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Original Research Paper

IN VIVO EVALUATION OF THE INFLUENCE OF VARIOUS DRUG CARRIERS WITH INCORPORATED HYPERAEMIC DRUG TO CHANGES IN THE PARTIAL PRESSURE OF OXYGEN IN RAT ORAL MUCOSA USING EPR OXIMETRY

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Summary: The purpose of this study was to select the best drug carrier among different types of liposomes for the topical treatment of oral mucosa lesions. A hyperaemic drug, benzyl nicotinate (BN), which increases perfusion and consequently also tissue oxygenation, was used as the active ingredient. Electron paramagnetic resonance (EPR) oximetry, using a lithium phthalocyanine paramagnetic probe, was used in vivo to measure the effects of the benzyl nicotinate which had been incorporated into liposomes of varying lamellarity and composition. The liposomes were made from either hydrogenated or non-hydrogenated soy lecithin. We used polymethyl methacrylate (PMMA) as the ointment for preparing the drug for application to the oral mucosa because of its good mucoadhesive properties.
EPR oximetry was used to measure the partial pressure of oxygen (pO_2) in the oral mucosa before and after the application of the liposomes. It was found that the most pronounced changes of pO_2 in oral mucosa and also the longest action of the drug occurred after the topical application of BN in multilamellar liposomes made from hydrogenated soy lecithin ($p < 0.0001$). Therefore, these liposomes proved to be the most appropriate for local drug delivery to oral mucosa.

Key words: mouth diseases; drug therapy; liposomes; *in vivo* EPR oximetry; oral mucosa; rat

Introduction

One of the characteristics of oral mucosa is its selective permeability, which provides a barrier to most chemicals. However, drugs can be used for local or systemic delivery (1, 2, 3, 4, 5, 6, 7). Local delivery of drugs to tissues of the oral cavity has numerous applications such as the treatment of aphthous stomatitis, lichen planus, bacterial and fungal infections and periodontal diseases (6, 8, 9). The success of a topical treatment of mucosal lesions via the application of a drug onto intact oral mucosa depends on the selection of a suitable active ingredient and an appropriate carrier, the rate of penetration through the mucosa and the residence time of the active ingredient in oral mucosa. Additionally, a major part of a successful application of a local drug delivery system into the oral cavity is the selection of an appropriate vehicle. For example, ointments that act as vehicles for local drug delivery to the oral mucosa need to have excellent mucoadhesive properties (10).

Topical treatments of ulcerative inflammatory diseases are associated with several general disadvantages such as the high permeability of the oral mucosa for drugs, which could result in the uncontrolled release of the drugs into the blood causing unwanted side effects (2, 10). Liposomal formulations have been used to regulate the release and localize the effect of incorporated drugs (11) and studies have shown that treatments with liposomal formulations result in an increase in the local and a decrease in the systemic concentration of a drug (11, 12).

The aim of this study was to investigate the effects of a liposome's composition and size on

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the delivery of the hyperaemic drug, benzyl nicotinate (BN), to the oral mucosa using in vivo EPR oximetry.

Materials and methods

The study protocol was submitted to and approved by the Veterinary Administration of the Republic of Slovenia (N° 323-02-76/01).

Animals, anaesthesia and implantation of the paramagnetic probe

Adult female Wistar rats, weighing 200-250 g and 7-9 weeks old were supplied by the Pathology Laboratory of Ljubljana's Faculty of Medicine, Slovenia. Anaesthesia was induced using an intraperitoneal injection of a mixture containing xylazine hydrochloride, 10 mg/kg (Rompun, Bayer, Leverkusen, Germany) and ketamine hydrochloride, 75 mg/kg (Ketanest 50, Parke-Davis, Berlin, Germany) and when indicated, anaesthesia was prolonged by the administration of a further amount of each drug at half the initial dosage. Twenty-four hours prior to starting electron paramagnetic resonance (EPR) measurements, the rats were anaesthetized and crystalline particles of lithium phthalocyanine (LiPc), the paramagnetic probe, were implanted beneath the buccal mucosal epithelium through a 23 gauge injection needle (Microlance, Becton Dickinson, Fraga, Spain). The approximate volume of the crystals (a generous gift from the EPR Centre for Viable Tissues, Dartmouth College of Medicine, Hanover, New Hampshire, USA) was 0.5 μm^3. The needle tip was filled with approximately 0.1 mm^3 of LiPc microcrystals. The needle was inserted 2 mm laterally from the injection site and the microcrystals deposited about 1 mm below the mucosa surface. Measurements began 24 hours after the implantation of the paramagnetic probe, permitting time for oxygen concentrations to balance between the paramagnetic crystals and the surrounding tissue and to minimize the risk of the results being affected by the initial stress and tissue injury from the implantation of the probe. The delay also permitted the injection site to start healing, reducing the likelihood that the hyperaemic drug – benzyl nicotinate (BN) – would permeate directly through the wound caused by the implantation of the LiPc microcrystals. Each rat was anaesthetised and the EPR spectra measured for 15 minutes to ensure that both the equipment and the implant were functioning correctly and to establish the baseline value for the partial pressure of oxygen (pO_2) in the tissue before proceeding further.

