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ABSORPTION OF THERAPEUTIC PEPTIDES

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BACKGROUND

Advances in molecular biology and synthetic chemistry have now made possible the development, production, and purification of a wide number of novel peptide and protein compounds. These new peptide and protein compounds provide new opportunities for drug therapy. They also pose a particular challenge to our ability to develop new systems for drug administration.

While oral administration is the most attractive route for the administration of any drug, oral delivery of this class of compounds has been extremely difficult. Enzymatic hydrolysis both in the gastrointestinal lumen and at the epithelial surface destroys much of an administered dose. Peptides and proteins are also poorly absorbed across the gastrointestinal epithelium. Removal from the portal circulation prior to reaching the systemic circulation is also significant.(1)

In contrast to many traditional drugs, many peptides will have their optimal therapeutic benefit when they are administered with a goal other than steady state kinetics. The endogenous secretion of most peptides and proteins does not follow steady state kinetics and the traditional goal of steady state concentrations of drug may be innappropriate for therapy with peptides. Most endogenous peptides are secreted in frequent small pulses. These pulses may vary considerably in their frequency and amplitude over the course of a 24 hour period. When pharmacologic therapy is designed to replace or mimic the endogenous secretion pattern, optimal pharmacologic effect may only be achieved when

a similar pattern of administration is achieved. The administration of a peptide with a pharmacokinetic profile different than the endogenous compound, may produce a paradoxical effect.(2)

NEW DEVELOPMENTS

Optimal exploitation of the therapeutic opportunities offered by peptide drugs will require the development of novel techniques for administration of these agents. These techniques should ideally provide reliable and efficient absorption of peptides with a capacity for both steady infusion of compound as well as intermittent bolus administration. A variety of efforts have been made to respond to this need. The areas of development are outlined in Table I. While promising work has been done in each of the highlighted areas, in each case significant problems remain.

The ideal route for administration of any drug would be the oral route. Ease of administration and compliance make this extremely attractive. The problems of oral bioavailability can be partially alleviated by alterations in peptide structure designed to render them resistant to hydrolysis, or by disguising peptides in more readily absorbable forms (1). Enalapril and cyclosporin are two peptide compounds in which oral absorption has proved practical. Recent research has suggested that insulin in "chylomicron"-like emulsions accompanied by protease inhibitors may make oral administration of this compound feasable.(3) The use of bioadhesive polymers to cause administered drug to "stick" to the gastrointestinal mucosa, or the use of protective agents to allow delivery of the peptide or protein to the colon, where enzymatic degradation is diminished, and mucosal absorption may be achieved, may allow the development of other oral preparations.(4-6) To date these efforts are very preliminary. It is also worth noting that even if successful, the temporal characteristics of the delivery of the drug might be less than ideal.

TABLE I

Potential Techniques for the Administration of Peptide and Protein Drugs

Route of Administration	Strengths	Weaknesses
Parenteral		
Depot	Predictable	Zero Order
	Long Duration	Irreversible
	Zero Order	
Pumps	Demand ± Infusion	Expensive
	Predictable	Cumbersome
		Needles
Transdermal	Compliance	Variability/Depots
	Variable Rates	Cutaneous Toxicity
		Antigenicity
Nasal	Compliance	Local Toxicity
	Rapid Onset	Variability
Inhalational	Compliance	Unexplored
Rectal	?	Compliance /Variability
		Needs Enhancers
Opthalmic	Compliance	Variability
Oral	Ideal Route	Time Course/Efficiency
		Is it possible ?

All of the alternatives to the oral route have produced some successes. All also have significant limitations. Depot preparations are more predictable and efficient, but suffer substantially from an inability to vary the rate of drug delivery over time. The use of depot

preparations has the attraction of single dose administration for prolonged therapy of 30-90 days, but has the major disadvantage of inflexibility in dosing and committment to steady state kinetics. Infusion pumps avoid this problem, but are expensive, require a committed and sophisticated patient, and despite advances in miniaturization, will remain somewhat cumbersome. Nasal administration has been looked at extensively, and both ADH and LHRH analogues are now commercially available for administration by this route. The extension of this technique to other peptides remains in question. Many larger peptides appear to require the use of an enhancer for significant nasal absorption.(7) A recent report of the successful inhalational administration of the LHRH agonist leuprolide to human volunteers raises the interesting possibility of using the pulmonary alveolar bed for peptide administration.(8) This area merits further investigation.

