Pharm Res (2015) 32:1417–1424
DOI 10.1007/s11095-014-1546-5

RESEARCH PAPER

Noninvasive Sampling of Gabapentin by Reverse Iontophoresis

Anroop B. Nair • Rachna Kumria • Bandar E. Al-Dhubiab • Mahesh Attimarad • Sree Harsha

Received: 17 June 2014 / Accepted: 6 October 2014 / Published online: 16 October 2014

ABSTRACT

Purpose Transdermal reverse iontophoresis offers a noninvasive tool for clinical and therapeutic monitoring of drugs and endogenous molecules. This study investigated the viability of reverse iontophoresis as an alternative technique to blood sampling for the monitoring of gabapentin.

Methods *Ex vivo* studies assessed the influence of polarity, applied current (0.064–0.32 mA) and subdermal concentration (0.5–20 μg/mL) on the recovery of gabapentin. These experiments were carried out in vertical Franz diffusion cell for a period of 3 h using rat skin. *In vivo* experiments examined the versatility of this method to extract gabapentin from the subdermal region following intravenous administration of gabapentin (30 mg/kg) in rat model.

Results Preliminary studies demonstrate that greater amount of gabapentin was extracted in the cathodal chamber due to the contribution of electroosmosis. Increasing the current intensity significantly enhances the extraction flux ($P < 0.005$) and shown linear relation ($r^2 = 0.84$) between the applied electrical dose (mA*h) and the amount of gabapentin recovered (μg). Indeed, transdermal iontophoresis of gabapentin was found to be concentration dependent in the range studied (0.5–20 μg/mL), which includes clinically relevant level. Further, a linear relationship was established between the iontophoretically recovered gabapentin 3 h flux values and the subdermal concentrations studied. The linear correlation with good regression value ($r^2 = 0.92$) observed in the *in vivo* studies infers that the drug in the plasma is proportionally extracted through the skin and potentially represents the subdermal drug concentrations.

Conclusions Given the promising results, this study concludes that the transdermal reverse iontophoresis technique could be a promising alternative for the noninvasive monitoring of gabapentin.

A. B. Nair (✉) • B. E. Al-Dhubiab • M. Attimarad • S. Harsha
Department of Pharmaceutical Sciences, College of Clinical Pharmacy
King Faisal University, Al-Ahsa 31982, Kingdom of Saudi Arabia
e-mail: anair@kfu.edu.sa

R. Kumria
Swift School of Pharmacy, Village-GhaggarSarai, Rajpura, Patiala, Punjab
India

KEY WORDS Epilepsy · Iontophoresis · Gabapentin · Noninvasive · Rat · Skin

INTRODUCTION

Epilepsy is a chronic neurologic disorder characterized by recurrent seizures which causes serious medical, psychological and social consequences for patients and their families. This disease is reported to affect over 50 million people worldwide although the prevalence is much higher in children [1, 2]. The effective treatment of this disorder mainly hinge on the prospective of maintaining therapeutically effective concentration of antiepileptic agents in plasma, which prevent seizures without causing side effects [3]. Currently, several new generation antiepileptic agents are used in the treatment of this disorder [4]. Gabapentin is one of the new generation antiepileptic agents used in the management of seizures associated with drug resistant partial epilepsy of adult and children aged 12 years and above [5, 6]. Although this drug was initially approved for the management of seizures, but later it showed promise in the treatment of various forms of chronic pains and is widely used for neuropathic pain [7]. However, this drug exhibit extensive pharmacokinetic variation leading to fluctuation in the plasma concentration due to the dose dependent absorption, which in turn varies the response widely within and between patients [8, 9]. It has also been reported that the efficiency of gabapentin to control seizures generally requires definite plasma concentrations [10]. In addition, the dose optimization of gabapentin is beneficial in patients with renal impairment [9]. Consequently, monitoring for gabapentin is valuable for personalizing drug therapy in the treatment epilepsy and has been widely studied [11–13].

Therapeutic drug monitoring constitutes a helpful tool for the clinician to bypass the uncertainty of drugs dose response relationships and use the better correlations. This approach has made it possible to study the individual variations in drug

utilization and to reveal noncompliance in patients. This in turn offers a better opportunity for the physician to adjust each patient's dosage and schedule to achieve better clinical effect. In general, the drug concentration in the plasma/serum is determined by frequent blood sampling. The existing methods for drug collection include blood sampling from vein (venipuncture) and finger stick method. However, both these methods are associated with discomfort, pain, risks of infection and possess low patient compliance especially very often sampling are required [14]. Alternatively, salivary concentration analysis is also employed in drug monitoring, however, the very low gabapentin concentration in saliva has limited its utility in clinical practice [15]. All these issues have provided considerable impetus for the development of alternative noninvasive methods for monitoring of gabapentin in epileptic patients.

