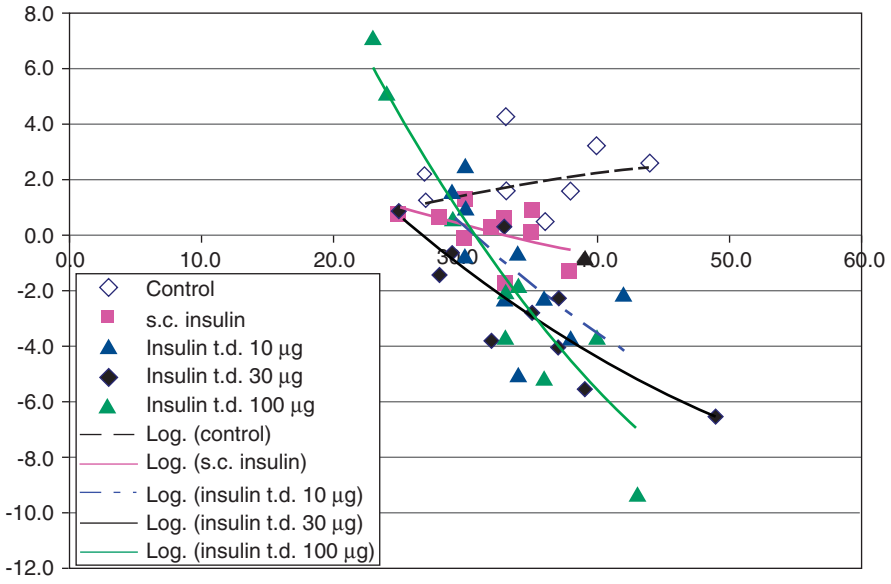

Figure 5. Effect of administration of insulin formulated in saline and given subcutaneously, or formulated in a water-in-oil microemulsion and administered topically. Female Swiss mice received formulations daily for 14 days, after which the mice were killed and their tissues dissected and weighed. Data are plotted as the change in body weight of the mice following treatment. Log-linear regression lines are plotted for with R^2 values. Control $R^2 = 0.0843$; insulin s.c. 10 μg $R^2 = 0.2081$; insulin t.d. 10 μg $R^2 = 0.4543$; insulin t.d. 30 μg $R^2 = 0.7255$; insulin t.d. 100 μg $R^2 = 0.8254$. The *x*-axis represents starting weight of mice (grams), the *y*-axis is the change in weight (grams).
s.c.: Subcutaneous; t.d.: Topical.

as anti-TNF, or TNF-Fc-fusion proteins for the treatment of inflammatory conditions such as psoriasis, rheumatoid arthritis, osteoarthritis, dermatitis, and so on, and imaging of sentinel lymph nodes, without the need for invasive surgery, which has particular relevance to the treatment of breast and skin cancer. Also, the prolonged profile of delivery using this route means that this mode of delivery has the potential to replace many of the depot delivery systems that are injected at present.

The major hurdle to transdermal delivery of proteins has thus been overcome with this mode of delivery, hence it has been demonstrated that it is possible to deliver pharmaceutically relevant doses of peptides and proteins using a vehicle that is pharmaceutically acceptable, cheap and easy to manufacture, and which is stable on storage. Foreseeable minor problems revolve around the stability of proteins when placed in solution. Thus, for some of the commercially available anti-TNF preparations, it was found that the proteins were not stable on reconstitution in buffer alone, presumably owing to the presence of small amounts of contaminating enzymes, which eventually degraded the protein. This was more a problem of the protein solution itself than the formulation into a water-in-oil microemulsion. Similarly, some proteins, such as human insulin utilized in the studies outlined above, tend to crystallize slowly on standing, particularly when stored at the concentration used in the microemulsions. This is a relatively minor formulation issue that can be overcome through the use of a different insulin analogue (Lispro, or insulin Aspart), or by means of slight modifications to the insulin formulation. This disadvantage is offset by the ability to deliver poorly water-soluble peptides and proteins, which can be more readily solubilized by the dual water/oil formulation. Thus, many peptide antagonists are poorly water-soluble and must be injected in dispersing agents such as corn oil; these peptides are ideally suited for delivery in the much more acceptable water-in-oil formulation.