NEW DEVELOPMENTS IN TRANSDERMAL DELIVERY

Our research has investigated the feasibility of the utilization of the transdermal route for peptide drug administration. We have investigated this route because it offers the attraction of ease of application and good patient acceptance, the possibility of modulation of drug effect by varying the rate of drug absorption, and ease of rapid discontinuation of therapy.

The outermost layer of the human skin is the stratum corneum. This portion of the skin is approximately 10 to 40 cells in thickness, and is composed of a rich extracellular matrix of lipids arranged in a highly ordered fashion. The keratinocytes are immersed like bricks in this extracellular lipid mortar work. The stratum corneum is pierced by hair follicles and eccrine glands, which comprise less than one tenth of one percent of the total skin surface area. The structure of the stratum corneum establishes it as a very effective barrier to the absorption of

most chemical entities(9).

Traditional transdermal absorption techniques have used as the driving force for absorption, the passive diffusion of drug along a concentration gradient established across the stratum corneum. Successful passive absorption of drug requires a potent molecule which partitions equally between lipid and water, has a wide toxic therapeutic ratio, and a very low molecular weight. Peptides do not generally fit this description. Therefore, successful transdermal administration will require the modification of the traditional techniques for transdermal absorption. We have sought to overcome these limitations by using an electrical current to alter the pattern of cutaneous absorption, and to provide a means to actively manipulate and control the transdermal absorption of peptides and proteins.

The use of electrical fields to enhance the transdermal transport of a compound is generally referred to as "iontophoretic" delivery. The technique of iontophoretic transport was first described over a century ago, and has been reported on intermittently since that time.(10,11) The traditional understanding of that process held that the technique was effective because the electrical field directly induced the movement of the charged solute (drug) across the stratum corneum. The full utility of this technique was not explored at least in part because an analysis of most drugs suggested that they were not highly mobile in electrical fields, and that successful administration of therapeutic quantities across the skin would require the use of levels of electrical energy that would produce severe and unacceptable cutaneous injury.

Gangarosa and colleagues were among the first to suggest that this simple interpretation of iontophoretic transport was inadequate. He noted that a variety of non-ionic solutes were transported across mouse skin during the process of water iontophoresis. This transport of neutral

compounds could not be explained by the use of routine iontophoretic transport theory. Gangarosa termed the process "iontohydrokinesis".(12) Subsequent investigations by Barry,(13) Burnette,(14,15) and most recently by Pikal(16-18) have clarified the nature of the process that is present.

In situations where passive diffusion is small, and where iontophoretic transport of solute is small, the induction of water flow across the skin occurs by electroosmotic and by transport number effect. The transdermal transport of water appears to have two major effects upon transdermal absorption. Hydration of skin alters cutaneous permeability. This occurs separate and apart from the direct effect of an electrical field. Thus, electrically mediated transport can function as a technique for cutaneous hydration, and may produce primary changes in the passive permeability characterstics of the skin. In addition, the magnitude of water flow induced by electroosmosis is sufficient to induce the "convective" transport of solutes dissolved within the water.

Burnette demonstrated that significant transdermal flux of the tripeptide TRH occurred in solutions at pH's of 4 and 8, with current values ranging between 0.1 and 0.5 mA/cm^2. Transport was greater at pH 8 (where TRH is without charge) as compared to pH 4, (where a small portion of TRH is in a charged form). However, even at the lower pH, ionic flow appeared to account for less than one tenth of one percent of the observed charge transport.(19) Similar data for other neutral compounds is also now available.(18)

We have attempted to investigate the practical utility of this technique for the transdermal administration of peptides and proteins. To investigate this technique, we needed a self contained and easily applied electrically powered transdermal patch. This patch (POWERPATCH (R), Drug Delivery Systems Inc., New York, N.Y.) has small, flexible batteries as a source of electrical power, an integrated circuit system to control

current, two drug reservoirs at the positive and negative electrodes, and a peripheral adhesive to keep it adherent to the skin. Using this iontophoretic patch system, we have investigated the feasibility of iontophoretic administration of therapeutic doses of peptides to humans.