Recently, the research has been focused on noninvasive methods for frequent clinical and therapeutic drug monitoring which could avoid blood sampling and improve patient compliance. In this context, skin provides an alternative way for clinical monitoring of drugs and biomarkers and the reverse iontophoresis technique has now been shown to monitor various endogenous molecules as effectively as the finger stick method [14, 16, 17]. Typically, transdermal reverse iontophoresis is a noninvasive approach whereby the application of a small low electric current across the skin increases the percutaneous extraction of charged ions and polar, neutral molecules from within or beneath the skin [18, 19]. There are two major mechanisms such as electromigration and electroosmosis contribute to the extraction of the substance of interest [20]. Gabapentin is a hydrophilic small molecular weight compound exist as a highly charged zwitterion at physiological pH with very low plasma protein binding (<3%) and could be a candidate for iontophoretic extraction. The aim of this study was to carry out a systematic investigation on the feasibility of transdermal reverse iontophoresis as an alternative noninvasive method for monitoring of gabapentin in rat model. To our knowledge, this is the first study which investigates the feasibility of transdermal reverse iontophoresis in therapeutic drug monitoring of gabapentin.

MATERIAL AND METHODS

Materials

Gabapentin was received as gratis sample from Ind Swift Ltd, Parwanoo, India. Sodium chloride, sodium acetate and phenylisothiocyanate were purchased from Loba Chemie, Mumbai, India. Levodopa, carbidopa, baclofen, polyvinylpyrrolidone, acetonitrile and methanol were purchased commercially from Sigma Aldrich, St. Louis, MO. All other chemicals and reagents used were of analytical grade. All solutions were prepared in ultrapure Millipore water (resistivity >18.2 MΩ/cm^2).

Analytical Method

The quantification of gabapentin in samples was carried out by high performance liquid chromatography (HPLC) method described by Zhu and Neirinck [21]. The HPLC system (Shimadzu, LC-10ATVP) consisting of a Symmetry C18 analytical column (4.6 mm x 150 mm, 5.0 μm) with a detector LC UV-100 was used to determine the amount of gabapentin. The drug sample was mixed with internal standard (baclofen) and extracted using a reverse phase solid extraction cartridge (Bond-Elut, 100 mg, 1 ml; Agilent Technologies, Santa Clara, CA) and derivatized with phenylisothiocyanate (0.35% in methanol) for 20 min at room temperature. A chromatographic separation was achieved by mobile phase consisted of sodium acetate buffer (0.04 M): methanol: acetonitrile (45:40:15, *v/v*). The flow rate was 1.2 mL/min and the separation was carried by eluting the solvent isocratically at 25°C. Samples of 25 μL were injected and the separation was observed at 252 nm. The retention time of internal standard and gabapentin were found to be 6.8 min and 8.2 min, respectively. The method was linear in the concentration range of 25–5000 ng/mL.

Skin Preparation

Skin membranes were prepared from the abdominal region of the Sprague–Dawley rats by carefully removing the hair without damaging the skin using trimmer. The full thickness skin was excised and the fat adhering to the dermis side was removed using a scalpel and isopropyl alcohol. Then the skin was washed with water, wrapped with aluminium foil, stored at -20^0 C and was used within a week [22]. The membrane resistance was measured before the experiment as described in our earlier studies [23] and the skin which had a resistance >20 kΩ.cm^2 was used.

Ex vivo Transdermal Reverse Iontophoresis Studies

Ex vivo experiments were carried out in a two chambered Franz diffusion cell using full thickness Sprague–Dawley rat skin freshly excised from the abdominal region. The excised skin with specific integrity was mounted between the two halves of a vertical cell such that the dermis was in contact with donor compartment while the upper chamber acted as the receiver. Both chambers were then filled with physiological phosphate buffered saline (PBS; pH 7.4) and equilibrated for 30 min. Then the entire PBS solution was removed and new buffer was added to the upper chamber (500 μL), while the lower chamber of the diffusion cell was filled with solution containing gabapentin in PBS (5 mL) and maintained at 37±

0.5°C. The area available for transport was 0.64 cm^2. Silver-silver chloride electrodes (0.5 mm diameter) were fixed at a distance of 2 mm from the skin surface in donor and receiver chambers. Constant direct current iontophoresis was applied using Iomed Phoresor II dose controller (Iomed Inc., Salt Lake City, UT). The amount of gabapentin extracted in the receiver compartment was measured by HPLC. Similarly, control experiments were also carried out at 0 mA current intensity.

Preliminary *Ex vivo* Study

The effect of anodal (drug extracted from the subdermal anodal chamber) iontophoresis on the extraction of gabapentin was carried out as described in the *ex vivo* transdermal reverse iontophoresis studies. The extraction was performed for a period of 3 h by applying a direct current (0.32 mA) by connecting anode electrode to the subdermal compartment containing gabapentin (20 μg/mL), while the opposite electrode in the extraction chamber completed the circuit. The electric current was stopped at intervals of every 1 h and both donor and receiver compartments were entirely removed and replaced with fresh media. The receptor solution was further derivatized and analyzed by HPLC.

Effect of Current Intensity

To study the effect of applied current on gabapentin extraction, iontophoresis was carried out at three current intensities (0.064, 0.192, and 0.32 mA) for different time periods (1, 2 and 3 h). The experimental set-up was similar to that described in the *ex vivo* transdermal reverse iontophoresis studies. The subdermal compartment contained gabapentin solution (20 μg/mL), which bathed the dermal side of the skin, worked as the anodal chamber while the buffer in the outer surface of the skin was the cathodal compartment. The amount of gabapentin recovered into the receiver compartment was plotted against the applied electrical dose.