Anaesthesia interferes with temperature homeostasis and changes in temperature have been reported to significantly influence the linewidth of the EPR spectra (15, 16), so a preliminary study was performed to develop a method to counter such effects.

Benzyl nicotinate

Benzyl nicotinate indirectly increases local blood flow through the release of nicotinic acid, which is followed by the formation of prostaglandin D2 (13, 14). The increased blood flow leads to an elevation of tissue oxygenation, pO_2, which can be measured by EPR oximetry. The gradual increase in pO_2 after the application of the formulations containing BN was measured and the overall effectiveness of the incorporated drug in different types of liposomes was determined.

Liposomes with benzyl nicotinate

Liposomes were prepared using the thin-film method from cholesterol and either hydrogenated or non-hydrogenated soy lecithin (HSL or NSL) in a weight ratio of 3:7. The lipophilic phase containing phospholipid, together with cholesterol and the benzyl nicotinate (Lek; Ljubljana, Slovenia), was dissolved in dichloromethane for the NSL or in chloroform:methanol (1:1) for the HSL. The solvent was removed in a rotary evaporator, which left a thin film clinging to the evaporator's wall. Any remaining solvent was removed completely under vacuum (10 to 15 minutes at 40 °C and pressure 100 Pa). The dry film was then hydrated with distilled water at approximately 80 °C for the HSL (i.e. above its phase transition temperature) and at room temperature (22 °C) for the NSL. The flask was shaken until the film was completely removed from its walls. The dispersion was then stabilized by stirring it for 2 hours on a magnetic stirrer (300 rpm) at room temperature. A 1-mL sample of the dispersion contained 25 mg of lipids and 12.5 mg of BN. A higher concentration of BN would not permit the liposomes to form.

A portion of the multilamellar liposome (MLV) was extruded with a Liposofast extruder (Avestin, Ottawa, Canada) using polycarbonate membranes with defined pore diameters (Nucleopore Corporation, Pleasanton, CA, USA), which started at 800 nm and decreased in diameter to 100 nm. The liposomes were extruded at temperatures slightly above their phase transition temperature.

Characterization of liposomes

The size of the liposomes and their polydispersity index (PI) rating were determined by photon correlation spectroscopy (PCS; Zetasizer 3000, Malvern, Malvern, UK) at a fixed angle of 90ş. The samples were diluted in dust-free water to give the recommended scattering intensity of 100,000 counts s^{-1}. The diameter was calculated from the autocorrelation function of the intensity of light scattered from the particles, assuming that the particles were spherical in form. For mean size calculation, the cumulant algorithm, which took into account only one population of particles was used. The PI is a measure of a dispersion's homogeneity, which ranges from 0 (homogeneous dispersion) to 1 (high heterogeneity). In our study, the mean diameters of the NSL and the HSL were respectively 400 ± 40 and 1,000 ± 100 with PI's close to 1; after extrusion it was 250 nm ± 20 with a PI of 0.7 ± 0.2 and 250 ± 10 with a PI of 0.3 ± 0.1.

Formulation for application

A mucoadhesive ointment, polymethyl methacrylate (PMMA) (a neutralized co-polymer of methacrylic acid and methyl methacrylate) was used in the application of the liposomal formulation to the tissues of the oral cavity. The PMMA (Sigma-Aldrich, Steinheim, Germany) was prepared as described elsewhere (7, 8). The liposomes encapsulating BN were mixed with PMMA in a weight ratio of 2:3, resulting in a final concentration of BN in the PMMA formulation of 0.5 wt %.

For a negative control, a PMMA without BN was used.

EPR measurements

The measurements taken in the oral cavity were conducted by in vivo EPR oximetry, which has been described in detail elsewhere (16). Briefly, 0.05 ml of the prepared liposomal formulation was applied to the surface of the buccal mucosa over the site where the LiPc had been implanted using a syringe (Plastipak, Becton Dickinson, Fraga, Spain).

The surface coil of an extended loop resonator, which was 11 mm in diameter, was placed over the implanted area and the EPR spectra were recorded on a Varian E-9 EPR spectrometer with a custom-made low-frequency microwave bridge (designed by Dr. T. Walczak, Dartmouth College of Medicine, Hanover, NH, USA), operating at 1.1 GHz. The spectra were recorded under the following conditions: magnetic field density 44-45 mT, modulation amplitude 2.5×10^{-3} mT and the microwave power 20 mW. The linewidth of the EPR spectra, which is proportional to local pO_2 changes in the tissue, was measured and converted to mucosal pO_2 according to the calibration curve for the LiPc (17).