Leuprolide is nine amino acid polypeptide with a molecular weight of 1209. It is neutral or positively charged at most pH values, having a pka above 8. The carboxyl terminus of the molecule has been replaced with an ethyl amide group. The compound is an LHRH agonist, and is extremely safe for acute administration. This makes the use of human volunteers feasible.

Our initial investigation was a randomized double-blind cross over trial in 13 normal male volunteers. These volunteers were studied on 2 days, 7 days apart. On one study day, they received electrically powered patches containing 5 mg of leuprolide added to the positive electrode. The patches were calibrated to deliver a constant direct current of 0.22 mA. This current was distributed over a surface area of 40 cm^2 at the positive electrode, giving a current density at the positive electrode of 0.005 mA/cm.

On the alternate study day, the subjects received an identical appearing patch containing 5 mg of leuprolide, but without a completed electrical current. The patches were left in place for 8 hours, during which time serum LH levels were obtained to monitor for pharmacologic effect. Mean serum LH levels (Figure 1) showed significant drug effect from active, but not from passive patches. Significant differences between the two arms were seen by 90 minutes (using a significance level of 0.01) and were maintained for the duration of the study. ANOVA also showed highly significant differences ($p = 0.0084$) and a "therapeutic" response (doubling of baseline LH levels) was seen in 12 of 13 subjects studied.(20)

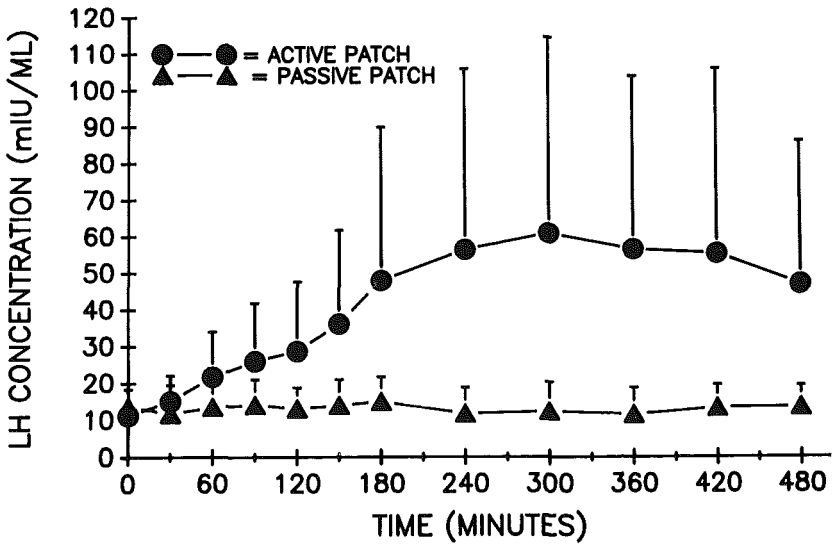

Figure 1: Mean serum LH response (± standard deviation) to transdermal leuprolide administration by "passive" or "active"(iontophoretic) technigues. Reprinted from reference (20) with permission.

