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Iontophoretic delivery of an insulin-mimetic peroxovanadium compound

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Abstract

The element vanadium has been shown to have insulin mimetic properties which gives it potential for use in the management of diabetes. Oral administration of vanadium has resulted in toxic side effects. Recently, a series of more potent vanadium compounds (peroxovanadium) have been synthesized which should be less toxic. However, there is no evidence that these compounds are orally active. Therefore, the feasibility of transporting peroxovanadium by iontophoretically enhanced transdermal delivery has been studied using several different donor conditions. The molecule was successfully delivered cathodally. Flux was linearly related to donor concentration and current density. The use of $CaCl_2$ instead of NaCl as the donor salt significantly increased drug penetration. Transport increased with decreasing buffer concentration. Hairless mouse skin is a reasonable model for peroxovanadium flux across human skin, since penetration across the two membranes was within a factor of two. This work demonstrates that transdermal delivery of peroxovanadium compounds is feasible and that in vivo studies to test its efficacy in regulating blood glucose levels are warranted.

Keywords: Iontophoresis; Transdermal drug delivery; Vanadium; Peroxovanadium; Hairless mouse skin; Human skin

1. Introduction

Vanadium is an element with a complex chemistry and a variety of physiological and pharmacological actions (Chasteen, 1990). It has be shown to have effects similar to fibroblast growth factor, epidermal growth factor and insulin in cell cultures (Canalis, 1985; Kato et al., 1987; Lau et al., 1988). Vanadium can mimic insulin by increasing glucose uptake (Dubyak and Kleinzeller, 1980; Schechter and Karlish, 1980), glycogen synthesis (Degani et al., 1981; Tamura et al., 1984), glucose oxidation (Duckworth et al., 1988), potas-

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sium uptake (Dubyak and Kleinzeller, 1980), lipogenesis (Schechter and Ron, 1986), and antilipolysis (Degani et al., 1981; Duckworth et al., 1988) in adipocytes. Similar effects have also been seen in vivo using animal models of Type I diabetes (Heyliger et al., 1985; Meyerovitch et al., 1987).

Oral administration of vanadium, however, is not without its drawbacks. Toxic side effects have been noted, including some deaths, decreased weight gain, and increased serum urea and creatinine concentrations (Domingo et al., 1991). Recently, a series of more potent vanadium compounds have been synthesized which should be less toxic (Posner et al., 1994; Bevan et al., 1995). Peroxovanadium compounds contain a central vanadium atom, an oxo group, one or two peroxo ligands and an ancillary bidenate ligand. In the crystalline state, these compounds are 5-coordinate distorted pentagonal bipyramidal molecules. A peroxo ligand occupies one site of the coordination sphere of the vanadium atom. There are no reports that these compounds are orally active. Given the chemistry of vanadium in its various forms, it is not unexpected that peroxovanadium compounds would break down upon enteral administration. Therefore, alternate delivery methods must be explored.

Transdermally delivered compounds by-pass the gasterointestinal tract, allowing these molecules to reach the systemic circulation intact (Guy and Hadgraft, 1987). The success of transdermal delivery is dependent on molecular size, charge and lipopholicity. Charged or polar molecules can have difficulty penetrating the skin successfully without the aid of enhancement techniques. Transdermal peroxovanadium delivery would be most effective with an enhancement method which can control delivery to correlate the insulin mimetic action of the drug with food intake. Techniques such as iontophoresis (Green et al., 1993), electroporation (Edwards et al., 1995), or ultrasound (Mitragorti et al., 1995), have these capabilities. The technique of iontophoresis was chosen as a means of testing the feasibility of transdermal $VO(O_2)_2$ 1–10 phenanthroline (bpV(phen)) delivery.

The purpose of this study was to test the iontophoretic delivery of peroxovanadium compounds, using bpV(phen) as a model drug and to determine the influence of donor conditions on its penetration.

2. Materials and methods

2.1. Chemicals

The peroxovanadium compound $(VO(O_2)_2$ 1–10 phenanthroline), bpV(phen), was synthesized as described by Posner et al. (1994). All chemicals used for synthesis and buffers were at least reagent grade and were purchased from Sigma (St. Louis, MO), Fisher Scientific (St. Louis, MO), or Alfa Aesar (Ward Hill, MA).