Each experimental formulation, including the negative control, was tested in 8 or 9 rats. In order to obtain the basal pO_2 of the oral mucosa, five EPR spectra were recorded before the application of the test formulation and the mean value was taken as the basal pO_2. The local pO_2 changes were then measured over 90 minutes (at 2 to 5 minute intervals) following the application of the test material. As the basal pO_2 varied from animal to animal the difference in pO_2 with respect to the basal value was measured. Time points and pO_2 measurements, which represent the efficiency of the drug's absorption and action, were evaluated for each type of liposome as follows: onset of increasing tissue oxygenation (lag time, t_{lag}), the maximal pO_2 (ΔpO_{2max}), the time when pO_{2max} was reached (t_{max}), the return to the basal pO_2 levels (t_{end}) and the area under the curve (AUC).

Statistical analysis

The hypothesis of average equality in different groups was tested with one-way single factor ANOVA. The GLM procedure for unbalanced data was used for the analysis of data. If the analysis of variance test was significant, a post-test analysis using a Duncan test was used to find the specific difference and P values of less than 0.05 were accepted as statistically significant.

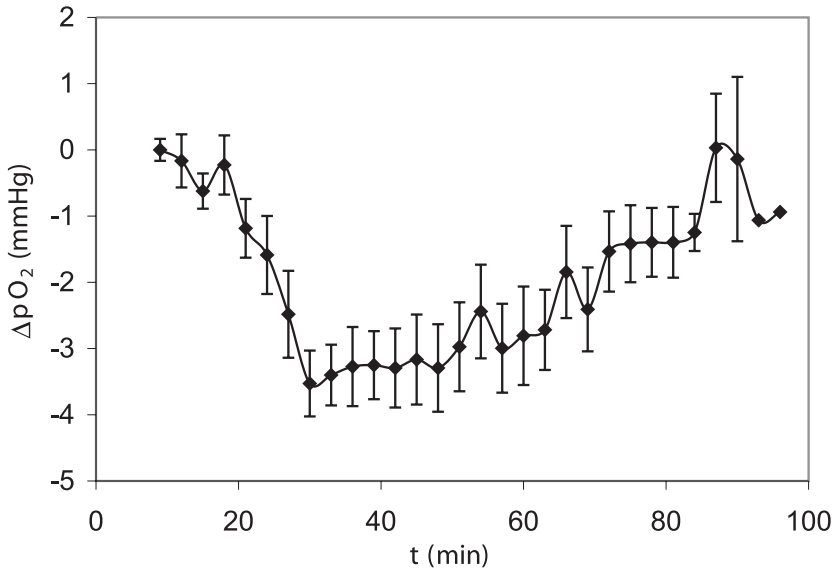

Figure 1: Dependence of partial pressure of oxygen (pO_2) on anaesthesia in rat oral mucosa. For the first 30 minutes after the application of the anaesthetic and sedative, the pO_2 in the oral mucosa decreased, then remained fairly steady for the next 30 minutes and then started to rise after one hour - when the animal began to wake up

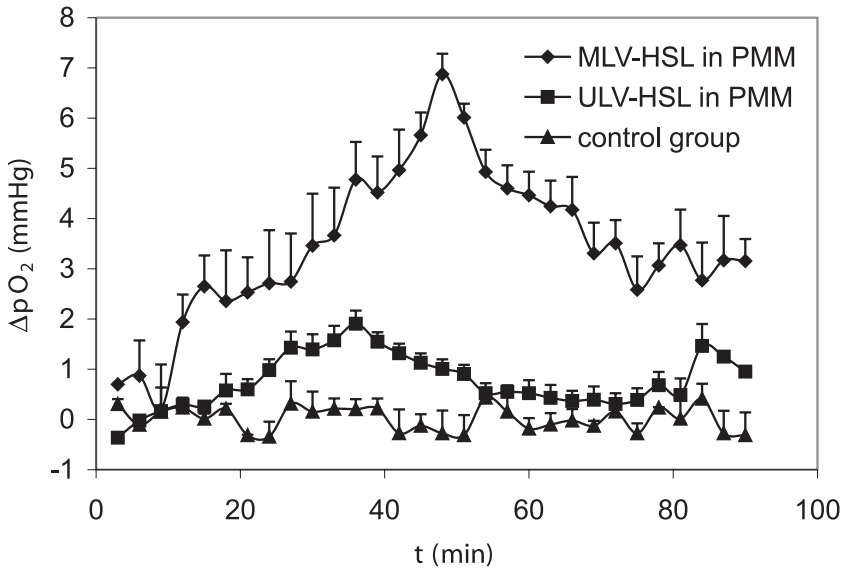

Figure 2: The time-course of oxygen level variation (ΔpO_2) in rat oral mucosa after the application of benzyl nicotinate in HSL liposomes of different sizes: (◆) multilamellar HSL liposomes, (■) unilamellar HSL liposomes and (▲) control group. Each point represents mean value ± SD of 8-9 measurements