In subsequent investigations we have compared the LH response seen with this transdermal administration technique, to the response observed after sub-cutaneous administration. The LH response to sub-cutaneous drug is more rapid peaking as early as 30-60 minutes after the administration of drug. However, the magnitude of LH response as judged by the height of the peak LH response or the AUC for LH response, does not differ between the transdermal and sub-cutaneous routes.(21,22)

Subsequent studies have analyzed serum leuprolide concentrations in individuals receiving patches containing leuprolide at concentrations of 10 mg/ml in either 0.5 or 0.05 molar sodium acetate buffering solution. Again, an electrical current of 0.22 mA and an approximate current density at the positive electrode of 0.005 mA/cm^2 was used. Mean serum leuprolide concentrations were between 400 and 1100 ng/ml for the duration of a 10 hour study. LH response (a doubling of LH levels was seen in 7 of

11 subjects.(23)

TABLE II

Serum Leuprolide Concentrations In Male Volunteers

Current 0.2 mA Current Density 0.0005 mA/cm^2

Leuprolide 10 mg/ml (0.4 ml) Na Acetate Buffer 0.5 M or 0.05 M

Responders		Non-Responders
N = 7		N = 4
	Mean LH	
32 mIU/ml	Response	15 mIU/ml
1.18 ± 0.65 ng/ml	Leuprolide Concentration at 150 minutes	0.19 % 0.12 ng/ml
517 ± 179 ng-min/ml	AUC_{0-600}	166 ± 34 ng-min/ml

The patches have been well tolerated by the over 40 individuals we have studied. The level of current used for these studies is well below the cutaneous threshold for pain. Some individuals reported a tingling sensation at the time of patch application. This was always transient in nature. At the time of patch removal small amounts of erythema were noted in approximately one-third of the subjects studied. This erythema resolved in a period of minutes to hours after patch application. The erythema frequently appeared most marked at the periphery of the patch, suggesting that the adhesive rather than the electrical field was the cause of the reaction.

We have also used these types of patches to investigate the iontophoretic transport of insulin.(24) Initial studies in animals investigated the transdermal transport of insulin in albino rabbits with

alloxan-induced diabetes mellitus. These animals had patches applied to their backs for periods up to 24 hours. The animal's skin was prepared for patch application by close clipping of the hair. Care was taken not to disrupt the skin and no irritant soaps were applied. Patches delivering an electrical current of 0.4 mA were used. Serial blood glucose and serum insulin levels were obtained. A significant rise in insulin concentration with an accompanying decline in blood glucose levels was observed. Blood glucose levels had returned to normal within 10 hours. The animals tolerated the patch well. (Figures 2 and 3) No cutaneous injury was observed.

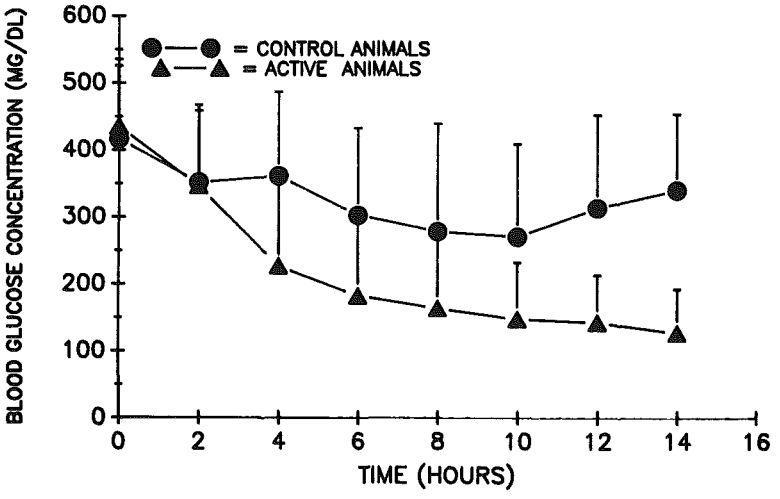

Figure 2: Mean blood glucose levels (± standard deviation) in rabbits with alloxan-induced diabetes after application of passive or active (iontophoretic) transdermal patches. (N = 16 active and 8 passive) Reprinted from reference (24) with permission.

