Changes in Skin A.C. Impedance Parameters *In Vivo* During the Percutaneous Absorption of Local Anesthetics

A. D. Woolfson,[1,4] G. P. Moss,[1,2] D. F. McCafferty,[1] A. Lackermeier,[3] and E. T. McAdams[3]

Received June 15, 1998; accepted December 1, 1998

KEY WORDS: a.c. impedance spectroscopy; percutaneous local anesthesia; tetracaine; lidocaine-prilocaine eutectic mixture; reservoir effect.

INTRODUCTION

Percutaneous local anesthesia (1) occurs when a local anesthetic agent penetrates the intact *stratum corneum* and desensitises the underlying nociceptors. Clinical applications of the technique include painless venepuncture, especially in children, harvesting of split-skin grafts and miscellaneous topical surgical procedures.

The ideal percutaneous local anesthetic formulation should produce rapid, deep and long-lasting cutaneous anesthesia (2). There are currently two products licensed for the provision of percutaneous local anesthesia. Ametop™ gel is based on the discovery of the tetracaine phase-change system (3) and EMLA® cream (4) comprises an eutectic mixture of lidocaine and prilocaine as their free bases. The tetracaine system produces profound anesthesia of the treated skin site following a 30–40 minute application, with a duration of action lasting from 4–6 hours, whereas the lidocaine-prilocaine eutectic system requires a 1 hour application period, producing effective anesthesia for 60–90 minutes (4).

The differences in duration of action observed clinically between the two percutaneous anesthetic systems may be related to the relative lipophilicities of the local anesthetic bases employed, which in turn may influence not only the duration of binding at the proteinaceous receptor in the sodium channel of the nerve axon (5) but also the ability of the anesthetic agent to remain bound to the keratin component of the *stratum corneum*, thereby exerting a reservoir effect within the skin. There is some clinical evidence for a reservoir effect in the case of the tetracaine system (6). Thus, the present study seeks to investigate the relative differences in duration of activity seen with these preparations by determining their effects, both during application and for a period after their removal from the treated skin site, on the electrical properties of human skin *in vivo* as determined by a.c. impedance spectroscopy.

MATERIALS AND METHODS

Materials

Tetracaine base USP was supplied by Orgamol Ltd. (Switzerland). Op-Site™ polyurethane film dressings were obtained from Smith and Nephew Ltd. (Hull, UK). EMLA® cream (Astra Pharmaceuticals Ltd., Hertfordshire, UK) was purchased commercially. Natrosol 250 HHX M Pharm grade of hydroxyethylcellulose was obtained from Hercules Ltd. (Salford, UK), microcrystalline cellulose (Avice®) was purchased from ISP Co. Ltd. (Manchester, UK). ECG-type electrodes were obtained from Medicatest Ltd. (Cambridge, UK). Adhesive foam sheet, type 9751 was a gift from 3M Inc. (St. Paul, Mn., USA). Conductive, silver-coated nylon mesh was screen-printed at The Northern Ireland Bio-Engineering Centre, University of Ulster (Newtownabbey, UK). All other chemicals and reagents were of analytical grade.

Preparation of Gels and Creams

Tetracaine base USP was formulated as an aqueous gel (4%w/w), as described previously (3). EMLA® cream was used as supplied. Placebo aqueous gels were prepared with hydroxyethylcellulose (1% w/w) and Avicel® microcrystalline cellulose (4% w/w).

Electrode Construction

The novel impedance electrode (Fig. 1) was prepared using wells (4, each of 2 × 2 cm) that were cut from an adhesive foam sheet. Thus, four sensing electrodes were used simultaneously. Each well was filled with active or placebo gel (1 g). A fifth, oval central aperture was cut to locate a standard ECG electrode as reference. Conductive, silver-coated nylon mesh was located across each gel-filled well and was in direct skin contact when the adhesive foam was applied to the upper surface of the thigh. Drug diffused from aqueous gel to skin through the mesh electrode. A further ECG-type electrode was applied to the back of the thigh to act as the third electrode in order to cancel impedance contributions from electrode-skin interfaces other than at the treated sites.