Results

Effect of body temperature and anaesthesia on pO2 in oral mucosa

Preliminary EPR measurements were performed without the application of medications to investigate the effect of changes in body temperature and duration of anaesthesia on the oxygenation of the oral mucosal. It was found that the tissue oxygenation in oral mucosa is influenced by changes in body temperature. The pO_2 of oral mucosa decreased, as expected, with the reduction of body temperatures below that considered normal (16). Minimal changes were observed when temperature homeostasis was maintained at 36.5 ± 0.5 °C throughout anaesthesia by a flow of hot air; this was measured rectally with a thermocouple inserted into a glass capillary. This method was therefore used during the liposome evaluation. Measuring the temperature of oral mucosa is invasive and interferes with its pO_2, therefore, it was not performed.

Furthermore, tissue oxygenation is also influenced by anaesthesia. After the application of the anaesthetic combination, the pO_2 in the oral mucosa decreased before stabilizing after

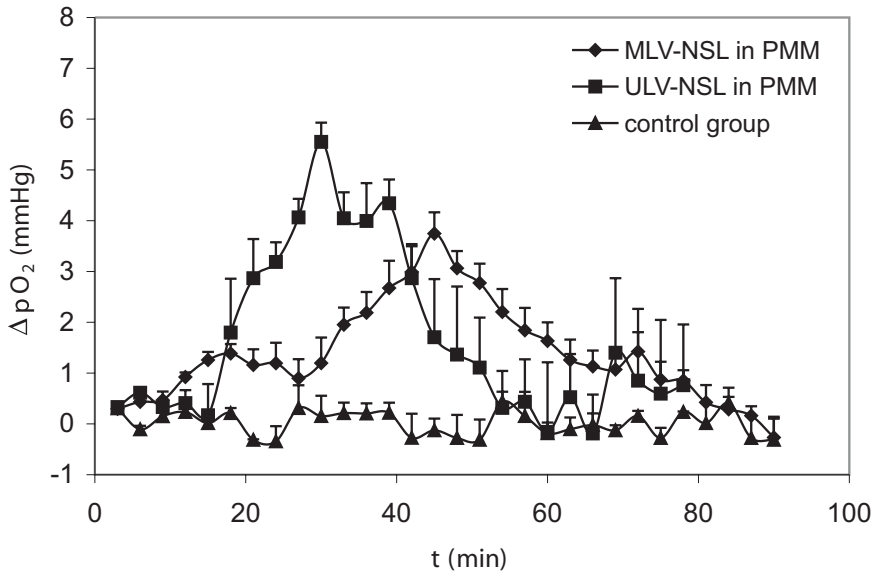

Figure 3: The time-course of oxygen level variation (ΔpO_2) in rat oral mucosa after the application of benzyl nicotinate in NSL liposomes of different sizes: (◆) multilamellar MLV liposomes, (■) extruded MLV liposomes and (▲) control group. Each point represents mean value ± SD of 8-9 measurements

about 30 minutes. After one hour, as the anaesthesia lightened, the pO_2 started to rise again (Figure 1.). As the measurements of the local pO_2 changes after the application of the liposomal formulations were performed over 90 minutes, an additional half of dose of the anaesthetic combination was administered (without the animal being moved) one hour after the initial dose. This was found to maintain the anaesthesia adequately and to stabilize the pO_2 over a longer period of time. With respect to the initial influence of anaesthesia, the formulations were not applied to the mucosa until 30 minutes after anaesthesia – when the basal pO_2 levels had been confirmed as having stabilized.