Other investigators have also reported successful "iontophoretic" transdermal delivery of insulin. Kari utilized an identical animal model, similar electrical conditions, and achieved substantially higher concentrations than those that we were able to achieve. However, in this

study, the stratum corneum was partially abraided prior to patch application.(25) This may have substantially altered the permeability characteristics of the skin. Chien and colleagues have also reported a substantial amount of work in a smaller animal model, the hairless rat. Using a system which delivers pulsed direct current, they were able to achieve between 10 and 40 fold changes in calculated permeability coefficients for insulin in the stratum corneum.(26)

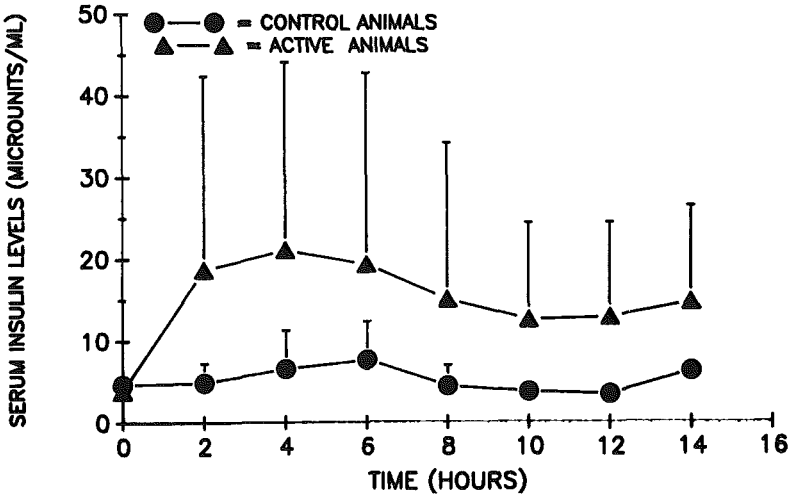

Figure 3: Mean serum insulin levels (± standard deviation) in rabbits from Figure 2. Reprinted from reference (24) with permission.

FUTURE DEVELOPMENT:

The current data support the conclusion that electrically mediated transdermal delivery of peptide drugs is possible. The actual development of a practical system for transdermal peptide delivery still requires considerable investigation. We require a more thorough understanding of the mechanism of transport and the variables in the patch formulation that effect the efficiency and efficacy of delivery. Current understanding suggests that several variables are particularly important.

Current:

The optimal strength of the applied electrical field is not clear. Higher current levels, in most studies, and in theoretical calculations,

are associated with improved flow. Chien's data (26) (noted earlier in this discussion) suggest that the use of intermittent pulsed DC current may allow the use of higher current levels with improved cutaneous tolerance.

pH:

Optimal pH conditions for delivery will be dependent upon the specific peptide chosen for delivery. Since most cutaneous pores appear to have a fixed negative charge, the optimal electrode for delivery will generally be the positive electrode, and the optimal pH will have the compound in a neutral or slightly positive state. Once an optimal pH is determined, however, a system for maintaining pH in the appropriate range at each electrode needs to be designed. The use of soluble buffers, such as we have used, will tend to limit the efficacy of drug delivery by providing additional solute molecules which will compete with the drug of interest in the transport process. The ideal buffer may be a system in which buffer is immobilized in a gel matrix at the electrode site.

Concentration:

The concentration of drug at the site of contact with the skin should be maintained at as high a level as practical.

Programmability:

The addition of minor changes in the patch system should allow for the initiation of electrical current (and hence delivery) according to pre-set times, or according to patient activation. Programmable delivery with intermittent bolus therapy is therefore a feasible goal.

LIMITATIONS OF TRANSDERMAL DELIVERY

Transdermal delivery has significant limitations. An examination of the data presented here shows large variability in absorption. No doubt further development and optimization of patch formulations will allow significant reductions in the variability seen in these early studies.

However, significant inter and intra patient variation as compared to traditional parenteral routes of administration may remain. Inter-patient variability is not as critical as intra-patient variability in absorption. Inter-patient variability may be dealt with by the development of several dosage forms, and use of the basic principles for individualization of dose established for oral dosing regimens. A more serious and difficult problem is the issue of intra-patient variability. There is very little information on how individual absorption of drug is effected by repeated applications, environmental conditions such as temperature and humidity, patient activity, and site of patch application.

