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International Journal of Pharmaceutics 116 (1995) 211–216

international journal of pharmaceutics

Transdermal iontophoretic delivery and degradation of vasopressin across human cadaver skin

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Received 26 July 1994; modified version received 13 September 1994; accepted 14 September 1994

Abstract

Transdermal iontophoretic transport and degradation of a peptide, vasopressin, across human cadaver skin, was investigated. Modified Valia-Chien cells were supplied with 0.5 mA/cm^2 current density via silver/silver chloride electrodes from a Scepter® power supply. Vasopressin (0.25 mg/ml) spiked with [^{3}H]vasopressin was transported across skin with anode in donor. Samples were analyzed by HPLC using a radio-chromatography detector. Degradation of vasopressin in contact with intact skin and in skin homogenates was also studied. Greater degradation was observed in receptor where the peptide was in contact with the dermal side of the skin. The cumulative amounts of intact vasopressin permeated during 8 h of iontophoretic transport was 15.37 ($\pm$5.31; $n = 3$) μg/cm^2, which corresponds to only about 1% permeation. Of the total radioactivity permeated, only about 40% was intact vasopressin by 12 h. Several degradation peaks could be seen in the chromatogram. No intact vasopressin was found to permeate under passive conditions. The enzymatic activity of cadaver skin is likely to be less than that expected in an in vivo drug delivery situation. Therefore, delivery to patients would be even less than that predicted by this study.

Keywords: Transdermal; Iontophoresis; Vasopressin; Peptide

1. Introduction

With the recent advances in biotechnology, peptide/protein drugs are rapidly being made available as therapeutic agents. However, the safe and efficacious delivery of such drugs is faced with formidable barriers (Banga and Chien, 1988a). Transdermal delivery could provide a non-invasive viable route but peptide/protein drugs are too large and hydrophilic to pass through the skin. However, it is possible to assist the delivery of such drugs through the skin by iontophoresis (Banga and Chien, 1988b; Lattin et al. 1991; Cullander and Guy, 1992; Sage, 1993).

Vasopressin, a nonapeptide with a molecular weight of 1084, was chosen as the model peptide for investigation, because: (a) its molecular size make it a good candidate as it is too large for

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SSDI 0378-5173(94)00306-8

passive skin delivery but still small enough to be electrically transported through skin; (b) it has therapeutic use in the treatment of diabetes insipidus and emergency treatment of bleeding from esophagogastric varices; (c) vasopressin may require a pulsed delivery for therapeutic effect rather than steady-state levels. This is due to the possibility of tolerance or desensitization of receptors by its continual presence at a receptor site (Koch and Lutz-Bucher, 1985). Iontophoretic delivery can provide for such pulsed delivery by controlling the current.

Delivery of vasopressin through the skin using penetration enhancers under passive conditions has been reported (Banerjee and Ritschel, 1989a,b). There is also published work (Lelawongs et al. 1989, 1990; Banga and Chien, 1993a) which has investigated the feasibility of iontophoretic transport of vasopressin through hairless rat skin. However, most of these studies have not carefully evaluated the stability of vasopressin during or following in vitro transport through skin. This could perhaps be due to the lack of analytical techniques that can be sensitive enough to pick up small amounts of vasopressin permeating into the receptor solution. HPLC assay has been used to investigate stability in donor or in buffer solutions, while thin-layer chromatography has been used to show the qualitative presence of vasopressin in the receptor compartment. These studies used rat skin, which may also differ in enzymatic activity and other characteristics from human skin. The studies described in this paper used human cadaver skin and an improved experimental design with silver/silver chloride electrodes to prevent pH shifts during the study. Donor solutions were spiked with tritiated vasopressin and receptor was analyzed by HPLC assay, made possible by a sensitive radio-chromatography detector. Arginine-vasopressin (AVP) has a relatively high isoelectric point (p*I* 10.9), so that more than 99% of the drug is protonated in a buffer solution with pH lower than 9.0. Since vasopressin is mostly protonated at the pH (7.20) of Hepes buffer during the study, only anodal delivery was investigated as maximum delivery is expected under the driving force from anode to cathode.