In Vivo A.C. Impedance Spectroscopy

The impedance electrode was placed on the ventral surface of the volunteer's upper thigh. The appropriate active or placebo preparation (1 g) was placed in each of the wells. The ECG electrodes were placed in the oval aperture of the electrode and on the dorsal surface of the thigh. All were then occluded with Op-Site™ dressings. Crocodile clips were attached to the electrode sites and connected to the four-channel parallel interface (designed and built at The Northern Ireland Bio-Engineering Centre, University of Ulster, UK).

The a.c. impedances of the four adjacent skin sites were simultaneously monitored for two hours during and after the

[1] School of Pharmacy, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK.

[2] Present address: Department of Pharmaceutical Sciences, School of Pharmacy, University of Strathclyde, 204 George Street, Glasgow G1 1XW, Scotland, UK.

[3] Northern Ireland Bio-Engineering Centre, University of Ulster, Shore Road, Newtownabbey, Northern Ireland, UK.

[4] To whom correspondence should be addressed. (e-mail: d.woolfson@qub.ac.uk)

0724-8741/99/0300-0459$16.00/0 © 1999 Plenum Publishing Corporation

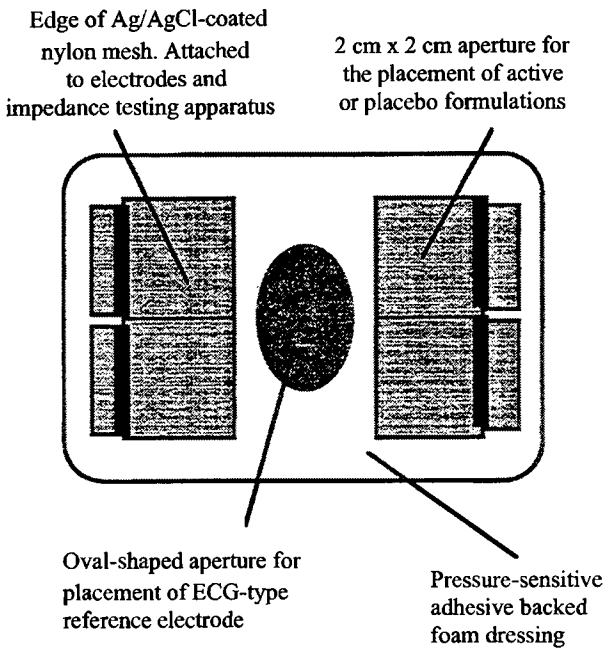

Fig. 1. Schematic diagram of the novel a.c. impedance drug delivery electrode.

application of active or placebo formulations using the techniques previously developed by McAdams and co-workers (7). In all experiments, channel 1 of the instrument was used to monitor sites treated continuously with placebo. At channels 2, 3 and 4, placebo replaced the active formulations after, respectively, 30, 45 and 60 minutes. Impedances of the four treated skin sites were monitored using an applied current amplitude of 1 mA. The applied frequency was varied in five logarithmic steps between 1 Hz and 2000 Hz in order to obtain the complete impedance loci. A complete sweep was obtained for each electrode at one minute intervals for two hours. Results were analysed by the impedance software and output, respectively, as plots of resistance (R_p), pseudocapacitive impedance (K) and α, a function of the phase angle of the impedance loci, against time.

THEORETICAL CONSIDERATIONS

McAdams and co-workers (7,8) characterised the changes induced upon the resistive and capacitive properties of intact, healthy skin by a.c impedance spectroscopy during the *in vivo* application of topical medicaments. The electrical properties of human skin, which may be modelled by means of an equivalent electrical circuit, are governed by the physiological nature of the *stratum corneum*, a non-conductive, insulating lipophilic membrane that forms the outermost skin layer and constitutes the major barrier to the cutaneous penetration of exogenous agents.

The electrical properties of deep layers of the skin, including the dermis and subcutaneous regions, are similar to other tissues within the human body in terms of their electrical properties and may be represented by a simple resistance with a typical value of 0.1 to 1 kΩ (9). The *stratum corneum* provides a high impedance to the passage of electrical current (10). However, the thinness (15–20 μm) of this insulating and predominately non-conductive layer permits capacitive coupling between an electrode placed on the skin and the underlying conductive tissues. Despite the insulating properties of the *stratum corneum*, it has been widely demonstrated that chemical entities can traverse intact skin by various routes. This flow of charged species may be represented electrically by a large resistance, R_p, shunting the skin's capacitance, C_p.