2.2. Iontophoretic studies

Dorsal skin from male hairless mice CRL:SK1 ages 8–20 weeks old was removed after the animals were killed by CO_2 asphyxiation. Full thickness skin was used immediately for transport studies. Excess human breast or abdominal skin was obtained from surgeries. The epidermis was separated by placing the skin in a 60°C water bath for 2 min and then peeling it away from the dermis (Klingman and Chrostophers, 1963). The epidermis was frozen at 20°C for later use.

Skin patches were placed in a flow-through diffusion cell system (Brand and Iversen, 1996) based on the design of Glikfeld et al., 1988. These cells allowed both the anodal and cathodal chambers to be located on the epidermal side of the skin. The receptor compartment had a volume of approximately 200 μl and the flow rate was 1000 μl/h. Approximately 1/2 ml of bpV(phen) solution was placed in the donor chamber. Control experiments were done with buffer alone. Several different donor conditions were studied in order to determine which were most effective. These included: (1) bpV(phen) at concentrations ranging from 2.5 to 250 mM; (2) pH from 5 to 9; (3) buffer concentrations from 0 to 25 mM; (4) 133 mM NaCl or 66.5 mM $CaCl_2$; and (5) current densities ranging from 0 to 0.5 mA/cm^2. The skin

was allowed to equilibrate for 180 min prior to current introduction. The vanadium compound readily binds to the Ag/AgCl electrodes, so electrical contact was maintained via salt bridges made from 3% Agarose in 1 M NaCl. The Ag/AgCl electrodes were then connected to a BioRad Model 1000/500 Power Supply, in constant current mode. The current was set to 0.5 mA/cm^2 (Ledger, 1992) and applied for 9 h. The receptor chamber was perfused with 25 mM Hepes, 133 mM NaCl at pH 7.4 which then passed to a fraction collector. The efflux was collected at 90 min intervals.

2.3. Assays and analysis

Vanadium in the form bpV(phen) was assayed using UV spectrophotometry by quantitating absorbance at 260 nm. Skin which has been placed in an in vitro diffusion chamber, however, will also release molecules which have absorbance at this wavelength. To control for this, iontophoresis was performed with only buffer in the donor compartment and samples were then measured on the UV spectrophotometer at 260 nm. The data were plotted as control output versus time and an equation was generated from the results. This curve was subtracted from the spectrophotometric data obtained when bpV(phen) was present in the donor solution. To verify this technique, a series of data points were assayed by UV spectroscopy and then by atomic absorption spectroscopy (Mongold et al., 1990). The two sets of data differed by less than 20%, indicating that the spectrophotometric technique was acceptable for use in maximizing flux.

All data have been expressed as mean ± S.E. When comparisons between experiments were conducted, significant differences were assessed by analysis of variance and a Bonferroni post test at the level $P < 0.05$ using the program Instat (Graph Pad Software, San Diego, CA). Each experiment was repeated at least three times.

3. Results

3.1. Current polarity

The peroxovanadium compound bpV(phen) was dissolved in 25 mM Hepes, 133 mM NaCl, pH 9.

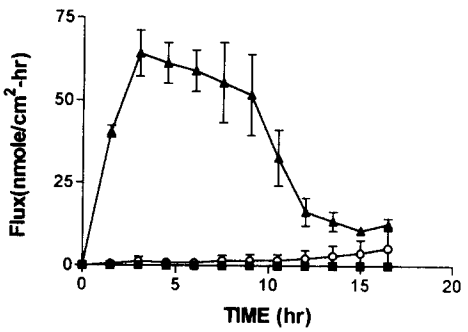

Fig. 1. Comparison of anodal (▲), cathodal (■) and passive (○) delivery of bpV(phen) across hairless mouse skin in vitro. Current was turned on at $t = 0$ and off at $t = 9$ h.

This solution was used to determine if bpV(phen) could penetrate the skin when applied passively, at the anode, or at the cathode. Fig. 1 demonstrates that iontophoresis increased the bpV(phen) flux when it was delivered cathodally. A steady-state flux of 62 nmol/cm^2 h was achieved after approximately 3 h of current. Passive and anodal transport were minimal.