At present it is believed that most of the technical issues involved in formulation and delivery of peptides and proteins through the skin have been resolved. Continuing issues will therefore involve those usually associated with clinical trialing and registration with the relevant regulatory bodies, such as the Therapeutic Goods Administration (TGA) and FDA. There may also be some more hurdles owing to the change in bioactivity of the topically applied material when compared with already registered subcutaneously administered material, particularly in light of the different pharmacokinetic profiles and the difference in biological response observed with the altered pattern of distribution of the applied material (see Section 3.3).

Apart from the obvious advantages that will eventuate from needle-free injection of peptides and proteins, particularly exciting possibilities exist in true site-directed targeting of peptides and proteins for use in the many skin conditions that must at present use the subcutaneous route of administration. It is believed that much more effective

treatment will be achieved with far fewer side effects. Thus, there are many adverse side effects observed with continued subcutaneous administration of the many monoclonal antibodies now being developed or sold for the treatment of inflammation, the most serious sequela of this being death! Hopefully these adverse effects will be greatly reduced or even eliminated by topical delivery in water-in-oil microemulsions.

Perhaps even more benefit will be seen in the areas of breast and skin cancer, both for imaging of the primary lesion and the sentinel lymph nodes and also in the treatment of these insidious diseases. Thus, the lymph-seeking behavior of the water-in-oil microemulsion should be of particular benefit in the detection and treatment of both of these conditions.

In addition to the delivery of water-soluble proteins, the use of water and oil-soluble surfactants in the formation of the microemulsion has the extra advantage of being able to solubilize poorly soluble molecules [83,84]. For this reason, delivery of many hydrophobic molecules that have limited commercial utility, owing to delivery problems, will be able to achieve commercial reality through both the solubilizing power of the water-in-oil microemulsions as well as the transdermal delivery potential shown by such formulations. The biphasic nature of the water-in-oil microemulsion has the further advantage of being able to deliver both water and oil-soluble molecules simultaneously [85,86].

An exciting area of potential development is weight control management. The results using insulin, IGF-I and GHRP-6 given topically are particularly intriguing. Whether these results can be replicated in humans and whether the use of these drugs for potential treatment of obesity will be commercially viable will be particularly interesting to observe.

Declaration of interest

The authors declare no conflict of interest. G Russell-Jones is employed by Mentor Pharmaceutical Consulting Pty Ltd.