Effect of liposome composition

In the control experiments it was determined that PMMA alone has no influence on the oxygenation of oral mucosa (Figures 2 and 3). For the test substances, the lag time (t_{lag}), the maximal relative increase of pO_2 after the application of the liposomes and the time when it was reached (ΔpO_{2max}, t_{max}), the area under the curve (AUC), and the time when BN stopped acting (t_{end}) were determined from the individual pO_2 curves. The influence on the oxygenation of the oral mucosa of BN incorporated in the HSL or NSL liposomes is shown in Figures 2 and 3. The influence of the different carriers investigated was significant. The major changes to the pO_2 of the oral mucosa occurred after the application of the multilamellar liposomes made from hydrogenated soy lecithin (MLV-HSL) ($p<0.0001$), which is expressed in maximal changes of pO_2 as well as in the AUC. The drug also had the longest lasting effects (the average exceeded the measurement time) for this type of liposome ($p<0.0001$). The least effective were the extruded liposomes from hydrogenated soy lecithin (ULV-HSL), this being lower than for liposomes from non-hydrogenated soy lecithin. When BN was incorporated in liposomes made from non-hydrogenated soy lecithin, their effectiveness was more pronounced when incorporated in multilamellar (MLV-NSL) rather than the extruded (ULV-NSL) liposomes, although the differences were much less marked than with the HSL liposomes. Maximal pO_2 is greater in extruded liposomes while the time when maximal increase in pO_2 was achieved as well as the duration of the effect was longer when using the non-extruded liposomes.

The analysis of variance test for the unbalanced data showed significant differences ($F=54.37$, $p<0.0001$) in the average values of the maximal increase of pO_2 (ΔpO_{2max}) for all four carriers (Table 1). Statistically significant differences ($F=4.47$, $p=0.01$) in the values of t_{max} were observed between the extruded and non-extruded liposomes of both types (NSL and HSL). The maximal change in pO_2 in the oral mucosa was achieved sooner in extruded rather than non-extruded NSL and HSL liposomes, while the differences in t_{lag} between the different carriers were

Table 1: The effect of topical applications of benzyl nicotinate (BN) incorporated in liposomes of varying lamellarity and composition on the oxygenation of rat oral mucosa

Liposomes	N	$\Delta pO_{2\ max}$ (mmHg)	t_{max} (min)	t_{lag} (min)	AUC (mmHg x min)	t_{end} (min)
MLV-HSL	8	7 ± 0.8^{a}	48 ± 11^{e}	14 ± 7.6	$270 \pm 103^{*}$	**
ULV-HSL	9	2 ± 0.6^{b}	36 ± 7^{f}	12 ± 4.50	54 ± 37	50 ± 23
MLV-NSL	9	4 ± 1.0^{c}	45 ± 10	16 ± 8.4	110 ± 49	57 ± 15
ULV-NSL	8	6 ± 1.0^{d}	30 ± 6^{f}	12 ± 7.4	90 ± 8.0	40 ± 10.4

Each value represents the mean ± SD of measurements.
Key:
N - Number of measurements
ΔpO_{2max} - the maximal relative increase of pO_2 after application of the liposomes
tmax - the time when pO_{2max} was reached
tlag - the time when the BN starts to act (lag time)
AUC - area under the curve
tend - the time when BN stops acting (after the application of BN incorporated in different carriers)
a,b,c,d - statistically significant difference between different carriers (Analysis of variance, Duncan's test; $p < 0.0001$)
** - statistically significant difference between different carriers (Analysis of variance, Duncan's test; $p < 0.0001$)*
e,f - statistically significant difference (Analysis of variance, Duncan's test; $p<0.01$)
*** - the effect lasted longer than the time of measurements*

not significant. However, the most pronounced effect was observed for MLV-HSL liposomes, where the pO_2 remained above the baseline longer, exceeding the time of measurement. The effectiveness of BN, expressed as AUC, was most pronounced when applied in MLV-HSL ($F=17.65$, $p<0.0001$) (Table 1), while in other formulations the differences in AUC were not significant.

Discussion

Oral mucosa is a stratified squamous epithelium, whose intercellular spaces are filled with lipids extruded from the membrane coating granules (MCG). The lipids may be organised into lamellae and they constitute the principal barrier against molecular diffusion through the mucosa. Keratinized areas in the oral cavity are generally more permeable than the skin because the intercellular lipids are less well structured. They exist mainly in discrete lamellar domains and there are fewer structural contributions from lipids (ceramides) covalently bound to the corneocyte surface (18, 19, 20). There are considerable differences in the permeability of different oral mucosae (9). In non-keratinized regions (e.g. cheek, floor of the mouth and lips) the chemical nature of the intercellular material is less well defined and the barrier is less efficient than that in the keratinized epithelia (19). In general, the permeability of oral mucosae decreases in the order of sublingual is greater than buccal, which in turn is greater than palatal (6). The permeability of the oral mucosa is estimated to be 4 to 4,000 times greater than that of the skin (3, 6, 9).

The oral epithelia of a number of experimental animals are entirely keratinized (6), and the rat has a buccal mucosa with a very thick, keratinized surface layer (9). Human oral mucosa is thin and non-keratinized. From the point of view of human mucosal drug delivery, the carriers tested in rats are expected to be even more effective in the thin non-keratinized human buccal mucosa. An inflammatory infiltrate in connective tissue increases epithelial permeability (2), and an ulcerated surface, i.e. without the epithelial barrier, provides an easier entry as well as an easier exit for the drug (11).