Effect of Gabapentin Concentration

Influence of subdermal drug concentration on the extraction of gabapentin was determined by following the same procedure described in the *ex vivo* transdermal reverse iontophoresis studies above. Different concentrations (0.5, 2.5, 5, 7.5, 10, 15 and 20 μg/mL) of gabapentin covering the therapeutic range of drug was used in the subdermal compartment and an iontophoresis (0.32 mA) was carried out for a period of 3 h. The upper half of the cell acted as the cathodal collecting chamber. Entire donor and receiver compartments were removed and replaced with new samples at every 1 h. The cumulative amount of gabapentin extracted into the receiver chamber normalized to surface area exposed to the drug was expressed as μg/cm^2.

In vivo Studies

Wistar rats (150–200 g), maintained on a 12/12 h light/dark cycle with unlimited access to food and water were anaesthetized by administering phenobarbitone (30 mg/kg) intraperitoneally were used in the study (Institutional Animal Ethical committee). Gabapentin (30 mg/kg) was injected into the lateral tail vein using 27 gauge needle. The dose of gabapentin (30 mg/kg) was calculated based on the human equivalent dose of gabapentin used for seizures, using the equation described by Reagan-Shaw *et al.* [24]. Abdominal region of rat was carefully shaved without damaging the skin using trimmer [25]. The application area (1 cm^2) was demarcated and two custom made cylindrical glass cells (1 × 1 cm inner diameter) were fixed using Premier cello tape (Premier Industries, New Delhi) with 1 cm distance between the chambers. Both chambers were filled with same volume (500 μL) of PBS. Silver-silver chloride electrodes (0.5 mm diameter) were inserted into the chambers and fixed at a distance of 2 mm above the skin surface. Constant direct current iontophoresis (0.5 mA) was applied using Iomed Phoresor II dose controller (Iomed Inc., Salt Lake City, UT) for a period of 3 h. The electric current was stopped at intervals of every 30 min and both donor and receiver compartments were entirely removed and replaced with fresh buffers. The receiver solution was further analyzed for the amount of gabapentin extracted. In parallel, the blood plasma samples were collected (~200 μL) from retro orbital plexus using dry heparinized tubes at the same time points when reverse iontophoresis extraction were sampled. Intraperitoneal injections of dextrose (250 μL) were given to rats after collection of each blood sample to minimize changes in volume of the central compartment. Plasma (200 μL) was thoroughly mixed by vortexing (2 min), subjected to protein precipitation using acetonitrile: 2-propanol (1:1), followed by centrifugation at 10,000×g for 10 min. The supernatant was further derivatized and the amount of gabapentin in plasma was estimated using the same chromatographic conditions as described above.

Data Analysis

The cumulative amount of gabapentin extracted per unit skin surface area was plotted against time and the slope of the linear portion of the plot was estimated as the flux [26]. The data were tested by one-way analysis of variance (ANOVA) and Unpaired *t*-test using graphpad prism 5 (graphpad software, Inc., CA, USA) to test the effects of various treatments. The data points provided in the graph is the mean of six replicates and the error bars represents the standard

deviation, unless otherwise specified. $P<0.05$ was considered as the level of significance.

RESULTS AND DISCUSSION

Extensive studies in the past two decades have identified numerous potentials of transdermal reverse iontophoresis which include clinical chemistry and therapeutic drug monitoring [14]. This noninvasive technique has proven its potential in extracting a diverse range of analytes including various endogenous molecules (urea, prostaglandin, glucose, lactate, mannitol, phenylalanine, amino acids etc.) and drugs (valproate, lithium, phenytoin, amikacin, caffeine, theophilline etc.) [14, 27]. Indeed, low molecular weight compounds of both charged and polar, neutral molecules are excellent candidates for noninvasive monitoring using transdermal reverse iontophoresis. In the current study, the physicochemical properties of gabapentin such as low molecular weight (171.34 Da) and hydrophilic nature (solubility in water; ~10 mg/mL) suggest the prospective of this molecule to be extracted through the skin. However, this molecule possess two pKa values (3.7 and 10.7) with an isoelectric point of 7.14, which signifies that at physiological pH (7.4) gabapentin is essentially zwitterionic [28]. Preliminary studies were carried out to assess the feasibility of transdermal reverse iontophoresis in the extraction of gabapentin by carrying out *ex vivo* skin recovery experiments. To simulate the *in vivo* condition, physiological phosphate buffer of pH 7.4 was used as the subdermal solution and a current intensity of 0.32 mA was applied for 3 h. Under the conditions of the reverse iontophoretic experiments performed (pH 7.4), it was observed that a greater amount of gabapentin was extracted towards the cathodal chamber, which increases with duration. Indeed, the cumulative amounts of gabapentin recovered at 1, 2 and 3 h in the cathodal chamber were found to be 0.26 ± 0.13 μg/cm^2, 0.66 ± 0.25 μg/cm^2 and 1.02 ± 0.25 μg/cm^2, respectively, when a low intensity current (0.32 mA) was applied. The possible reasons for the enhancement in gabapentin extraction (in cathodal chamber) could be primarily due to electroosmosis, which is a convective solvent flow, in response to the preferential passage of counter ions, when the electric field is applied. This solvent flow in the anode to cathode direction, due to the fact that the skin possess net negative charge at physiological condition, in turn increases the transport of zwitterionic species of gabapentin. Indeed the data observed here is in agreement with the earlier reports wherein the iontophoretic transport of zwitterionic compounds, under the physiological pH conditions, are predominantly electroosmosis [29–31]. On the other hand, no detectable amount of gabapentin was measured in control experiments, in the current experiment conditions.