3.2. pH

The influence of pH on iontophoretic flux is presented in Fig. 2. Flux was examined at pH 5, 7.4 and 9. The pH was maintained with an appropriate buffering system; 6.6 mM glycine at pH 9, 6.6 mM Hepes at pH 7.4, and 6.6 mM acetate at

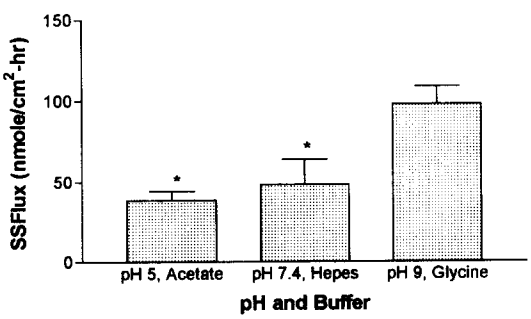

Fig. 2. Steady state flux as a function of donor pH. Transport of bpV(phen) at pH 5.0, 7.4 and 9.0 are compared. * indicates a significantly different flux ($P < 0.05$) from pH 9.0. Receptor solution was maintained at pH 7.4 for all experiments.

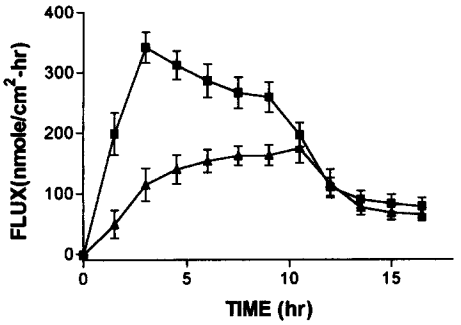

Fig. 3. Cathodal flux of bpV(phen) in the presence of NaCl (▲) or $CaCl_2$ (■). Current was turned on at $t = 0$ and off at $t = 9$ h. Receptor solution was maintained at 25 mM Hepes, 133 mM NaCl, pH 7.4.

pH 5. Flux was pH sensitive with the greatest transport occurring at the most basic pH ($P < 0.05$ vs. pH 7.4; $P < 0.01$ vs. pH 5). There was a downward trend in flux associated with decreasing pH, however, flux at pH 7.4 and 5 were not significantly different from each other.

3.3. Salt type

The influence of the cation species in the donor solution on bpV(phen) flux is shown in Fig. 3. The bpV(phen) was dissolved in either 65 mM $CaCl_2$ or 133 mM NaCl to a final concentration of 25 mM. Drug flux was greater in the presence of $CaCl_2$ than NaCl (113 ± 10.5 vs. 62.3 ± 7.1 nmol/cm^2 per h, $P < 0.01$). Furthermore, transport kinetics were different with $CaCl_2$ having a more rapid rise (slope different, $P < 0.05$), followed by a small flux decrease and NaCl causing a slower rise followed by steady state.

3.4. Buffer concentration

Transport was compared between 25 mM bpV(phen) with either 0, 6.6 or 25 mM glycine, pH 9, at the cathode (Fig. 4). The buffer also contained 66.5 mM $CaCl_2$ at the anode. There is an inverse correlation between buffer concentration and bpV(phen) flux, with the change becoming statistically significant by 25 mM ($P < 0.05$).

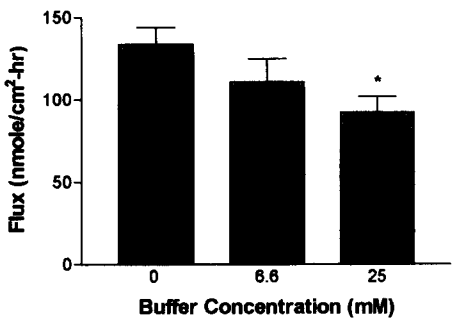

Fig. 4. Cathodal flux of bpV(phen) as a function of buffer concentration. Steady state flux is inversely related to buffer concentration. * indicates statistically significant difference from flux at 0 mM ($P < 0.05$). Receptor solution was maintained at 25 mM Hepes, 133 mM NaCl, pH 7.4.

3.5. Concentration

The correlation between donor concentration and bpV(phen) transport was determined over two orders of magnitude, from 2.5 to 250 mM. The drug was placed at the cathode which contained 6.6 mM Glycine, pH 9. The anode was also maintained at pH 9.0 with a 6.6 mM glycine buffer, containing 66.5 mM $CaCl_2$. Flux increased linearly with increasing concentration (Fig. 5).