Bibliography

1. Subedi RK, Oh SY, Chun MK, et al. Recent advances in transdermal drug delivery. Arch Pharm Res 2010;33(3):339-51
2. Williams AC, Barry BW. Penetration enhancers. Adv Drug Deliv Rev 2004;56:603-18
3. Walker RB, Smith EW. The role of percutaneous penetration enhancers. Adv Drug Deliv Rev 1996;18:295-301
4. Cevce G. Lipid vesicles and other colloids as drug carriers on the skin. Adv Drug Deliv Rev 2004;56:675-711
5. Leichtnam ML, Rolland H, Wuthrich P, et al. Formulation and evaluation of a testosterone transdermal spray. J Pharm Sci 2006;95:1693-702
6. Ainbinder D, Touitou E. Testosterone ethosomes for enhanced transdermal delivery. Drug Deliv 2005 12:297-303
7. Gooren LJG, Bunck MCM. Transdermal testosterone delivery: testosterone patch and gel. World J Urol 2003;21:316-19
8. El-Kamel AH, Al-Fagih IM, Alsarra I. Testosterone solid lipid microparticles for transdermal drug delivery. Formulation and physicochamical characterization. J Microencapsul 2007;24(5):457-75
9. Fang J-Y, Yu S-Y, Wu P-C, et al. In vitro skin permeation of estradiol from various proniosome formulations. Int J Pharm 2001;215:91-9
10. Ghafourian T, Fooladi S. The effect of structural QSAR parameters on skin penetration. Int J Pharm 2001;217:1-11
11. CeveCevc G, Blume G, Schatzlein A, et al. The skin: a pathway for systemic treatment with patches and lipid-based agent carriers. Adv Drug Deliv Rev 1996;18:349-78
12. Choi MJ, Maiback HI. Liposomes and niosomes as topical drug delivery systems. Skin Pharmacol Physiol 2005;18:209-19
13. Mezei M, Gulasekharam V. Liposomes – a selective drug delivery system for the topical route administration. I. Lotion dosage forms. Life Sci 1980;26:1473-7
14. Mezei M, Gulasekharam V. Liposomes – a selective drug delivery system for the topical route administration: gel dosage form. J Pharm Pharmacol 1982;34:473-4
15. Jain S, Jain P, Umamaheshwari RB, et al. Transfersomes – A novel vesicular carrier for enhanced transdermal delivery: development, characterization, and performance evaluation. Drug Dev Ind Pharm 2003;29:1013-26
16. Walters KA. Penetration enhancers and their use in transdermal therapeutic systems. In: Hadgraft J, Guy RH, editors, Transdermal drug delivery, developmental issues and research initiatives, Marcel Dekker, New York; 1989. p. 197-246
17. Langer R. Transdermal drug delivery; past progress, current status, and future prospects. Adv Drug Deliv Rev 2004;56:S57-8
18. Denet A-R, Vanbever R, Preat V. Skin electroporation for transdermal and topical delivery. Adv Drug Deliv Rev 2004;56(5):659-74
19. Kalia YN, Naik A, Garrison J, et al. Iontophoretic drug delivery. Adv Drug Deliv Rev 2004;56:619-58
20. Giudice EL, Campbell JD. Needle-free vaccine delivery. Adv Drug Deliv Rev 2006;58:68-89
21. Krestos K, Kasting GB. Dermal capillary clearance: physiology and model. Skin Pharmacol Physiol 2005;18:55-74
22. Aukland K, Reed RK. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. Physiol Rev 1993;73:1-78
23. Ikomi F, Hunt J, Hanna G, et al. Interstitial fluid, plasma protein, colloid, and leukocyte uptake into initial lymphatics. J Appl Physiol 1996;81:2060-7
24. Reed RK, Ishibashi M, Townsley MI, et al. Blood-to-tissue clearance vs. lymph analysis in determining capillary transport characteristics for albumin in skin. Am J Physiol 1993;264:H1394-401
25. Renkin EM, Wiig H. Limits to steady-state lymph flow rates derived from plasma-to-tissue uptake