The capacitive properties of most biological tissues, including skin, can be represented by an empirical constant phase angle impedance Z_{CPA} (8), as defined by equation 1.

$$Z_{CPA} = K(j\omega)^{\alpha} \quad (1)$$

K is a constant and represents the magnitude of the pseudocapacitive impedance, ω is the angular frequency, j is a complex operator ($\sqrt{-1}$) and α, a measure of the deviation from pure capacitive behaviour, has a value $1 < \alpha < 0$. The impedance locus of the $Z_{CPA} - R_p$ parallel combination gives rise to a depressed arc (8) whose centre lies below the real axis (Fig. 2). The phase angle θ of the pseudocapacitance, Z_{CPA}, is described by equation 2.

$$\theta = \frac{\alpha\pi}{2} \text{ radians} \quad (2)$$

RESULTS AND DISCUSSION

Data was processed by the custom software and presented as plots of each measured parameter as a function of time. Figures 3 to 8 show the effects on Rp, K and α of applying the placebo and active systems to human skin. In most cases, the results obtained exhibited a high degree of variability for the first ten to fifteen minutes of each experiment. This was attributed to skin hydration effects and anxiety of the volunteer during initial setup, the latter resulting in increased perspiration and, consequently, an initial decrease in skin resistance. These

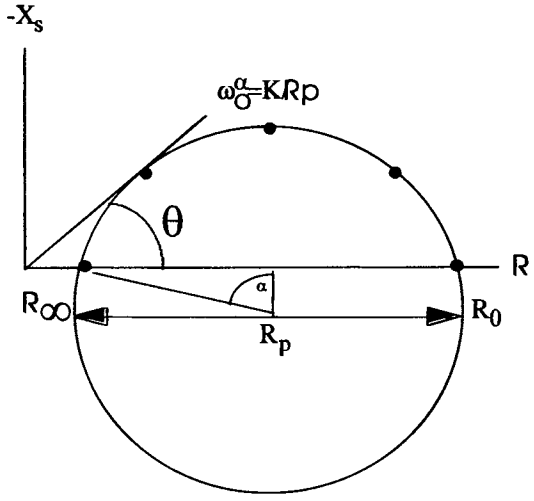

Fig. 2. The classical a.c. impedance locus, where the impedance signal is divided into its real (resistive) and imaginary (capacitive) parts. This results in a plot of reactance (a complex function of capacitance) against resistance. This reactance value is negative and is usually plotted as $-X_S$ for convenience. The impedance locus is obtained by measuring the impedance of the system at various frequencies. A best fit is then applied to these points, resulting in the circle shown above.

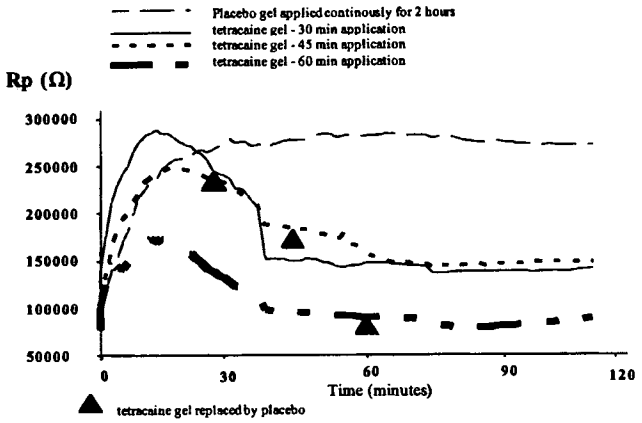

Fig. 3. Typical plot of R_p against time during and after application of percutaneous tetracaine gel.

Throughout the duration of the study, the results for all experiments carried out upon the same volunteer demonstrated a high degree of reproducibility. However, inter-subject variation was relatively high. This was predominately due to variations commonly associated with biological tissue. This problem has been encountered in previous *in vivo* impedance studies by Oh and Guy (11,12) who performed impedance measurements on four patients but, for purposes of clarity, presented the results obtained from a single individual. The results of this study have been treated similarly.