The continuous flow of saliva and the mechanical movements of the tongue may prevent the long-term adhesion of carriers to oral mucosa. Among the different hydrophilic polymers that have been investigated, PMMA has been found to be the most appropriate mucoadhesive ointment for local liposome applications in the oral cavity. They are most stable in this polymer and the penetration of the incorporated

substance into the oral mucosa or gingiva is greatest when PMMA was used (8). Therefore we chose PMMA as the vehicle for the liposomes with the entrapped BN. We found that multilamellar liposomes made from hydrogenated soy lecithin were the most effective carriers of the liposomes investigated in this study. Several studies have shown that a liposome's composition and, to a lesser extent, its size influences the rate of transport and effectiveness of a drug's action in skin (21, 22, 23, 24). The effect of free BN in PMMA has been evaluated before, the drug's effect increasing linearly with a BN concentration up to 3 %; higher concentrations having no greater effect indicating that the saturation level had been achieved (16). If the concentration of the hyperaemic drug was less than 1 % then no local changes of pO_2 were observed (16). As we achieved significant effects with each of the four liposomal formulations in our study when the concentration of BN in the formulation was 0.5 % we can conclude that the encapsulation of a drug into liposomes enhances its delivery into oral mucosa.

Multilamellar liposomes made from HSL were the most effective carriers for the hyperaemic drug used in this study and also produced the greatest duration of action (Table 1). The HSL liposomes cause a greater effect than NSL liposomes. These results are in agreement with in vitro and in vivo results previously obtained, which show that a hydrophilic probe, when applied entrapped in a NSL liposome, does not penetrate deeper than 100 μm, while the HSL liposomes enable penetration into the deeper layers of the skin (22, 24). We studied the influence of a lipophilic substance that can penetrate into the skin even if it is not entrapped in a liposome. Therefore, the enhanced effect of all four formulations is not surprising. However, multilamellar liposomes from HSL caused the most pronounced increase in pO_2 and prolonged the effect of the BN action in accordance with previous findings, which show that liposomes allow a controlled and continuous release of a drug over a longer period of time (25, 26). The pO_2 was still above the baseline when we stopped taking measurements after 90 minutes. The populations of multilamellar liposomes are very heterogeneous in size and lamellarity; therefore, they release the entrapped substance more evenly over a prolonged period while penetrating the oral mucosa. In contrast, after an initial increase in pO_2 a decrease was observed after 35 minutes with the extruded liposomes. We suggest that the extruded liposomes, which are smaller, more homogeneous in size and with less layers in their structure, release a drug more uniformly and rapidly, therefore the effect is shorter. We assume that most unilamellar liposomes break down on the surface of the oral mucosa and the released free BN then penetrates the mucosa as if it had been applied directly.

The drop in pO_2 after the administration of the anaesthetic's agents is likely to be related to the depressed respiration rate and altered circulation induced by the anaesthetics. Anaesthesia affects the tissue pO_2 directly through its effect on the respiratory centre and indirectly due to peripheral vasoconstriction (33). Xylazine hydrochloride is a sedative and muscle relaxant commonly used in veterinary medicine (34). Being an agonist for α2-adrenoceptors, xylazine hydrochloride decreases the heart rate, causes a biphasic change in mean blood pressure (transient hypertension followed by hypotension), decreases venous cerebral blood volume and intracranial pressure, and depresses the central nervous system (33, 35, 36). During the initial stage of hypertension there is peripheral vasoconstriction, which reduces the blood flow through the capillaries. This varies with the type of α2-adrenoceptors present in the tissue, the dose and the route of administration. This initial stage is followed by a lowering of blood pressure, circulation and the peripheral resistance of blood vessels. The reduced peripheral blood flow, together with the decreased arterial oxygen content, account for the remarkable reduction in the oxygenation of the oral mucosa in ketamine-xylazine hydrochloride anaesthetised rats. This explains the decrease of pO_2 that was observed after the application of the anaesthetic and sedative. It is therefore impossible to avoid a certain influence of the anaesthesia during in vivo measurements. There was also fluctuation of pO_2 around the basic value of pO_2, which was obtained as an average of the EPR spectra linewidths taken before the application of the ointment.

Conclusions

In this study we have proven that in the rat, a topical application of a liposome preparation

facilitates the penetration of BN through the oral mucosa in vivo, the drug increasing the oxygenation of the oral mucosa. The liposome's composition and size plays an important role in the penetration of this lipophilic drug through the oral mucosa. The present study has indicated that multilamellar liposomes made from hydrogenated soy lecithin are much more effective carriers for BN in oral mucosa than are the non-hydrogenated liposomes or the extruded liposomes of the same type. EPR oximetry in vivo as a non-invasive method can be used to precisely monitor the penetration of BN into oral mucosa by following the physiological response of the body to the drug's action. In addition, our results indicate that pO_2 in the oral mucosa is altered by body temperature and anaesthetics.