In the next phase, we examined the effect of current intensity on the extraction of gabapentin across the skin membrane. Iontophoresis was carried out by applying three different current intensities (0.064, 0.192 and 0.32 mA for a period of 3 h) and the amount of gabapentin extracted are depicted in Fig. 1a. It was observed that the increase in current intensity proportionately enhance extraction of drug through the skin, irrespective of the sampling period. The amount of gabapentin extracted were found to be 0.13 ± 0.04 μg/cm^2/h, 0.29 ± 0.06 μg/cm^2/h and 0.47 ± 0.11 μg/cm^2/h with 0.064, 0.192 and 0.32 mA current, respectively, implies that the increase in current intensity significantly ($P<0.005$) enhances the flux of drug recovered. In addition, the cumulative amount of gabapentin extracted through the skin also increased with duration, suggest that the steady state flux has not been achieved in the current experimental condition (Fig. 1a).

In general, the efficiency of extraction (γ) is determined for zwitterionic compounds as an apparent solvent flow. In the current study we calculated the efficiency of extraction in the *ex vivo* studies at three different time intervals (1, 2 and 3 h) with the highest current intensity applied (0.32 mA) as described by Leboulanger *et al.* [32]. It was observed that the efficiency of gabapentin extraction values varied marginally between ~8–18 μL/h, and was found to increase with the duration. The possible reason for the variation in the efficiency of extraction value is likely due to the short study period. Hence an accurate correlation between the fluxes to the subdermal level of gabapentin is not anticipated in the current experimental condition. However, one can relate the fluxes to the subdermal levels once the efficiency of extraction achieves plateau, which is possible with a long term study.

The same data was also used to assess the effect of electrical dose on the gabapentin transdermal extraction by plotting the amount extracted (μg) as a function of the applied electrical dose (current multiplied by time; mA*h) and depicted in Fig. 1b. It is apparent from the Fig. 1b that the relationship between the applied electrical dose (applied to 0.64 cm^2) and the amount of gabapentin recovered possess linear relation ($r^2=0.84$). However, it is known that the therapeutic drug monitoring occurs in kinetic conditions wherein rapid extraction is anticipated to measure the actual subdermal drug concentration. Thus a short time extraction would be more advantageous than the long time extraction as the latter is likely to provide more average value about the subdermal drug concentration. One the other hand, the slope value observed in this study signifies that unit increase in electrical dose is likely to extract ~0.78 μg of gabapentin, which may be much higher once the extraction efficiency become constant. From the results of this experiment, it appears that the transdermal reverse iontophoresis technique could be a feasible alternative approach to extract gabapentin from the subdermal region.