It was observed in all cases that those skin sites treated continuously with placebo exhibited higher values of R_p, K and α than any of the drug-treated sites, which all decreased by varying degrees. This indicated that the presence of percutaneously absorbed drug in the skin, predominately the *stratum corneum*, exerted a significant effect on the electrical properties of skin. After an initial period, distinct trends developed in measurements of R_p and K of skin sites treated with either placebo or drug-containing systems. After the equilibration period, all measurements recovered rapidly towards distinct positions.

It has been demonstrated that the electrical properties of skin vary greatly from one individual to the next and are affected by factors including race and sex (13). Jossinet and McAdams (8) determined that these variations are predominately due to changes in R_p rather than to C_p or K. Further, the electrical properties of skin will vary depending upon the region of the body being examined, with regions of thicker skin generally exhibiting higher impedance. The presence and quantity of sweat glands and hair follicles will also influence the electrical impedance of the skin.

Figures 3 to 8 illustrate changes induced in the electrical properties of skin during and after treatment of the skin site with percutaneous tetracaine gel and lidocaine-prilocaine (EMLA) cream. They also show the effect of the duration of application of each active formulation. It is apparent that applying the active formulations for sixty minutes resulted in a substantially greater decrease in R_p by comparison to thirty and forty-five minute application times at the end of the experiment. These changes were maintained both after the removal of active formulations and their replacement with placebo, and at the end of the two hour experiment. Duration of drug application did not significantly affect the values of K or α. This would suggest that the longer application time results in more drug passing into the *stratum corneum*, and that this further decrease in R_p is only significant between application times of less than 45 minutes and 60 minutes, but is not significant between application times of 30 and 45 minutes. These values reflect the clinical efficacy of both percutaneous anesthetic systems.

Figures 5 and 8 show the effect of drug application on changes in α. Typically, α has a value of approximately 0.8 for human skin (8). Drug-treated sites all demonstrated a slight decrease in α compared to sites treated continuously throughout the experiment with placebo. This decrease was maintained beyond the removal of active formulations and at the end of the experiment, with subsequent assessment and interpretation proving more difficult. In all cases, decreases in α were insignificant and highly variable. These results will warrant further investigation and development of suitable experimental methodologies.

Comparison of the magnitude of decreases in R_p (Figs. 3 and 6) and K (Figs. 4 and 7) for skin sites treated with both gels indicates that percutaneous tetracaine gel induced significantly greater decreases in R_p than the lidocaine-prilocaine cream. A greater reduction in R_p indicates that the *stratum corneum* contains relatively more exogenous charged species. A decrease in K represents an increase in the capacitance of the skin, or

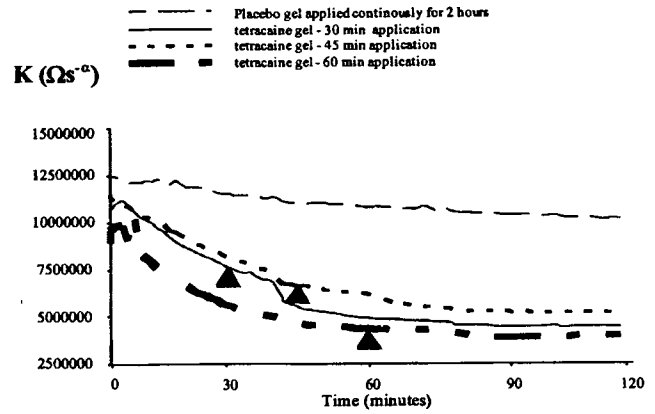

Fig. 4. Typical plot of K versus time during and after application of percutaneous tetracaine gel.

