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Transdermally delivered peroxovanadium can lower blood glucose levels in diabetic rats☆

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Abstract

The element vanadium can have insulin mimetic properties and therefore has been suggested as a possible therapeutic agent for treatment of diabetes. A series of peroxovanadium compounds that are more potent at lowering blood glucose levels than sodium metavanadate, sodium orthovanadate and vanadyl sulfate have recently been synthesized. These compounds probably will not be orally active so transdermal administration is a potential option. A patch containing either the peroxovanadium compound [$VO(O_2)_2$ 1–10 phenanthroline], abbreviated bpV(phen), or placebo was placed on the back of streptozotocin induced diabetic rats and was delivered either passively (16 h) or iontophoretically (0.5 mA/cm^2 for 4 h). Blood samples were analyzed for glucose and vanadium levels. Mean blood glucose levels were $83 \pm 1\%$ and $109 \pm 1\%$ of the starting values for animals iontophoretically treated with bpV(phen) and vehicle, respectively. The compound's insulin mimetic properties were evident within 60 min of current initiation. Blood glucose levels were reduced to $74 \pm 14\%$ of the original level after 16 h of passive treatment. The compound was ineffective when fed to animals. Transdermal delivery of bpV(phen) resulted in significantly greater blood levels of vanadium than the orally delivered compound ($P < 0.05$). Overall these experiments demonstrate that peroxovanadium delivered through the skin can lower blood glucose levels in rats. Further experiments are warranted to better characterize the nature of the response and to determine the potential for using these compounds in humans.

Keywords: Transdermal drug delivery; Iontophoresis; Vanadium; Peroxovanadium; Rat; Blood glucose; In vivo

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1. Introduction

The element vanadium has a complex chemistry and will form a variety of compounds (Butler, 1990; Willsky, 1990). Some of these compounds have insulin mimetic properties in vitro (Dubyak and Kleinzeller, 1980; Schechter and Karlish, 1980) and in vivo (Heyliger et al., 1985; Meyerovitch et al., 1987; Pederson et al., 1989; Brichard et al., 1990). Vanadium has, therefore, been suggested as a possible therapeutic agent for the treatment of diabetes (Shechter, 1990; Brichard et al., 1991; Shechter and Shisheva, 1993). Three vanadium compounds, vanadyl sulfate, sodium metavanadate and sodium orthovanadate have been used in human studies. These vanadium compounds increased insulin sensitivity in patients with type 2 diabetes (non-insulin dependent) and may also have benefits in treating type 1 diabetics (insulin dependent). There were only minor side effects associated with vanadium treatment in these studies, but they were relatively short in duration (Cohen et al., 1995; Goldfine et al., 1995a,b; Halberstam et al., 1996).

Despite these successes, there is considerable concern regarding the long-term toxicity of vanadium compounds, especially within the reproductive and immunological systems (Domingo et al., 1991; Cohen et al., 1993; Leonard and Gerber, 1994). One possible solution is to find more potent insulin mimetic compounds which require less vanadium to be administered. A series of peroxovanadium compounds have been synthesized that are 10–100 times more effective than vanadyl sulfate (Posner et al., 1994). These compounds are acutely effective at lowering blood glucose levels i.v. and do not require long term (days) administration of relatively high doses as do vanadyl sulfate, sodium metavanadate or sodium orthovanadate (Bevan et al., 1995a,b). However, given the chemistry of vanadium (Butler, 1990; Willsky, 1990), these compounds are unlikely to have good enteral bioavailability, and to our knowledge there are no reports of peroxovanadium being orally active.

Transdermal penetration allows compounds to by-pass the gastrointestinal tract, thereby eliminating degradation in the stomach and hepatic first-pass metabolism (Cullander and Guy, 1992). It is, therefore, a potential method for delivering peroxovanadium compounds. The skin, however, is a very effective barrier so penetration enhancement techniques such as iontophoresis are frequently used to improve transport across it (Singh and Maibach, 1994).

The feasibility of transdermal delivery enhanced by iontophoresis of the negatively charged peroxovanadium compound [$VO(O_2)_2$ 1–10 phenanthroline], bpV(phen), has been previously demonstrated in vitro (Brand et al., 1997). This work has now been extended to include in vivo studies. This manuscript reports on the use of iontophoretically enhanced and passive percutaneous administration of a peroxovanadium compound as an effective means of lowering blood glucose levels in diabetic rats.

2. Materials and methods

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 Chemical Company (St. Louis, MO), Fisher Scientific (St. Louis, MO), or Alfa Aesar (Ward Hill, MA).