2. Materials and methods

2.1. Materials

[Arg^8]Vasopressin (acetate salt) and sodium azide were obtained from Sigma Chemical Co. (St. Louis, MO), while [*phenylalanyl*-3,4,5-^{3}H(N)]Arg^8-vasopressin was purchased from Du Pont (Boston, MA). Hepes from Kodak (Rochester, NY) was supplied by Fisher Scientific Co. (Fair Lawn, NJ). Silver wire (0.5 mm diameter, 99.99%) and tetrabutylammonium hydroxide were obtained from Aldrich Chemical Co. (Milwaukee, WI). Silver/silver chloride electrodes were obtained from In Vivo Metric (Healdsburg, CA). Scintillation cocktail (Ultima-Flo^{TM}) for the radio-chromatography detector was purchased from Packard (Meriden, CT). Human cadaver skin was obtained from National Disease Research Interchange (Philadelphia, PA). Autopsy skin was obtained from healthy normal, HIV negative donors. The skin was frozen within 12 h of death and supplied either as dermatomed to 250 μm or as full-thickness skin. Once received, the skin was stored at -80°C and then thawed just before use.

2.2. Analytical techniques

The HPLC setup used was from Micromeritics (Norcross, GA) and consisted of a Solvent Delivery System (Model 750) and an Autosampler (Model 728). An Integrator (Model 3394 A) from Hewlett Packard Co. (Avondale, PA), Radio-chromatography detector (Radiomatic Flo-One\ Beta Series A-500) from Packard Instruments (Meriden, CT), HPLC glass vials and plastic caps from Alcott Chromatography (Norcross, GA) were used. The column used was C_{18} NOVA-PAK® 3.9 × 150 mm, pore size 125 Å (Waters Technology Group, Hightstown, NJ) and filter paper 0.22 μm GVWP was obtained from Millipore (Bedford, MA). The radio-chromatography detector was operated by the software loaded on a 386 Windows-based computer.

The mobile phase used for HPLC assay of vasopressin consisted of 0.05 M ammonium acetate buffer (pH 6.4)-acetonitrile (82:18) at a flow rate of 0.5 ml/min. The buffer was filtered

through a 0.22 μm filter. Detection was carried out using a radio-chromatography detector. The ratio of scintillation cocktail (Ultima-Flo™) through the detector to the HPLC flow rate within the analytical cell was 3:1.

2.3. Solution preparation

The buffer used contained 25 mM Hepes along with 0.133 M sodium chloride and 0.02% of sodium azide. The pH of the buffer was raised to 7.2–7.4 with tetrabutylammonium hydroxide. Hepes buffer was chosen as it is a physiological buffer with a high buffering capacity at pH 7.4, is zwitterionic at this pH and thus has reduced charge carrying ability. Addition of sodium chloride provides one primary cation (Na^+) and one primary anionic (Cl^-) charge carrier. Chloride also participates in the electrochemical reaction to form silver chloride. Tetrabutylammonium hydroxide was used for pH adjustment as it avoids the addition of small positive ions which would otherwise carry a large fraction of the charge (Burnette and Ongpipattanakul, 1987). The donor solution was prepared in Hepes buffer containing vasopressin (0.25 mg/ml) and spiked with [^{3}H]vasopressin (1 μCi/ml). Human serum albumin, 0.1% was also added to prevent any adsorption of vasopressin to glass or other contact surfaces.

2.4. Transdermal iontophoresis studies

Valia-Chien cells, modified to have two ports on each half-cell, were used. One port served as the sampling port while the other port accommodated the electrode. Excised human skin was thawed and mounted between the two half-cells. An external water bath maintained the temperature of the circulating water in the jackets at 37°C, and star-headed magnetic stirrers were used to stir the solutions in both compartments continuously. A silver wire was used as the anode and silver/silver chloride wire was used as the cathode. Silver-silver chlorides are reversible electrodes and were used as these do not cause electrolysis of water, which would otherwise lead to pH shifts in solution. The current was controlled from a PC-based software and the resistance of the skin was continually monitored. Samples taken from the receptor were analyzed by HPLC using a radio-chromatography detector.

Experiments were performed in triplicate and the three sets of experiments (nine cells) could be run simultaneously. The receptor compartment was filled with 4 ml of Hepes buffer and the donor compartment was filled with the donor solution under study. Teflon caps were positioned over the sampling ports and electrodes were placed through holes in the caps, to avoid evaporation of the solutions bathing the electrodes. Constant (D.C.) current of 0.5 mA/cm^2 was supplied through a Scepter® (Keltronics, Oklahoma City, OK) power supply for 8 h but sampling was carried out for 12 h.

2.5. Skin homogenate studies

Human cadaver skin equivalent to the exposed surface area in V-C cells was weighed and homogenized in Hepes buffer. Homogenized skin was then centrifuged at 2500 rpm for 15 min and the supernatant was filtered through 0.45 μm filter. The filtrate was added to a vasopressin solution (0.25 mg/ml) spiked with 2 μCi/ml of tritiated vasopressin. The resulting solution was allowed to stand at 37°C. Samples were taken every 2 h and immediately analyzed by HPLC.