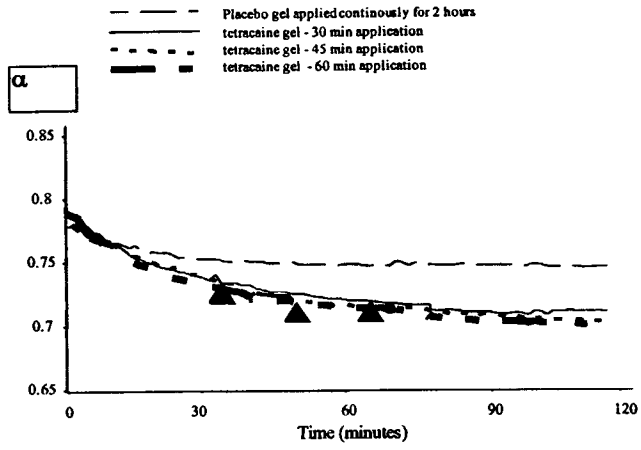

Fig. 5. Typical plot of α against time during and after application of percutaneous tetracaine gel.

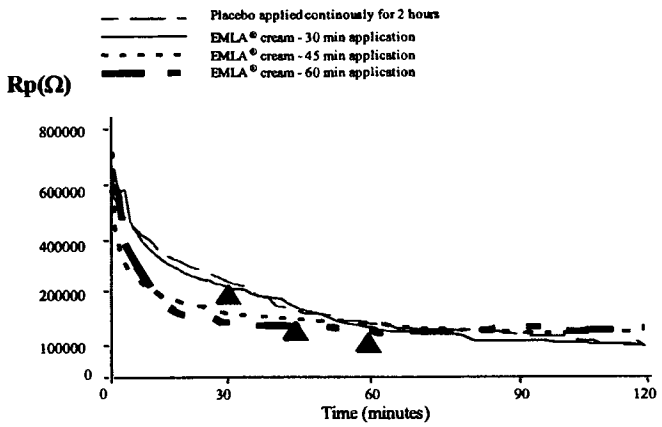

Fig. 6. Typical plot of R_p against time during and afterapplication of lignocaine-prilocaine eutectic (EMLA®) cream.

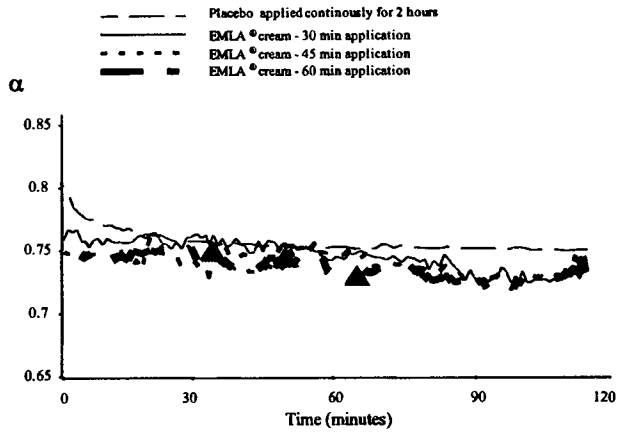

Fig. 8. Typical plot of α against time during and after application of lignocaine-prilocaine eutectic (EMLA®) cream.

a change in its dielectric properties. This would indicate that decreases in K relative to placebo may provide an indication of the amount of drug present in the *stratum corneum* during and after administration of a percutaneous dosage form.

The clear differences observed between the tetracaine and lidocaine-prilocaine systems, particularly after the active formulations were replaced by placebo, strongly suggests that tetracaine, following its percutaneous penetration, remains in the *stratum corneum* for a prolonged period of time, thus maintaining an effective drug concentration at the nerve receptor sites (nociceptors), a contributory factor in the prolonged duration of local anesthetic action that is characteristic of percutaneous tetracaine gel. Tetracaine is a substantially more lipophilic local anesthetic than either lidocaine or prilocaine and thus exhibits a greater protein binding tendency. It is, for example, strongly bound to plasma proteins (14). This is probably the major factor in its increased residence time (reservoir) effect in the *stratum corneum*. The prolonged anesthesia obtained with percutaneous tetracaine gel offers a distinct clinical advantage (6), allowing patients to be treated well in advance of the intended procedure without loss of efficacy due to unforeseen changes in clinic or office routine, such as prolonged waiting periods.