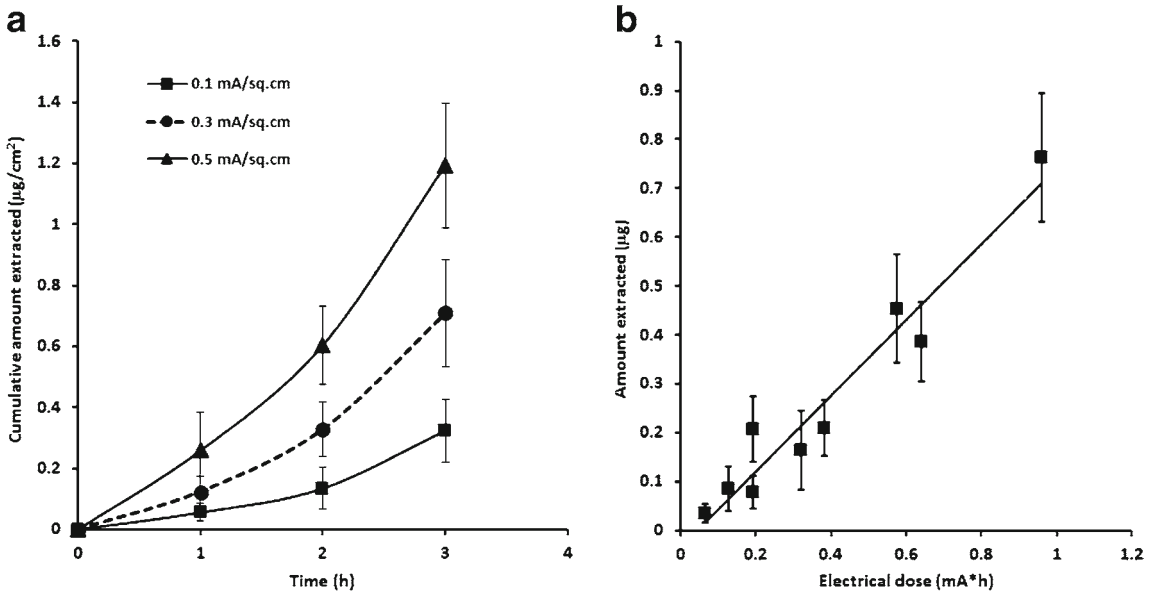

Fig. 1 Comparison of the extraction profiles of gabapentin (**a**), relationship between applied electrical dose (mA*h) and the amount of gabapentin extracted (µg) ($r^2 = 0.8432$; $F = 279.6$; $p < 0.0001$) (**b**) in the *ex vivo* studies using rat skin. Iontophoresis was carried out for 3 h by applying different current intensities (0.064, 0.192 and 0.32 mA). All values are mean ± SD ($n = 6$).

Gabapentin exhibit pharmacokinetic variation which causes fluctuation in the plasma concentrations during the therapy. Therefore the viability of the proposed transdermal reverse iontophoresis technique primarily rely on its prospective to extract gabapentin through the skin at different subdermal concentrations and is expected to be predictable in order to individualize in clinical practice. In the next phase, *ex vivo* extraction studies were carried out by iontophoresis (0.32 mA) using known subdermal gabapentin concentrations (0.5–20 µg/mL) to establish the relation between subdermal gabapentin flux and the amount extracted by the reverse iontophoresis. The amount of gabapentin extracted into the receiver compartment at different time intervals (1–3 h) is represented in Fig. 2a. It is apparent from Fig. 2a that the application of transdermal iontophoresis (0.32 mA) could extract significant amount of gabapentin in all the subdermal concentrations studied. Further, it was observed that the increase in subdermal gabapentin concentration proportionately enhanced the extraction, which denotes that the transdermal reverse iontophoresis of gabapentin is concentration dependent. Indeed, the flux values measured for the highest gabapentin concentration studied (20 µg/mL) was an order of magnitude greater than that observed for the lowest (0.5 µg/mL), under similar experimental conditions. Moreover, it is also evident from the Fig. 2a that the lowest drug concentration studied (0.5 µg/mL, which is well below the therapeutic range of gabapentin), could also be successfully extracted by the transdermal reverse iontophoresis technique. The efficiency of gabapentin extraction with different subdermal concentration (0.5–20 µg/mL) was found to be varied between 12–17 µL/h. Further, the observed iontophoretic 3 h flux values of gabapentin were plotted as a function of subdermal drug concentration in order to assess their relation (Fig. 2b). A linear relationship ($r^2 = 0.71$) was observed between the iontophoretically recovered gabapentin 3 h flux values and the subdermal concentrations studied, which include clinically relevant level (1–10 µg/mL). However, a better linear correlation with greater regression is possible once the extraction efficiency reaches a plateau, wherein the amount of gabapentin extracted would potentially reflects the underneath compartment. It should also be mentioned that the linear flux observed in the current study also signified that any further increase in subdermal concentration could eventually lead to enhancement in the flux. This will help in monitoring the gabapentin in any situation wherein the plasma concentration is greater than 20 µg/mL. The above observations indicate that the transdermal reverse iontophoresis technique could be a promising approach which is predictable and could be successfully utilized as a noninvasive approach for the therapeutic monitoring of gabapentin in clinical situation.

Given the encouraging results in the *ex vivo* studies, further experiments were carried out *in vivo* using rat model. This preliminary *in vivo* study was carried out in order to examine the versatility of current technique to extract gabapentin and evaluate the possible relationship between the subdermal concentration and amount recovered by transdermal reverse iontophoresis. An intravenous bolus dose of 30 mg/kg body weight was administered through the tail vein of Wistar rats and applied iontophoresis (0.5 mA) immediately. The amount of gabapentin extracted into the cathodal chamber as well as the blood plasma samples were collected simultaneously on