measurements. Microvasc Res 1994;47:318-22

26. Schmid-Schonbein GW. Microlymphatics and lymph flow. Physiol Rev 1990;70:987-1028
27. Leu AJ, Husmann JW, Held T, et al. Measurement of the lymphatic clearance of the human skin using a fluorescent tracer. J Vasc Res 2001;38(5):423-31
28. Uren RF, Howard-Giles R, Thompson JF. Patterns of lymphatic drainage in the skin of patients with melanoma. J Nucl Med 2003;44:570-82
29. Russell-Jones GJ, Ey PL, Reynolds BL. Inhibition of cutaneous anaphylaxis and arthus reactions in the mouse by antigen-specific IgA. Int Arch Allergy Appl Immunol 1981;66:316-25
30. Russell-Jones GJ, Lee SR, McMenigall K, et al. Topical delivery of proteins using microemulsions. Proceedings of 34th International Symposium on Controlled Release of Bioactive Materials held in Los Angeles, CA; 2007
31. Russell-Jones GJ, Luke MR, Himes SR. Transdermal Delivery of Pharmaceutical Agents. WO2007070983; 2007. USP 2007/0243132. (Priority Date 22/12/05), Eu, Au (PCT 2006/001999)
32. de Wilt JH, Thompson JF, Uren RF, et al. Correlation between preoperative lymphoscintigraphy and metastatic nodal disease sites in 362 patients with cutaneous melanomas of the head and neck. Ann Surg 2004;239(4):544-52
33. Uren RF, Howman-Giles RB, Chung D, et al. Role of lymphoscintigraphy for selective sentinel lymphadenectomy. Cancer Treat Res 2005;127:15-38
34. Uren RF, Thompson JF, Howman-Giles R, et al. The role of lymphoscintigraphy in the detection of lymph node drainage in melanoma. Surg Oncol Clin N Am 2006;15(2):285-300
35. Uren RF, Howman-Giles R, Chung DK, et al. The reproducibility in routine clinical practice of sentinel lymph node identification by pre-operative lymphoscintigraphy in patients with cutaneous melanoma. Ann Surg Oncol 2007;14(2):899-905
36. Thompson JF, Uren RF. Lymphatic mapping in management of patients with primary cutaneous melanoma [review]. Lancet Oncol 2005;6(11):877-85
37. Thompson JF, Uren RF, Saw RP, et al. Internal mammary node metastasis from primary cutaneous melanoma on the anterior chest. ANZ J Surg 2005;75(8):723-5
38. Reynolds HM, Smith NP, Uren RF, et al. Three-dimensional visualization of skin lymphatic drainage patterns of the head and neck. Head Neck 2009;31(10):1316-25
39. Reynolds HM, Walker CG, Dunbar PR, et al. Functional anatomy of the lymphatics draining the skin: a detailed statistical analysis. J Anat 2010;216(3):344-55
40. Mills PC, Cross SE. Transdermal drug delivery: basic principles for the veterinarian. Vet J 2006;172:218-33
41. Mills PC, Magnusson BM, Cross SE. The effects of vehicle and region of application on in vitro penetration of testosterone through canine skin. Vet J 2006;171:276-80
42. Russell-Jones GJ, Lee SR, McMenigall K, et al. Topical delivery of proteins using microemulsions. Recent advances in drug delivery systems. Salt Lake City, Utah; 2007; Elsevier, Georgia
43. Himes R, Lee S, McMenigall K, Russell-Jones GJ. Reduction in inflammation in the footpad of carrageenan treated mice following the topical administration of anti-TNF molecules formulated in a Microemulsion. JCR J Control Release 2010;145(3):210-13
44. Constantinedes PP, Scalart J-P, Lancaster C, et al. Formulation and intestinal absorption enhancement evaluation of water-in-oil microemulsions incorporating medium chain glycerides. Pharm Res 1994;11:1385-90
45. Paul BK, Moulik SP. Uses and applications of microemulsions. Curr Sci 2001;80:990-1001
46. Prince LM, editor, Microemulsions, theory and practice. Academic Press, New York; 1977
47. Schulman JH, Stoeckenius W, Prince LM. Mechanism of formation and structure of microemulsions by electron microscopy. J Phys Chem 1959;63:1677-80
48. Kumar P, Mittal KL, editors, Handbook of microemulsion science and technology. CRC, Dekker, New York; 1999
49. Kreilgaard M. Influence of microemulsions on cutaneous drug delivery. Adv Drug Deliv Rev 2002;54:S77-98
50. Kreilgaard M, Pedersen EJ, Jaroszewski JW. NMR characterisation and transdermal drug delivery potential of microemulsion systems. J Control Release 2000;69:421-33
51. Kreilgaard M, Kemme MJB, Burggraaf J, et al. Influence of a microemulsion vehicle on cutaneous bioequivalence of a lipophilic model drug assessed by microdialysis and pharmacodynamics. Pharm Res 2001;18:593-9
52. Kemken J, Ziegler A, Muller BW. Investigations into the pharmacodynamic effects of dermally administered microemulsions containing beta-blockers. J Pharm Pharmacol 1991;43:679
53. Kemken J, Ziegler A, Muller BW. Pharmacodynamic effects of transdermal bupranolol and timolol in vivo: comparison of microemulsion and matrix patches as vehicle. Methods Find Exp Clin Pharmacol 1991;13:361-5
54. Kemken J, Ziegler A, Muller BW. Influence of supersaturation on the pharmacodynamic effect of bupranolol after dermal administration using microemulsions as vehicle. Pharm Res 1992;9:554-8
55. Sintov AC, Shapiro L. New microemulsion vehicle facilitates precutaneous penetration in vitro and cutaneous drug bioavailability in vivo. JCR 2004;95:173-83
56. Sintov AC, Krymberk I, Gavrilov V, et al. Transdermal delivery of paracetamol for pediatric use: effects of vehicle formulations on the percutaneous penetration. J Pharm Pharmacol 2003;55:911-19
57. Delgado-Charro MB, Iglesias-Vilas G, Blanco-Mendez J, et al. Delivery of a hydrophilic solute through the skin from novel microemulsion systems. Eur J Pharm Biopharm 1997;43:37-42
58. Schmalfuss U, Neubert R, Wohlrab W. Modification of drug penetration into human skin using microemulsions. J Control Release 1997;46:279-85
59. Rhee Y-S, Choi J-G, Park E-S, et al. Transdermal delivery of ketoprofen using microemulsions. Int J Pharm 2001;228:161-70