Male Sprague Dawley rats 250–300 g were made diabetic by injecting 65 mg/kg streptozotocin into the penile vein. One week later blood glucose levels were determined and the animals were considered diabetic if their blood glucose levels were above 250 mg/dl. Rats were divided into five treatment groups: (1) transdermally delivered bpV(phen) with iontophoretic enhancement; (2) transdermally delivered distilled water with iontophoretic enhancement; (3) transdermally delivered bpV(phen) without iontophoretic enhancement (passive); (4) transdermally delivered distilled water without iontophoretic enhancement (passive control), and (5) orally delivered bpV(phen).

In order to prevent fur from interfering with dermal contact of the patch, the animals were anesthetized with pentobarbital at a dose of 50

mg/kg, 24 h prior to experimentation and their backs were shaved. Shaving the animals 1-day before the skin was exposed to a compound allows the stratum corneum time for repair of small nicks prior to experimentation. Rats fed peroxovanadium were not shaved prior to treatment.

For iontophoretic experiments (groups 1 and 2), the animals were anesthetized with 50 mg/kg Ketamine and 10 mg/ml Rompin prior to patch placement and given boosters of anesthetic as needed to maintain sleep. Animals were anesthetized during the experiment to reduce the stress associated with frequent blood withdrawal from the tail vein. The patch consisted of a 3-cm^2 Hilltop Chamber (Kenwood, OH) that had been modified to contain an Ag/AgCl electrode in the drug reservoir. Two patches were placed on the shaved region of the back and connected to a constant current power supply (Iomed, Salt Lake City,UT). One patch was filled with 0.25 ml of 1 M NaCl and connected to the anode, while the other containing 0.25 ml of 120 mM of the negatively charged bpV(phen) or control was connected to cathode. A blood glucose measurement was taken prior to the initiation of current and this was used as a baseline value for normalization. A current of 0.5 mA/cm^2 was then applied for 4 h and blood samples were taken from the tail vein every 30 min and assayed for their blood glucose levels via glucometer (One Touch, Lifescan, Milpitas, CA). Iontophoresis produces little skin damage or animal discomfort at this current density (Ledger, 1992). Data were normalized by dividing the blood glucose level after treatment by that measured before treatment, due to variations in starting glucose levels.

For passive experiments (groups 3 and 4), a small amount of blood was removed from the tail vein and glucose levels were determined at 8:30 AM on day 1. At 4:30 PM, rats were anesthetized by ether and a patch containing either 1 ml of 120 mM bpV(phen) or H_2O was placed on the rat's back. The patch consisted of an 8.5 mm piece of glass filter (Gellman Sciences, Ann Arbor, MI), covered with a piece of Parafilm to reduce evaporation and affixed to the animal with Polyskin Transparent Dressing (Kendall, Mansfield MA) and surgical tape. The glucose level was determined again on day 2 at 8:30 AM, after the patch had been in place for 16 h.

The rats which were fed bpV(phen) (group 5) had blood samples taken from the tail vein at 8:30 AM for 3 consecutive days. The first measurement was prior to bpV(phen) exposure. The animals were then fed 160 μM/kg per day (96 μmol total dose) of bpV(phen) for the remaining 2 days. This provided before and after exposure blood glucose levels so that data could be normalized as described for the transdermal experiments. All animal experiments adhered to the 'Principles of Laboratory Animal Care' (NIH publication no. 85-23, revised 1985).

Blood levels of vanadium were determined for the animals treated with passive transdermal and oral delivery. The radiochemical neutron activation analysis (RNAA) determination of vanadium content of the tissues was done as described elsewhere (Blotcky et al., 1989).

All data have been expressed as mean + SE. Significant differences were assessed by analysis of variance and a Newman-Kuels post test at the level $P < 0.05$ using the program Prism (Graph Pad Software, San Diego, CA).

3. Results

The ability of iontophoretically delivered bpV(phen) to lower blood glucose levels in diabetic rats is shown in Fig. 1. The insulin mimetic properties of the compound were evident within 60 min of the initiation of current ($P < 0.05$). The mean blood glucose levels relative to starting values during the effective portion of treatment were 0.83 ± 0.01 for bpV(phen) animals and 1.09 ± 0.01 for the controls (H_2O).