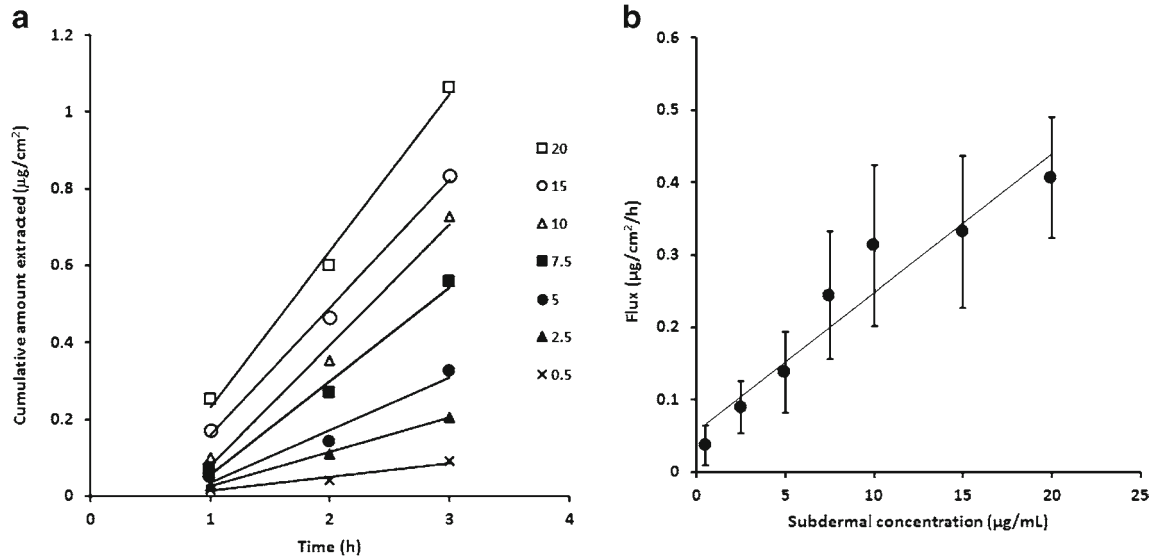

Fig. 2 Comparison of the amount of drug extracted from different subdermal gabapentin concentrations (0.5–20 µg/mL) (**a**), correlation between observed 3 h flux values of gabapentin and the subdermal concentrations ($r^2 = 0.7067$; $F = 96.39$; $p < 0.0001$) (**b**). Iontophoresis (0.32 mA) was carried for a period of 3 h. All values are mean ± SD ($n = 6$).

each rat at the same time points for a period of 3 h and analyzed by HPLC. The measured plasma drug concentration (µg/mL) was plotted as a function of the amount recovered by transdermal reverse iontophoresis (µg/cm^2) at various time intervals (1.5, 2, 2.5 and 3 h) following intravenous administration of gabapentin (30 mg/kg) and is illustrated in Fig. 3. The first phase of the extraction curve (0.5 and 1 h) were excluded as they corresponds to the time required for extraction fluxes to become stable. The linear correlation with good regression value ($r^2 = 0.92$) observed here also infers that the amount of drug extracted through the skin by transdermal reverse iontophoresis would potentially reflects the subdermal drug concentrations. The extraction of gabapentin during transdermal reverse iontophoresis could be likely due to the rapid availability of this unbound drug in the subdermal region, as this drug is reported to possess extensive distribution throughout the body including the peripheral tissues such as skin probably due to its very low protein binding [33]. However, the correlation observed here is limited to the gabapentin plasma concentration range of ~7–22 µg/mL, although it includes the therapeutic range. Nevertheless, Fig. 3 also signifies that the linear relation observed is likely to extend when the subdermal gabapentin concentration is low. Thus it is always possible that one can utilize a much

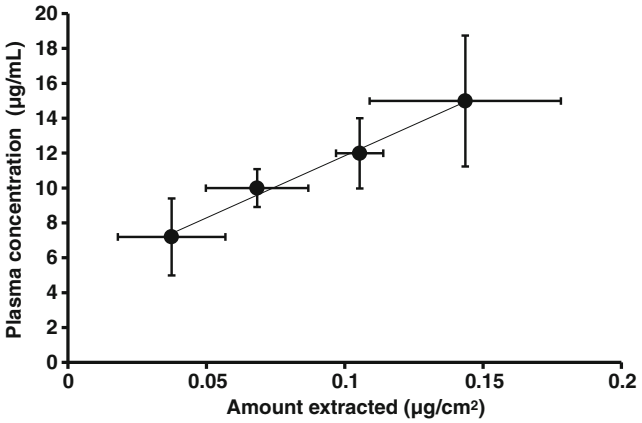

Fig. 3 Correlation between measured gabapentin plasma concentration (µg/mL) and the amount extracted by transdermal iontophoresis (µg/cm^2) at various time intervals (1.5, 2, 2.5 and 3 h) following intravenous administration of gabapentin (30 mg/kg) in Wistar rats. Iontophoresis (0.5 mA) was carried for a period of 3 h ($r^2 = 0.9226$; $F = 262.4$; $p < 0.0001$). All values are mean ± SD ($n = 6$).

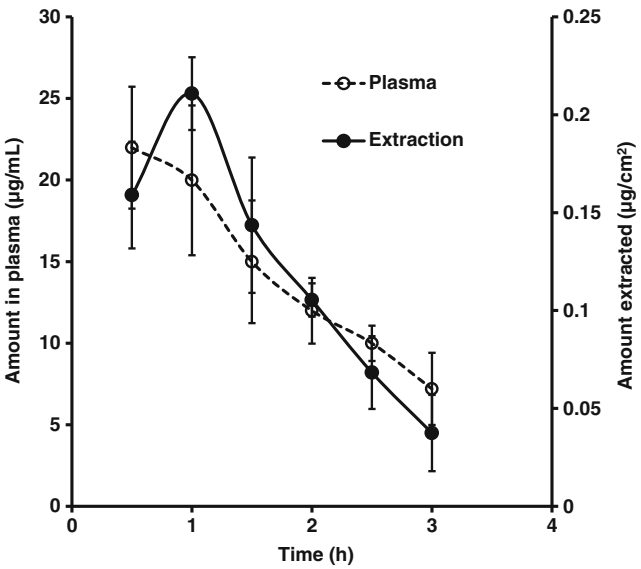

Fig. 4 Comparison of the concentration time profiles of gabapentin in plasma (µg/mL) and the amount extracted by transdermal iontophoresis (µg/cm^2) at different time intervals following intravenous administration of gabapentin (30 mg/kg) in Wistar rats. Iontophoresis (0.5 mA) was carried for a period of 3 h. All values are mean ± SD ($n = 6$).

sensitive analytical technique such as liquid chromatography mass spectrum to estimate even if the recovered gabapentin concentration is relatively low. On the other hand, greater correlation between the amount extracted and the subdermal concentration could be further achieved by optimizing the iontophoretic conditions which may result in an improved flux.