60. Valenta C, Schultz K. Influence of carrageenan on the rheology and skin permeation of microemulsion formulations. JCR 2004;95:257-65

61. Shukla A, Krause A, Neubert RHH. Microemulsions as colloidal vehicle systems for dermal drug delivery. IV. Investigation of microemulsion systems based on a eutectic mixture of lidocaine and prilocaine as the colloidal phase by dynamic light scattering. J Pharm Pharmacol 2003;55:741-8

62. Ambade KW, Jadhav SL, Gambhire MN, et al. Formulation and evaluation of flurbiprofen microemulsion. Curr Drug Deliv 2008;5:32-41

63. Kantarci G, Ozguney I, Karasulu HY, et al. Comparison of different water/oil microemulsions containing diclofenac sodium: preparation, characterization, release rate, and skin irritation studies. AAPS PharmSciTech 2007;2:E91-8

64. Osborne DW, Ward AJ, O'Neill KJ. Microemulsions as topical drug delivery vehicles. Part 1. Characterization of a model system. Drug Dev Ind Pharm 1988;14:1202-19

65. Osborne DW, Ward AJ, O'Neill KJ. Microemulsions as topical drug delivery vehicles: in vitro transdermal studies of a model hydrophilic drug. J Pharm Pharmacol 1991;43:450-4

66. Alvarez-Figueroa MJ, Blanco-Mendez J. Transdermal delivery of methotrexate: ionophoretic delivery from hydrogels and passive delivery from microemulsions. Int J Pharm 2001;215:57-65

67. Baroli B, Lopez-Quintela MA, Delgado-Charro MB, et al. Microemulsions for topical delivery of 8-methoxsalen. J Control Release 2000;69:209-18

68. Getie M, Wohlrab J, Neubert RHH. Dermal delivery of desmopressin actetate using colloidal carrier systems. J Pharm Pharmacol 2005;57:423-7

69. Goebel A, Neubert RHH. Dermal peptide delivery using colloidal carrier systems. Skin Pharmacol Physiol 2008;21:3-9

70. Steinman RM, Pope M. Exploiting dendritic cells to improve vaccine efficiency. J Clin Invest 2002;109:1519-26

71. Gupta PN, Mishra V, Rawat A, et al. Non-invasive vaccine delivery in transferosomes, niosomes, and liposomes: a comparative study. Int J Pharm 2005;293:73-82