The asessment of pain *in vivo* is highly subjective (15). The correlation of observed changes in skin a.c. impedance parameters with the known prolonged duration of anesthesia produced by tetracaine gel (6) suggests that a.c. impedance spectroscopy may be a useful, non-subjective and non-invasive tool in the further development of effective percutaneous anesthetic systems and, indeed, may have wider future applications for *in vivo* monitoring of transdermal drug penetration.

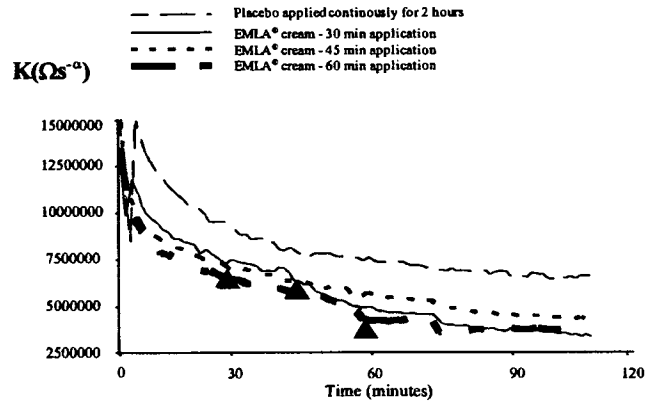

Fig. 7. Typical plot of K against time during and after application of lignocaine-prilocaine eutectic (EMLA®) cream.

REFERENCES

1. A. D. Woolfson and D. F. McCafferty *Percutaneous Local Anesthesia*, Ellis Horwood, Chichester, UK, 1993.
2. A. D. Woolfson and D. F. McCafferty. Local anaesthesia of the skin. *J. Clin. Pharm. Ther.* **14**:103–109 (1989).
3. A. D. Woolfson and D. F. McCafferty. Percutaneous local anaesthesia: Drug release characteristics of the amethocaine phase-change system. *Int. J. Pharm.* **94**:75–80 (1993).
4. G. M. E. Ehrenstrom-Reiz and S. L. A. Reiz. EMLA—a eutectic mixture of local anaesthetics for topical anesthesia. *Acta Anaesthesiol. Scand.* **26**:596–598 (1982).
5. J. A. W. Wildsmith, A. J. Gissen, J. Gregus, and B. G. Covino. Differential nerve blocking activity of amino ester local anesthetics. *Br. J. Anaesth.* **57**:612 (1985).
6. A. D. Woolfson, D. F. McCafferty, and V. Boston. Clinical experiences with a novel percutaneous amethocaine preparation: prevention of pain due to venepuncture in children. *Br. J. Clin. Pharmac.* **30**:273–279 (1990).
7. E. T. McAdams, A. Lackermeier, A. D. Woolfson, D. F. McCafferty, and G. P. Moss. A.c impedance monitoring of percutaneous drug delivery. *Proc. 9th. Int. Conf. on Electrical Bioimpedance.* Heidelburg, Germany, pp. 344–347 (1995).
8. E. T. McAdams and J. Jossinet. The importance of electrode-skin impedance in high resolution electrocardiography. *Automedica* **13**:187–208 (1991).
9. S. Grimnes. Impedance measurement of individual skin surface electrodes. *Med. Biol. Eng Comput.* **21**:750–755 (1983).
10. T. Yamamoto and Y. Yamamoto. Electrical properties of epidermal *stratum corneum. Med. Biol. Eng.* 151–158 (1976).
11. S. Y. Oh, L. Leung, D. Bommannan, R. H. Guy, and R. O. Potts. Effect of current, ionic strength and temperature on the electrical properties of skin. *J. Contr. Rel.* **27**:115–125 (1993).
12. S. Y. Oh and R. H. Guy. Effects of iontophoresis on the electrical properties of skin *in vivo. Int. J. Pharm.* **124**:137–142 (1995).
13. J. J. Almasi and O. H. Schmitt. Systemic and random variations of ECG electrode system impedance. *Ann. N. Y. Acad. Sci.* **170**:509–519 (1970).
14. B. G. Covino. Pharmacology of local anesthetic agents. *Br. J. Anaesth.* **58**:701–716 (1986).
15. R. Melzak. In: R. Melzak (ed.) *Pain Measurement and Assessment*, Raven Press, New York, pp. 1–6 (1983).