2.6. Degradation by intact skin

This experiment was designed to study the degradation of vasopressin while in contact with skin during the experiment. The donor solution was exposed to the stratum corneum side of a piece of skin while the receptor solution was exposed to the dermal side of another piece of skin. An aluminum foil was placed between these two pieces so that no transdermal transport of drug took place during the experiment. No electrodes were fitted and vasopressin solution (0.1 mg/ml) spiked with tritiated vasopressin (2 μCi/ml) was filled in both the donor and receptor half-cells. Samples were removed and immediately analyzed by HPLC.

3. Results and discussion

3.1. Iontophoretic delivery

When a current density of 0.5 mA/cm^2 was applied for 8 h, vasopressin was found to permeate through the skin (Fig. 1). Since a radiochemical detector was used for analysis, any free tritium or degradation products would not show up at the vasopressin retention time on the chromatogram. As can be seen in Fig. 1, the flux of vasopressin across the skin stopped as soon as the current was stopped. This shows the reversibility of delivery. Thus, modulation of peptide delivery by multiple applications of current should be possible. This can be used to achieve pulsatile delivery of peptides, which would be desirable since many physiological peptides are released in a pulsatile manner. No flux of vasopressin across was detected under passive conditions, i.e., when no current was applied.

The total cumulative amount permeated over 12 h (data not shown) was 15.37 ($\pm$5.31; $n = 3$) μg/cm^2. Commercial products of vasopressin currently in the market include Pitressin® (Parke-Davis, Morris Plains, NJ) which contains 20 pressor units of vasopressin. One adult human dose is about 10 units and this corresponds to about 25 μg. This suggests that therapeutic doses can easily be administered using a iontophoretic patch with a realistic surface area, even though the cumulative amounts permeated correspond to only about 1% permeation.

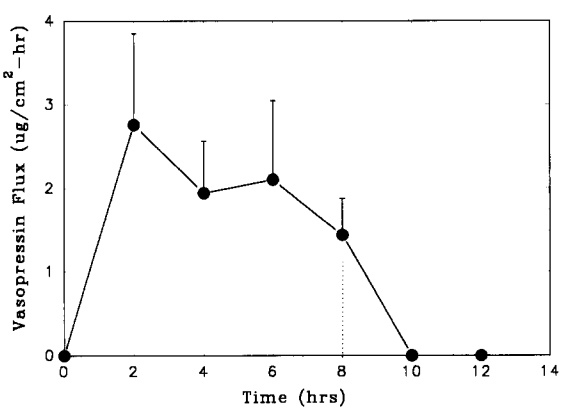

Fig. 1. Flux of vasopressin across human cadaver skin under anodal iontophoretic transport. Current density of 0.5 mA/cm^2 was applied for 8 h.

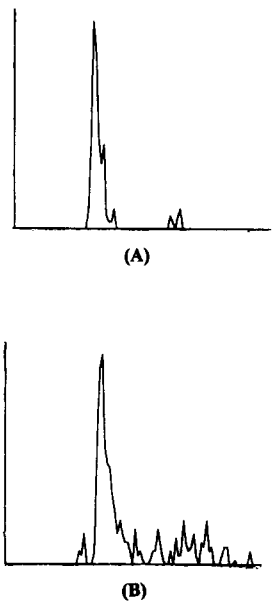

Fig. 2. HPLC chromatogram of vasopressin at (A) 2 h after iontophoresis and (B) 8 h after iontophoresis. The peak at 8 h was larger (Y-axis is on an automatic scale adjustment) but also had several degradation peaks.

3.2. Degradation during transport

Skin is known to contain both exo- and endopeptidases, with the aminopeptidases being the best known (Banga and Chien, 1993b). The use of frozen skin in iontophoresis research has been supported since its electrical behavior was found to be similar to that of fresh skin (Kasting and Bowman, 1990). However, the enzymatic activity of dermatomed cadaver skin used in this study could not be equivalent to an in vivo drug delivery situation. The enzymatic activity is likely to be higher under the in vivo situation. Therefore, less drug may be delivered to patients than what could be predicted from this study.