72. Ruiz-Torres A, Soares M, Kirzner M. Ageing and Longevity are related to Growth Hormon/insulin-like growth factor-1 secretion. Gerontology 2002;48:401-7

73. Scacchi M, Pincelli AI, Cavagnini F. Growth hormone in obesity. Int J Obese Relat Metab Disord 1999;23:260-71

74. Yang L, Morriello G, Patchett AA, et al. 1-[2(R)-(2-Amino-2-methylpropionylamino)- 3-(1H-indol-3-yl)propionyl]-3- benzylpiperidine-3(S)-carboxylic Acid Ethyl Ester (L-163,540): a potent, orally bioavailable, and short-duration growth hormone secretagogue. J Med Chem 1998;41:2439-41

75. Hillier TA, Fryburg DA, Jahn LA, et al. Extreme hyperinsulinemia unmasks insulin's effect to stimulate protein synthesis in human forearm. Am J Physiol 1998;274:E1067-74

76. Chow LS, Albright RC, Bigelow ML, et al. Mechanism of insulin's anabolic effect on muscle: measurements of muscle protein synthesis and breakdown using aminoacyl-tRNA and other surrogate measures. Am J Physiol Endocrin Metab 2006;291:E729-36

77. Nygren F, Nair KS. Differential regulation of protein dynamics in splanchnic and skeletal muscle beds by insulin and amino acids in healthy human subjects. Diabetes 2003;52:1377-85

78. Fujita S, Rasmussen BB, Cadenas JG, et al. Aerobic exercise overcomes the age-related insulin resistance of muscle protein metabolism by improving endothelial function and Akt/mammalian target of rapamycin signaling. Diabetes 2007;56:1615-22

79. Garvey WT, Olefsky JM, Marshall S. Insulin receptor down-regulation is linked to an insulin-induced postreceptor defect in the glucose transport system in rat adipocytes. J Clin Invest 1985;76:22-30

80. Berhanu P, Kolterman OG, Baron A, et al. Insulin receptors in isolated human adipocytes: characterization by photaffinity labeling and evidence for interanlization and cellular processing. J Clin Invest 1983;72:1958-70

81. Knutson VP, Donnelly PV, Balba Y, et al. Insulin resistance is mediated by a proteolytic fragment of the insulin receptor. JBC 1995;270:24972-40

82. Torossian K, Nower P, Schwartz T, et al. Phorbol esters inhibit insulin-induced receptor down-regulation in cultured human lymphocytes: association with diminished insulin receptor autophosphorylation. Biochem J 1993;290:151-8

83. Kawakami K, Yoshikawa T, Hayashi T, et al. Microemulsion formulation for enhanced absorption of poorly soluble drugs. II. In vivo study. J Control Release 2002;81:75-82

84. Kawakami K, Yoshikawa T, Moroto Y, et al. Microemulsion formulation for enhanced absorption of poorly soluble drugs. I. Prescription design. J Control Release 2002;81:65-74

85. Lee PJ, Langer R, Shastri VP. Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs. Pharm Res 2003;20:264-9

86. Hosmer J, Reed R, Bentley MV, et al. Microemulsions containing medium-chain glycerides as transdermal delivery systems for hydrophilic and hydrophobic drugs. AAPS PharmSciTech 2009;10(2):589-96

Affiliation

Gregory Russell-Jones[†1] & Roy Himes[2]
[†]Author for correspondence
[1]Mentor Pharmaceutical Consulting Pty Ltd,
23 Greenfield Ave, Middle Cove,
Sydney, New South Wales 2069,
Australia
Tel: +61 2 9417 6707;
E-mail: mentor127@optusnet.com.au
[2]University of Wollongong,
Northfields Ave, Wollongong,
New South Wales 2522,
Australia