One of the major issues in electrically-mediated transdermal transport is the occurrence of skin injury secondary to the applied electrical field. The use of current densities in the range reported here is well below the cutaneous pain threshold, and in acute dosing studies no unnacceptable toxicity has been seen. Chronic dosing studies have not been conducted, and will of course need to be performed before any definitive answer concerning the safety of technique can be entertained.

One area of concern with transdermal peptide administration is the potential antigenicity of foreign peptides administered transdermally. Physicians interested in the development of radioimmunoassays have for a long time recognized that the repeated intradermal administration of peptides in appropriate vehicles can lead to the production of antibodies and sensitization of the animal. Whether the transdermal administration of peptides to humans will replicate the experience with peptide and adjuvant administration to rabbits is not known. Whether the development of an antibody response to the administered peptide will effect its therapeutic role is not known, but a cause for concern.

What is the "best" technique for peptide delivery?

The optimal technique for peptide administration will no doubt vary

according to the peptide and the therapeutic goals of treatment. In some cases, such as the treatment of prostate carcinoma with an LHRH agent, the use of a once monthly or tri-monthly depot preparation may be a very adequate delivery technique. In other cases, such as the treatment of congenital growth hormone releasing hormone deficiency, optimal results can only be obtained with intermittent nocturnal bolus administration of the drug. We can expect to see a variety of new techniques come to fruition, each with its own particular clinical context and justification.

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Discussion - ABSORPTION OF PEPTIDES

B.P. du Souich

Could you explain the variability in the amount absorbed on the basis of different degrees of sweating?

B.R. Meyer

I don't know. I think that the technique basically is a technique to electrically induce the transport of water and to hydrate the skin. It may be that the amount of hydration of the skin that one achieves will vary with individuals. It may be that the pre-treatment hydration status of the individual will make a difference. I don't fully know what accounts for the inter-individual variation that we saw. I think we need to do further work to clarify exactly what contributes to that.

B.P. du Souich

In my view, a good candidate for this type of administration would be a peptide such as ANP for which one aims at achieving steady state levels, in the treatment of hypertension. Could you comment on that?

B.R. Meyer

In the long run about the first clinical use I would see of this kind of a system, which clearly needs further investigation and development, I would think of an indication where I was not worried about pulsatile delivery. I would want something where I had a steady infusion. I think that is technologically easier to achieve. And then after some sophistication is achieved, turning current on and off and modulating for pulsatile delivery would be a second step in that kind of a development.

J.A. Galloway

What was the charge on the insulin in your studies?

B.R. Meyer

The charge was negative. It was slightly negative and therefore instead of adding it to the positive electrode we actually added it to the negative. In this type of studies one has to consider the pH one is going to work at, the charge of the peptide, and then determine in that context what electrode one is going to add it to, positive or negative. It's something that has to be individualized for each particular peptide, I think.

A.J.H. Gearing

Have you ever tried the technique on patients prone to psoriasis or eczema?

B.R. Meyer

No, for these studies we chose people without known skin disease. Obviously, psoriasis would raise an issue. Most studies say that these patients have increased permeability of their skin rather than diminished, which one might have expected with the exfoliative process.

H.R. Röthig

Your data on leuprolide are quite interesting. We did the same experiments with buserelin. Unfortunately, we found out that the bioavailability is 1 0/00 or less. So this is only 1/10 or 1/20 of what we can administer intranasally. Do you have a good explanation? We think that there is probably a surface or area limitation for absorption. Did you ever try more patches?

B.R. Meyer

I have heard about the data with buserelin and transdermal delivery, but I have not seen the data. I don't know what the differences are between the two systems. In our case, the bioavailability is probably in the range of 5 - 10%. I don't have an explanation for the difference.

S. Erill

Does anyone know whether the transfer of water across the skin is regulated to any extent by ADH. ADH release is influenced by many factors, and this could affect the performance of these devices.

P. du Souich

I do not have precise data concerning the skin, but it is quite clear that the role of ADH is not limited to the kidney.