The comparison of time course of gabapentin in rat plasma measured by blood sampling and transdermal reverse iontophoresis (0.5 mA) following intravenous bolus administration of 30 mg/kg of drug is presented in Fig. 4. It can be seen from Fig. 4 that the plasma gabapentin concentration was maximum (21.98±3.74 μg/mL) at the initial sampling point (0.5 h), which decreases as the drug is eliminated with duration of time. In contrast, the amount of gabapentin extracted by transdermal iontophoresis was relatively low (0.16±0.03 μg/cm^2) in the initial period (0.5 h) but increased at 1 h (0.21± 0.02 μg/cm^2). A similar observation was also reported by Leboulanger *et al.* [30] when lithium was extracted. However, after 1 h both drug concentration profiles measured from plasma and transdermal extraction by reverse iontophoresis showed identical trend. It is apparent from the Fig. 4 that the extraction profile decreased in parallel with the subdermal gabapentin concentration which signifies that the amount extracted by transdermal iontophoresis is proportional to the subdermal concentration. Further, the efficiency of extraction values of gabapentin in the current experimental condition was found to be ~10–21 μL/h, which substantiate the *ex vivo* data. Indeed, the data observed here demonstrate that gabapentin could be quantitatively and proportionately extracted by the transdermal reverse iontophoresis, even when the subdermal concentrations of the analytes are varying with time. However, the drug concentration observed in the plasma was found to be two orders of magnitude higher than the corresponding drug concentration extracted by transdermal reverse iontophoresis, in the current experimental condition. This *in vivo* data also supported the *ex vivo* findings and suggest that the proposed technique could be viable alternative noninvasive approach for the monitoring of gabapentin at clinically relevant levels. Further, it must be borne in mind that the skin membrane of rat is different from the human as the regular rats have a very high hair follicle density compared to human skin. This is very important since the iontophoretic transport is presume to take place via an appendageal pathway [34] and therefore the rat skin membrane is more permeable than human. Thus one can expect slightly low extraction in human studies, although it need to be proved.

CONCLUSION

In summary, this study investigated the potential of transdermal reverse iontophoresis as an alternative noninvasive tool for the therapeutic monitoring of gabapentin. Initial studies indicate moderately high extraction of the zwitterions of gabapentin towards the cathodal chamber, due to the contributions of electroosmosis to electro-transport. The *ex vivo* extraction studies with different current intensities revealed that the increase in current intensity proportionally enhance the flux of gabapentin recovered. A reasonable correlation was observed when the applied electrical dose was plotted as a function of the amount of gabapentin recovered from the subdermal region. It was also found that the increase in subdermal drug concentration proportionately enhanced the extraction of gabapentin, which denotes that the transdermal reverse iontophoresis of gabapentin is concentration dependent. *In vivo* results shown good linear correlation with higher regression value, suggest that the transdermal reverse iontophoretic extraction is a feasible and promising noninvasive approach for the monitoring of gabapentin in epileptic patients. However, the efficiency of gabapentin extraction values in *ex vivo* and *in vivo* studies were found to vary marginally, probably due to the short study period. Further studies in human are required to demonstrate this finding in clinical practice.

ACKNOWLEDGMENT AND DISCLOSURES

The authors thank the Deanship of Scientific Research, King Faisal University for funding this research project No. 140069.

Declaration of Interest The authors report no conflict of interest.

REFERENCES

1. Jacoby A, Snape D, Baker GA. Epilepsy and social identity: the stigma of a chronic neurological disorder. Lancet Neurol. 2005;4(3):171–8.
2. WHO, World Health Organization Epilepsy: Key facts. Available at: www.who.int/mediacentre/factsheets/fs999/en Accessed June 3, 2014.
3. Goldenberg MM. Overview of drugs used for epilepsy and seizures: etiology, diagnosis, and treatment. Proc Natl Acad Sci U S A. 2010;35(7):392–415.
4. French JA, Gazzola DM. New generation antiepileptic drugs: what do they offer in terms of improved tolerability and safety? Ther Adv Drug Saf. 2011;2(4):141–58.
5. McLean MJ, Gidal BE. Gabapentin dosing in the treatment of epilepsy. Clin Ther. 2003;25(5):1382–406.
6. Al-Bachari S, Pulman J, Hutton JL, Marson AG. Gabapentin add-on for drug-resistant partial epilepsy. Cochrane Database Syst Rev. 2013;7:CD001415.
7. Rose MA, Kam PC. Gabapentin: pharmacology and its use in pain management. Anaesthesia. 2002;57(5):451–62.
8. Johannessen SI, Tomson T. Pharmacokinetic variability of newer antiepileptic drugs: when is monitoring needed? Clin Pharmacokinet. 2006;45:1061–75.
9. Krasowski MD. Therapeutic drug monitoring of the newer antiepilepsy medications. Pharmaceuticals. 2010;3:1909–35.