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References

1. Hicks DC. The buccal absorption of some adrenoceptor blocking drugs. Br J Pharmacol 1973; 47: 680-1.

2. Squier CA, Johnson NW. Permeability of oral mucosa. Br Med Bull 1975; 31: 169-75.

3. Galey WR, Lonsdale HK, Nacht S. The in vitro permeability of skin and buccal mucosa to selected drugs and tritiated water. J Invest Dermatol 1976; 67: 713-7.

4. Pimlott SJ, Addy M. A study into the mucosal absorption of isosorbide dinitrate at different intraoral sites. Oral Surg Oral Med Oral Pathol 1985; 59: 145-8.

5. Barsuhn CL, Olanoff LS, Gleason DD, Adkins EL, Ho NF. Human buccal absorption of flurbiprofen. Clin Pharmacol Ther 1988; 44: 225-31.

6. Harris D, Robinson JR. Drug delivery via the mucous membranes of the oral cavity. J Pharm Sci 1992; 81: 1-10.

7. Sveinsson SJ, Holbrook WP. Oral mucosal adhesive ointment containing liposomal corticosteroid. Int J Pharm 1993; 95: 105-9.

8. Petelin M, Sentjurc M, Stolic Z, Skaleric U. EPR study of mucoadhesive ointments for delivery of liposomes into the oral mucosa. Int J Pharm 1998; 173: 193-202.

9. Shojaei AH. Buccal mucosa as a route for systemic drug delivery: a review. J Pharm Sci 1998; 1 (1): 15-30.

10. Bremecker KD, Strempel H, Klein G. Novel concept for a mucosal adhesive ointment. J Pharm Sci 1984; 73: 548-52.

11. Harsayi BB, Hilchie JC, Mazei M. Liposomes as drug carriers for oral ulcers. J Dent Res 1986; 65: 1133-41.

12. Mazei M, Gulasekharam V. Liposomes: a selective drug delivery system for the topical route of administration. Gel dosage form. J Pharm Pharmacol 1982; 34: 473-4.

13. Wilkin JK, Fortner G, Reinhardt LA, Flowers OV, Kilpatrick SJ, Steeter WC. Prostaglandins and nicotinate-provoked increase in cutaneous blood flow. Clin. Pharmacol Ther 1985; 38: 273-7.

14. Morrow JD, Adwad JA, Oates JA, Roberts LJ. Identification of skin as a major site of prostaglandin D2 release following oral administration of niacin in humans. J Invest Dermatol 1992; 98: 812-5.

15. Šentjurc M, Kristl J, Abramovic Z. Transport of liposome entrapped substances into skin as measured by electronic paramagnetic resonance oximetry in vivo. Methods Enzymol. (2002) in press.

16. Petelin M, Pavlica Z, Bizimoska S, Sentjurc M. In vivo study of different ointments for drug delivery into oral mucosa by EPR oximetry. Int J Pharm 2004; 270: 83-91.

17. Sentjurc M, Krzic M, Kristl J, Grinberg O, Swartz HM. EPR Oximetry in vivo in mouse skin. Polish J Med Phys Eng 2001; 7: 165-74.

18. Wertz PW, Squier CA. Cellular and molecular basis of barrier function in oral epithelium. Crit Rev Ther Drug Carrier Syst 1991; 8 (3): 237-69.

19. Wertz PW, Swartzendruber DC, Squier CA. Regional variation in the structure and permeability of oral mucosa and skin. Adv Drug Dell Rev 1993; 12: 1-12.

20. Squier CA, Wertz PW. Structure and function of the oral mucosa and implications for drug delivery. In: Rathbone MJ, ed. Oral mucosa drug delivery. New York: Marcel Dekker, 1996: 1-25.

21. Kirjavainen M, Monkkonen J, Saukkosaari M, Valjakka-Koskela R, Kiesvaara J, Urtti A. Phospholipids affect stratum corneum lipid bilayer fluidity and drug partitioning into the bilayers. J Controll Rel 1999; 58 (2): 207-14.

22. Šentjurc M, Vrhovnik K, Kristl J. Liposomes as a topical delivery system: the role of size on transport studied by the EPR imaging method. J Controll Rel 1999; 59 (1): 87-97.

23. Coderch L, Fonollosa J, De Pera M, Estelrich J, De La Maza A, Parra JL. Influence of cholesterol on liposome fluidity by EPR. Relationship with percutaneous absorption. J Controll Rel 2000; 68 (1): 85-95.