3.6. Current density

The effect of current density on bpV(phen) flux was tested at 0.125, 0.25 and 0.5 mA/cm^2. BpV(phen) was dissolved in 6.6 mM glycine, pH

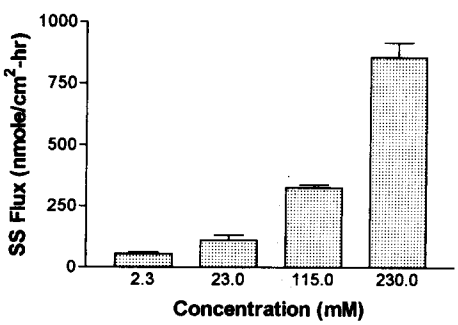

Fig. 5. Steady state flux of bpV(phen) as a function of donor concentration. The bpV(phen) was dissolved in 6 mM Glycine, 133 mM $CaCl_2$, pH 9.0.

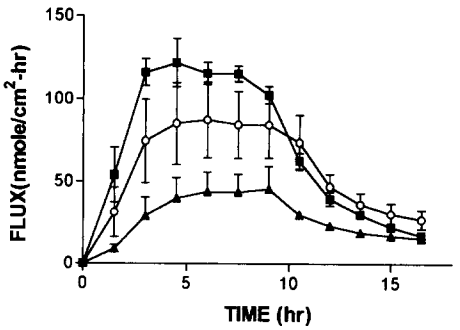

Fig. 6. Cathodal flux of bpV(phen) as a function of current density. Currents of 0.5 mA/cm^2 (■), 0.25 mA/cm^2 (○), or 0.12 mA/cm^2 (▲). Current was turned on at $t = 0$ and off at $t = 9$ h. Receptor solution was maintained at 25 mM Hepes, 133 mM NaCl, pH 7.4.

9, to a final concentration of 25 mM. The anodal chamber contained the same buffer plus 66.5 mM $CaCl_2$. As expected, larger current densities led to greater flux (Fig. 6), with transport at 0.5 mA/cm^2 being significantly different from that at 0.125 mA/cm^2 ($P < 0.05$).

3.7. Skin type

Flux across isolated human epidermis was compared with transport across full thickness hairless mouse skin under identical conditions. Penetration through human epidermis was approximately one half of that measured across mouse skin (Table 1).

4. Discussion

Vanadium compounds have been shown to lower blood glucose levels when given orally to diabetic animals. However relatively high doses are required to be effective and toxic side effects are frequently induced. A new series of peroxovanadium compounds with various ancillary ligands have been synthesized (Posner et al., 1994). These compounds are significantly more potent at altering blood glucose than vanadium. The molecule $VO(O_2)_2$ 1–10 phenanthroline (bpV(phen)), was chosen for transdermal studies because it is one of the more potent peroxovanadium compounds; 100 times more effective than the commonly used sodium orthovanadate (Posner et al., 1994).

Table 1
Influence of skin type on iontophoretic bpV(phen) delivery

Skin type	Steady-state flux (nmol/cm^2 per h)	n
Hairless mouse	97.98 ± 10.51	4
Human epidermis	50.13 ± 10.90	4

There are currently no reports of bpV(phen) compounds being orally active, and it is unlikely that these molecules would remain intact given the acidic nature of the stomach and the complex chemistry of vanadium (Chasteen, 1990). Therefore, the feasibility of providing it transdermally was examined. Iontophoretic flux was emphasized, because regulating current onset, offset, and duration can enable the drug to be given in a pattern more similar to insulin than would be possible with passive delivery. Posner et al. (1994) determined that a dose of 600 nmol/100 g body weight produced a change in blood glucose equivalent to 1.5 mg insulin/100 g body weight. Iontophoretic delivery resulted in a steady state flux of 855 + 60.7 nmol/cm^2 h with a donor concentration of 230 mM. Therefore, bpV(phen) can be transdermally delivered at therapeutic levels in rats. Peroxovanadium compounds are still experimental, so the necessary human dose is not known at this time.

Because transdermal delivery is highly dependent on the chemistry of the compound and its solvent, the iontophoretic flux of these molecules were examined for several different donor conditions (Gupta et al., 1994). The negatively charged bpV(phen) molecule was, as expected, primarily transported cathodally. Furthermore, flux was linearly related to both current density and concentration. The linear relationship with concentration indicates that the solubility limits of the compound have not been reached and that the ion-conducting pathways of the skin have not reached saturation (Brand and Guy, 1995).