Normalized blood glucose levels resulting from orally and transdermally delivered bpV(phen) are presented in Fig. 2. Transdermal treatment with bpV(phen) decreased normalized blood glucose levels while the orally delivered compound had no effect on blood glucose. Furthermore, simply patching the animal with water did not alter glucose levels. An ANOVA with post test revealed that the transdermally delivered compound was more effective at lowering blood glucose levels

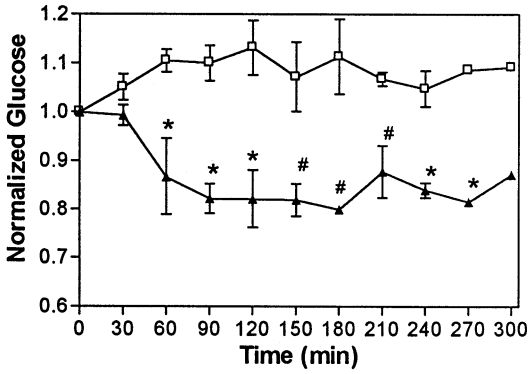

Fig. 1. Effect of iontophoretic delivery of 120 mM bpV(phen) (π) and H_2O ([×]) on the relative blood glucose levels of anesthetized rats. The current was 0.5 mA/cm^2 for both treatments. Data points for each animal were normalized by their blood glucose values prior to induction of current ($n=2$). Statistical differences were $^*P<0.05$ and $\#\ P<0.1$.

than either the ingested drug or the control patch ($P<0.05$). There were no statistical differences among the other treatment methods.

Upon completion of the experiment, the animals were sacrificed, their blood collected and vanadium levels were determined by neutron activation analysis. When bpV(phen) was delivered passively through the skin, blood levels of 313 ± 66 ng/g blood were detected (Fig. 3). This was significantly greater ($P<0.05$) than for either the transdermal control patch (7 ± 3) or the orally delivered compound (59 ± 4).

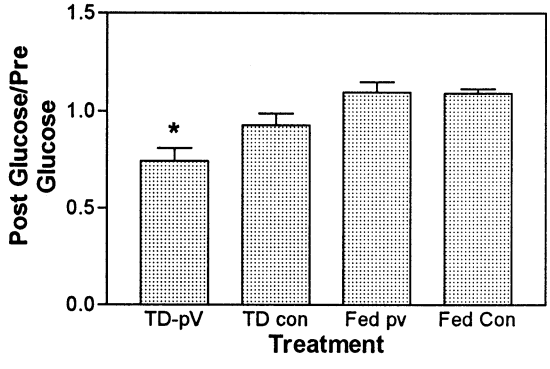

Fig. 2. Normalized glucose levels for animals given bpV(phen) (TD PV, $n=4$) or water (TD CON, $n=5$) transdermally. Rat food was either mixed with bpV(phen) (FED PV, $n=3$) or was left untreated (FED CON, $n=2$). There were significant differences between TDPV and all other groups ($^*P<0.05$).

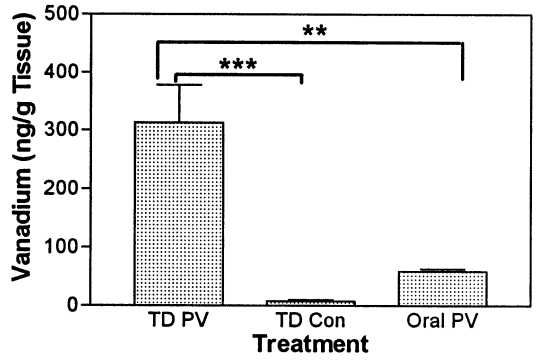

Fig. 3. Blood levels of vanadium after 2 days of treatment with bpV(phen). The compound was either passive transdermal delivery bpV(phen) (TD PV, $n=4$) or water (TD CON, $n=5$) or given in food) (FED PV, $n=3$). Statistical differences were $^{**}P<0.01$ and $^{***}P<0.001$.

4. Discussion

Vanadium compounds have been touted as possible therapeutic agents in the treatment of diabetes (Shechter, 1990; Brichard et al., 1991; Shechter and Shisheva, 1993); however, serious concern has been raised about the toxic effects of its long term administration (Domingo et al., 1991; Leonard and Gerber, 1994). Vanadium can accumulate in virtually every organ tested (Hamel and Duckworth, 1995) and the half-life of retention varies from organ to organ. This retention is dose-dependent (Hamel, 1998; Hamel and Duckworth, 1995). Thus, more potent compounds result in lower tissue loads and presumably less toxicity.

While sodium metavanadate, sodium orthovanadate and vanadyl sulfate are orally active at reducing blood glucose levels, no publications have demonstrated availability of peroxovanadium compounds after ingestion. The experimental results reported in this manuscript suggest that the peroxovanadium compound bpV(phen) is not well absorbed from the gut. An alternate mode of administration that avoids both the stomach's acidic environment and hepatic first-pass metabolism is therefore necessary to make use of these compounds as therapeutic agents feasible. This led us to hypothesize that transdermally delivered bpV(phen) would be more effective at

lowering blood glucose than when given orally. The data presented support this hypothesis.