10. Sivenius J, Kälviäinen R, Ylinen A, Riekkinen P. Double-blind study of Gabapentin in the treatment of partial seizures. Epilepsia. 1991;32(4):539–42.
11. Johannessen SI, Battino D, Berry DJ, Bialer M, Krämer G, Tomson T, *et al.* Therapeutic drug monitoring of the newer antiepileptic drugs. Ther Drug Monit. 2003;25:347–63.
12. Patsalos PN, Berry DJ. Therapeutic drug monitoring of antiepileptic drugs by use of saliva. Ther Drug Monit. 2013;35(1):4–29.
13. Tribut O, Bentué-Ferrer D. Verdier MC; therapeutic drug monitoring of gabapentin. Therapie. 2010;65(1):57–60.
14. Nair AB, Goel A, Prakash S, Kumar A. Therapeutic drug monitoring by reverse iontophoresis. J Basic Clin Pharm. 2012;3:207–13.
15. Berry DJ, Beran RG, Plunkeft MJ, Clarke LA, Hung WT. The absorption of gabapentin following high dose escalation. Seizure. 2003;12(1):28–36.
16. Nixon S, Sieg A, Delgado-Charro MB, Guy RH. Reverse iontophoresis of L-lactate: *in vitro* and *in vivo* studies. J Pharm Sci. 2007;96(12): 3457–65.
17. Sieg A, Jeanneret F, Fathi M, Hochstrasser D, Rudaz S, Veuthey JL, *et al.* Extraction of amino acids by reverse iontophoresis *in vivo*. Eur J Pharm Biopharm. 2009;72(1):226–31.
18. Paulley Y, Delgado-Charro MB, White KAJ. Modelling formation of a drug reservoir in the stratum corneum and its impact on drug monitoring using reverse iontophoresis. Comput Math Method M. 2010;11:353–68.
19. Wang CY, Maibach HI. Why minimally invasive skin sampling techniques? a bright scientific future. Cutan Ocul Toxicol. 2011;30:1–6.
20. Leboulanger B, Guy RH, Delgado CMB. Reverse iontophoresis for noninvasive transdermal monitoring. Physiol Meas. 2004;25:R35–50.
21. Zhu Z, Neirinck L. High-performance liquid chromatographic method for the determination of gabapentin in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2002;779(2):307–12.
22. Anroop B, Ghosh B, Parcha V, Kumar A, Khanam J. Synthesis and comparative skin permeability of atenolol and propranolol esters. J Drug Del Sci Tech. 2005;15(2):187–90.
23. Nair AB, Vaka SR, Gupta S, Repka MA, Murthy SN. *In vitro* and *in vivo* evaluation of a hydrogel-based prototype transdermal patch system of alfuzosin hydrochloride. Pharm Dev Technol. 2012;17: 158–63.
24. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J. 2008;22(3):659–61.
25. Nair AB, Singh K, Al-Dhubiab BE, Attimarad M, Harsha S, Alhaider IA. Skin uptake and clearance of ciclopirox following topical application. Biopharm Drug Dispos. 2013;34:540–9.
26. Anroop B, Ghosh B, Parcha V, Khanam J. Transdermal delivery of atenolol: effect of prodrugs and iontophoresis. Curr Drug Deliv. 2009;6:280–90.
27. Sieg A, Wascotte V. Diagnostic and therapeutic applications of iontophoresis. J Drug Target. 2009;17(9):690–700.
28. Kong VK, Irwin MG. Gabapentin: a multimodal perioperative drug? Br J Anaesth. 2007;99(6):775–86.
29. Sieg A, Guy RH, Delgado-Charro MB. Electroosmosis in transdermal iontophoresis: implications for noninvasive and calibration-free glucose monitoring. Biophys J. 2004;87(5):3344–50.
30. Leboulanger B, Fathi M, Guy RH, Delgado-Charro MB. Reverse iontophoresis as a noninvasive tool for lithium monitoring and pharmacokinetic profiling. Pharm Res. 2004;21(7): 1214–22.
31. Lopez RF, Bentley MV, Delgado-Charro MB, Guy RH. Iontophoretic delivery of 5-aminolevulinic acid (ALA): effect of pH. Pharm Res. 2001;18(3):311–5.
32. Leboulanger B, Aubry JM, Bondolfi G, Guy RH, Delgado-Charro MB. Lithium monitoring by reverse iontophoresis *in vivo*. Clin Chem. 2004;50(11):2091–100.
33. Johannessen Landmark C, Johannessen SI, Tomson T. Host factors affecting antiepileptic drug delivery-pharmacokinetic variability. Adv Drug Deliv Rev. 2012;64(10):896–910.
34. Nair A, Reddy C, Jacob S. Delivery of a classical antihypertensive agent through the skin by chemical enhancers and iontophoresis. Skin Res Technol. 2009;15:187–94.