The influence of pH on the iontophoretic delivery has been demonstrated for a variety of compound. Optimal delivery is generally found where the molecule has the most favorable charge (Sid-

diqui et al., 1985; Siddiqui et al., 1996) or where it can take greatest advantage of electroosmotic flow (Delgado-Charro and Guy, 1994). Iontophoresis improves molecular transport by inducing electrorepulsion and electroosmosis. Electroosmotic flux has been demonstrated to flow from anode to cathode (Kim et al., 1993), thereby inhibiting cathodal transport. This flux has been shown to decrease in magnitude as the pH approaches 4 and can actually reverse at lower pH. Optimal penetration of bpV(phen) was found at pH 9, indicating that electroosmosis is not a major mechanism for transport.

Iontophoretic transport has also been correlated with the ionic composition of the buffer (Yoshida and Roberts, 1996). By changing the buffer to obtain a given pH, the ionic composition, as determined by conductance, will change. Under constant current conditions, there is a specific amount of charge which will be carried across the skin. Greater ionic activity in donor buffer creates more competition for the vanadium molecules to carry that limited charge. This leads to reduced transdermal transport. We measured the conductance of bpV(phen) in ddH_2O, 6.6 mM acetate pH 5, 6.6 mM Hepes, pH 7.4 and 6.6 mM glycine, pH 9 and found that ddH_20 < glycine < Hepes < acetate (data not shown). Conductance, therefore, was inversely correlated with the measured steady state flux, indicating that ionic composition is a factor in influencing iontophoretic transport of bpV(phen). This mechanism can also account for the decreased flux seen with increased buffer concentration.

Transport of bpV(phen) was greater in the presence of $CaCl_2$ than of NaCl. Divalent cations have been shown to bind more readily to skin than monovalant ions (Phipps et al., 1989; Burnette and Ongpipattanakul, 1987). The increased cationic binding neutralizes the negative charge which the skin carries above pH 4. This alters cathodal drug flux in two ways. First, it decreases the electrorepulsive forces of the skin, which inhibit anionic transport, leading to improved cathodal penetration. Second, it reduces the magnitude of iontophoretically induced electroosmotic flux. Electroosmosis inhibits cathodal transport because it flows in the opposite direction (anode to cathode). Reducing the electroosmotic flux by substituting the divalent cation for the monovalant would, therefore, be expected to increase cathodally delivered bpV(phen) flux. This was found to be true, as steady state transport of bpV(phen) was 1.9 times greater when Ca^{2+} was used as the counterion in place of Na^+.

Flux kinetics were also different for the two salt systems (Fig. 3). BpV(phen) permeation from the $CaCl_2$ solution increased rapidly and then slowly decreased towards steady state. Transport from the NaCl solution, however, consisted of a gradual rise to steady state. Because the divalent Ca^{2+} binds more readily to the skin than the monovalent Na^+, it decreases the skin's net negative charge. This reduces the electrostatic repulsion for both Cl^- anions and bpV(phen), leading to the large increase in drug transport not seen for the NaCl donor solution. A rapid rise followed by a decrease to steady state has also been seen with amino acids and tripeptides (Green et al., 1991a,b). The lower steady state has been associated with the pattern of Cl^- flux. The chloride ion will have a greater transport number and therefore will travel across the skin more readily than the large molecules. Early in the experiment, Cl^- will be driven into the skin and therefore will be depleted from the donor chamber. Cl^- is also generated at the Ag/AgCl surface. Eventually, the electrochemically generated Cl^- becomes greater than what is being transported through the skin, leading to an increase in the chamber. The Cl^- ions then compete with the drug, causing decreased transport in bpV(phen). Optimum flux, therefore, occurs between the time the Cl^- is initially depleted and when it is regenerated.

Iontophoretic flux of bpV(phen) across full thickness hairless mouse skin and isolated human epidermis were within a factor of two of each other. This is consistent with transport of other similarly-sized compounds. Passive permeation of morphine, fentanyl and sufentanil was found to be one order of magnitude higher across hairless mouse skin than human epidermis (Roy et al., 1994). Iontophoresis of nicotine, however, resulted in equivalent flux across skin from the two species (Brady et al., 1992). The data, therefore, provide additional support for the use of hairless

mouse skin as a model transport system for human epidermis.

These results indicate that transdermal delivery of peroxovanadium compounds using peroxovanadium as a model drug is feasible. Percutaneous flux of other peroxovanadium compounds warrants further investigation. As appropriate donor conditions have now been determined, in vivo studies in which the ability of these molecules to lower blood glucose concentrations will be performed.

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