24. Honzak L, Sentjurc M, Swartz HM. In vivo EPR of topical delivery of a hydrophilic substance encapsulated in multilamellar liposomes applied to the skin of hairless and normal mice. J Controll Rel 2000; 66: 221-8.

25. Šentjurc M, Gabrijel?i? V. Transport of liposome-entrapped molecules into the skin as studied by electron paramagnetic resonance imaging methods. In: Lasic DD, ed. Handbook of non-medical applications of liposomes. Vol. 4. Boca Raton: CRC Press, 1996: 91-114.

26. Kržic M, Šentjurc M, Kristl J. Improved skin oxygenation after benzyl nicotinate application in dif-

ferent carriers as measured by EPR oximetry in vivo. J Controll Rel 2001; 70: 203-11.

27. Swartz HM, Boyer S, Gast P, et al. Measurements of pertinent concentrations of oxygen in vivo. Magn Reson Med 1991; 20 (2): 333-9.

28. Norby SW, Swartz HM, Clarkson RB. Electron and light microscopy studies on particulate EPR spin probes lithium phthalocyanine, fusinite and synthetic chars. J Microsc 1998; 192 (2): 172-85.

29. Liu KJ, Gast P, Moussavi M, et al. Lithium phthalocyanine: a probe for electron paramagnetic resonance oximetry in viable biological systems. Proc Natl Acad Sci USA 1993; 90 (12): 5438-42.

30. Smirnov AI, Norby SW, Clarkson RB, Walczak T, Swartz HM. Simultaneous multi-site EPR spectroscopy in vivo. Magn Reson Med 1993; 30 (2): 213-20.

31. Smirnov AI, Norby SW, Weyhenmeyer JA, Clarkson RB. The effect of temperature on the respiration of cultured neural cells as studied by a novel electron paramagnetic resonance technique. Biochim Biophys Acta 1994; 1200 (2): 205-14.

32. Smirnov AI, Norby SW, Walczak T, Liu KJ, Swartz HM. Physical and instrumental considerations in the use of lithium phthalocyanine for measurements of the concentration of oxygen. Magn Reson 1994; B103 (2): 95-102.

33. Liu KJ, Bacic G, Hoopes PJ, et al. Assessment of cerebral pO2 by EPR oximetry in rodents: effects of anesthesia, ischemia, and breathing gas. Brain Res 1995; 685: 91-8.

34. Greene SA, Thurmon JC. Xylazine – a review of its pharmacology and use in veterinary medicine. J Vet Pharmacol Ther 1988; 11: 295-313.

35. Hsu WH. Xylazine-induced depression and its antagonism by alpha adrenergic blocking agents. J Pharmacol Exp Ther 1981; 218: 188-92.

36. McCormick JM, McCormick PW, Zabramski JM, Spetzler RF. Intracranial pressure reduction by a central alpha-2 adrenoreceptor agonist after subarachnoid hemorrhage. Neurosurgery 1993; 32: 974-9.

SPREMLJANJE VPLIVA RAZLIČNIH NOSILCEV Z VGRAJENIM HIPEREMIKOM NA PARCIALNI TLAK KISIKA V PODGANJI USTNI SLUZNICI Z ELEKTRONSKO PARAMAGNETNO RESONANČNO OKSIMETRIJO

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Povzetek: Namen študije je bil izbrati med različnimi liposomi najboljši nosilec zdravilne učinkovine za lokalno zdravljenje bolezenskih sprememb na ustni sluznici. Kot zdravilno učinkovino smo uporabili hiperemik benzil nikotinat (BN), ki poveča prekrvitev in s tem oksigenacijo tkiva. Z elektronsko paramagnetno resonančno (EPR) oksimetrijo smo ob uporabi paramagnetne snovi (litijev ftalocianin) spremljali učinek benzil nikotinata, ki je bil vgrajen v liposome različnih oblik in sestave. Liposomi so bili pripravljeni bodisi iz hidrogeniranega bodisi iz nehidrogeniranega sojinega lecitina. Kot podlago za pripravo zdravila, ki smo ga nanašali na ustno sluznico, smo uporabljali polimetilmetakrilat (PMM), ki se na ustno sluznico dobro lepi. Z EPR smo merili parcialni tlak kisika (pO_2) v ustni sluznici pred nanosom liposomov in po njem. Ugotovili smo, da pride do največjega povečanja pO_2 v ustni sluznici po lokalnem nanosu benzil nikotinata, vključenega v večplastne liposome iz hidrogeniranega sojinega lecitina ($p<0.0001$), zato so ti liposomi najprimernejši za lokalno dovajanje zdravilnih učinkovin na ustno sluznico.

Ključne besede: ustne bolezni; zdravljenje z zdravili; liposomi; in vivo EPR oksimetrija; ustna sluznica; podgana