In vitro data demonstrated that bpV(phen) administered transdermally using iontophoresis has a lag time of less than 90 min, whereas measurable transport takes at least 8 h when the compound is applied passively (Brand et al., 1997). Two different types of experiments were therefore undertaken, given the wide range in kinetics. Rapid, short-term changes in blood glucose levels were studied with the iontophoresis experiments; a time course in which passive delivery would have no effect. Longer studies allowed time for sufficient quantities of bpV(phen) to be delivered passively. The two techniques resulted in similar reductions in blood glucose levels (17 and 26%, respectively).

Peroxovanadium compounds are still experimental and thus doses necessary for efficacy in humans have not been determined. Sodium orthovanadate was given to human volunteers at a dose of 125 mg/day (Goldfine et al., 1995a,b). This dose led to a statistically significant drop in insulin requirements of 14%. Blood glucose levels did not change in these subjects because glucose levels were controlled with insulin. A 20% reduction in blood glucose levels in humans would provide significant benefit. For example, lowering fasting blood glucose from 300 to 240 would reduce Hemoglobin A_{1c} content from approximately 11.6–9.8% (Nathan et al., 1984). A direct comparison between the blood glucose lowering ability of bpV(phen) versus sodium metavanadate, sodium orthovanadate or vanadyl sulfate is difficult because these compounds work on a much longer time frame and have to accumulate in tissue before significant activity. Indirect measurements of relative potency have therefore been employed to approximate relative potency. The compound bpV(phen) has been shown to be 100 times more effective at stimulating insulin receptor kinase (IRK) and inhibiting phosphotyrosine phosphatase activity (PTP) in vitro (Posner, JBC).

Based on these studies we can assume that bpV(phen) will be effective at 1% of the sodium orthovanadate dose; therefore, 1.25 mg/day would provide a therapeutic response in humans. With passive penetration of bpV(phen) across hairless mouse skin during a 24-h period, 39 nmol/cm^2-24 h was delivered, according to our in vitro data (Brand et al. 1997). Given that the donor concentrations were 23 and 120 mM in vitro and in vivo, respectively, that delivery is linearly related to donor concentration in this range, and that the molecular weight of bpV(phen) is 404 g/mol; a total of 82 μg/cm^2 is delivered after 24 h. Assuming that penetration across human skin is half that of mouse skin (Brand et al., 1997), the target dose of 1.25 mg/day would be successfully delivered using a 31-cm^2 patch. This is not an unreasonably large patch and thus the delivery of this dose to humans would be feasible. Furthermore, increasing the donor concentration or the use of penetration enhancers could reduce the patch size. Patch sizes required for iontophoretic delivery would be considerably smaller. Overall these calculations demonstrate that delivering potentially therapeutic doses of bpV(phen) to humans is possible.

Peroxovanadium compounds could potentiate the effects of endogenous insulin for type 2 diabetic patients (non-insulin dependent), or to exogenously given insulin for type 1 (insulin dependent) patients. This would reduce the required amount of insulin and help reduce the long-term adverse effects of hyperinsulinemia.

Since iontophoresis significantly increases the amount of peroxovanadium rapidly crossing the skin, it could potentially be a primary therapy for type 2 diabetic patients. Using a battery powered iontophoresis device, peroxovanadium levels could be increased during meal times to potentiate the effect of insulin, and thereby decrease the amount needed to be released by the pancreas. Iontophoretic delivery of peroxovanadium compounds, however, would not serve as the primary treatment for insulin dependent diabetics (type 1) because the drug is not as potent as insulin. Comparison of our results to those of transdermally delivered insulin (either iontophoretic or electroporated) reveal that bpV(phen) has similar lag times but is less effective in its ability to lower blood glucose levels (Tachibana, 1992; Bevan et al., 1995a,b; Mitragotri et al., 1995; Tomohira et al., 1997; Haga et al., 1997).

Passive delivery of bpV(phen), however, is probably a more realistic use. Chronic treatment could be used to take advantage of the insulin mimetic and increases in insulin sensitivity making this a potential treatment for either type 1 or 2 diabetics. Overall these experiments demonstrate that peroxovanadium delivered through the skin can lower blood glucose levels in rats. Additional experiments are warranted to further characterize the nature of the response and to determine the potential for using these compounds in humans.

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