Vasopressin was found to degrade during skin transport, as indicated by the degradation peaks in the HPLC chromatogram that were observed (Fig. 2). This suggests that the skin still had enough enzymatic activity left to cause enzymatic cleavage of vasopressin into several products. The

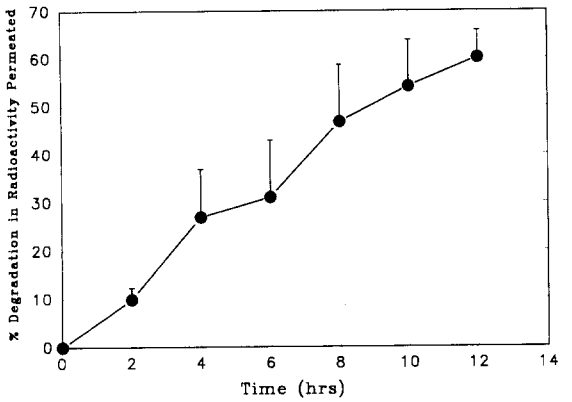

Fig. 3. Percent degradation peaks in the total radioactivity permeated as a function of time. The calculation is based on the removal of vasopressin peak from the total radioactivity permeated.

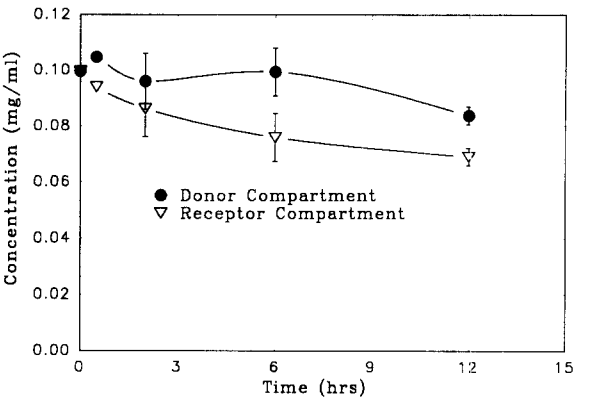

Fig. 4. Comparison of degradation of vasopressin (0.1 mg/ml) in donor vs receptor compartment. No transdermal transport was allowed in this experiment.

number of degradation products could be even higher than what would appear from such a chromatogram since any cleaved fragment that did not carry the tracer would not show up on the chromatogram. Since the detector summed up the radioactivity in the vasopressin peak and all the degradation peaks, the total radioactivity permeated and the distribution between intact vasopressin and degradation products could be calculated. Fig. 3 shows the percent degradation peaks in the chromatogram as a function of time. At 12 h, about 60% of the radioactivity was in the degradation peaks, i.e., only about 40% of the radioactivity permeated was intact vasopressin by 12 h.

3.3. Degradation by intact skin

The degradation of vasopressin by intact skin (Fig. 4) was investigated as described in section 2. It was seen that greater amount of vasopressin remained in the donor compartment than in the receptor compartment after 12 h. Higher enzymatic degradation in the receptor solution is most likely because more proteolytic enzymes would be present on the dermal side of the skin, as compared to the stratum corneum, which was in contact with the donor solution. As explained in section 2, the experiment was designed so that no transdermal transport took place during this experiment.

3.4. Degradation by skin homogenate

The subcellular compartmentalization of proteolytic enzymes is not well understood. It is possible that the skin may contain proteolytic enzymes within the cell that would never contact the peptide being transported across appendageal routes during iontophoresis. However, preparation of a skin homogenate would disrupt the cells and expose these enzymes. As can be seen in Fig. 5, skin homogenate caused degradation of vaso-

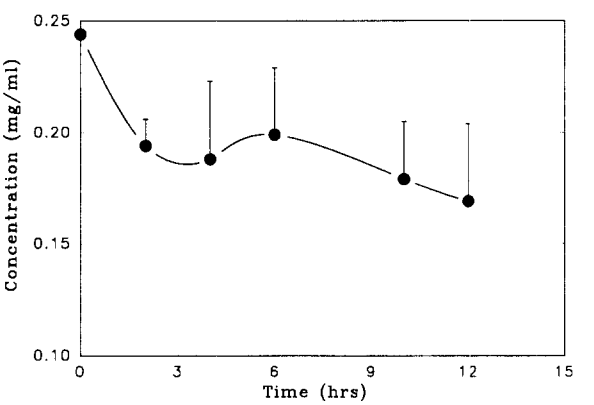

Fig. 5. Degradation of vasopressin (0.25 mg/ml) by skin homogenate as a function of time.

pressin. No degradation was observed in the controls that were incubated for 12 h at 37°C but without any skin homogenate. Although we started with the same skin/vasopressin ratio as in other studies, the percent loss of activity by skin homogenate does not seem much different from that by intact skin. This was most likely because our homogenization and filtration technique used was not able to extract all the proteolytic enzymes from the skin. This could be due to hydration of skin and adsorption to the filter.

Acknowledgements

This project was supported by the Auburn University Grant-in-Aid Program. Also, the supply of human cadaver skin from the National Disease Research Interchange is acknowledged.

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