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International Journal of Pharmaceutics 208 (2000) 35–39

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Permeability of antisense oligonucleotide through porcine buccal mucosa

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Received 7 April 2000; received in revised form 24 July 2000; accepted 26 July 2000

Abstract

Antisense oligonucleotides (AONs) that can modulate malfunctioning genes have a great potential to become future therapeutic agents. In this study, we investigated the feasibility of buccal delivery of AONs using ISIS 3082 as a model compound. An isocratic HPLC method was developed to quantify ISIS 3082. The permeability coefficient of this AON at 37°C, determined by using side-by-side diffusion cells, was 1.05×10^{-9} (cm/s). The flux of ISIS 3082 across buccal mucosa was dependent upon its concentration in the donor chamber. The permeation of ISIS 3082 was increased when 100 mM of sodium glycocholate was used as a permeation enhancer. The potential of delivering AONs via buccal route with the aid of permeation enhancers is explored in this study.

Keywords: Antisense oligonucleotides; Buccal; Permeability; Mucosal delivery

1. Introduction

Specific genes that result in disease conditions are being identified as molecular biology advances. In recent years, drug design and discovery have been moving towards the gene regulation. Since the antisense oligonucleotides may be tailor-made, they can be designed to disrupt gene expression related to a disease. Currently, there are several antisense molecules in different stages of clinical studies. The results from clinical trials of several phosphorothioate AONs reveal a promising future for this new class of therapeutic agents (Agrawal, 1996; Crooke, 1997; Bennett, 1998).

The potential for applying natural AONs to a clinical situation has been limited due to the degradation of these molecules when administered through traditional routes of administration. Chemically modified AONs show acceptable therapeutic effect when administered systemically. Administration of AONs via intravenous, subcutaneous, intraperitoneal, intracerebroventricular,

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0378-5173/00/$ - see front matter

PII: S0378-5173(00)00543-3

oral, and transdermal routes have been explored (Vlassov et al., 1993; Iversen et al., 1994; Sakai et al., 1994; Agrawal et al., 1995; Wang et al., 1996). AONs are mostly intended for therapeutic treatment of chronic diseases, such as cancer, AIDS, genetic disorders, and cardiovascular diseases, where repeated administration may be required. Development of alternate routes of administration and /or controlled release drug delivery systems would significantly improve patient compliance and provide convenience. Vlassov et al. reported that detectable amounts of AON penetrated the skin and mucosa when administered by transdermal and nasal routes, respectively (Vlassov et al., 1993). The feasibility of iontophoretic delivery of antisense oligonucleotides was investigated by Brand et al. (Brand and Iversen, 1996; Brand et al., 1998). Wang et al. showed that therapeutic levels of AON could be achieved in a study using stripped skin. Also, they found that chemical enhancers improved the permeation of AON through skin (Wang et al., 1996).

Transbuccal delivery of AONs using a controlled release delivery system has great potential because the buccal epithelium has higher permeability than skin and oral cavity is well suited for retentive systems. Delivery of macromolecules via this route has been reported (Ebert et al., 1994). Surfactants, particularly bile salts, were shown to increase the permeation of drugs through buccal mucosa into circulation (Ebert et al., 1994; Hoogstraate et al., 1996a,c). It is essential to characterize buccal transport of AONs in designing transbuccal delivery systems. In this study, we investigated buccal permeation of a model AON, ISIS 3082 (a 20-mer with a sequence of 5′-TGC ATC CCC CAG GCC ACC AT-3′). In addition, the effect of sodium glycocholate on the buccal permeation of ISIS 3082 was determined.

2. Materials and methods

2.1. Materials

Model AON, ISIS 3082 (molecular weight 6405), was obtained from ISIS Pharmaceuticals, Inc, Carlsbad, CA and other chemicals were used as received.

2.2. Buccal tissue preparation

Porcine buccal tissue was obtained from Long Ranch, Ripon, CA. The tissue was stored in Krebs buffer at 4°C and used within 2 h of decapitation. The buccal mucosal membrane was separated from the underlying connective tissue using surgical scissors. Buccal mucosa with an approximate area of 1 cm^2 was then mounted between the donor and the receiver chambers of modified Franz-type diffusion cells.

2.3. In vitro permeation studies

The experiments were conducted using modified Franz-type diffusion cells having a diffusional area of 0.95 cm^2 at 37°C. ISIS 3082 is freely soluble in double distilled water and its solution was then charged into the donor chamber. Distilled water was also used as receiver medium as phosphate buffer interfered with AON detection. Two milliliter samples were collected at specified times and fresh medium was added to the receiver chamber. ISIS 3082 was quantified by HPLC. All the measurements were made in triplicate and expressed as mean $\pm$ standard deviation.

The steady-state flux was calculated from the slope of the linear region of the cumulative amount of ISIS 3082 permeated versus time plot. The apparent permeability coefficient (P) was calculated using the following expression:

$$P = \frac{(\mathrm{d}Q/\mathrm{d}t)}{C_0 A},$$

where $\mathrm{d}Q/\mathrm{d}t$ is the steady state flux, C_0 is the initial donor concentration, and A is the diffusional area.

2.4. Analytical methods

An isocratic HPLC method for quantification of the model AON, ISIS 3082 was developed. The HPLC system consisted of two Waters 6000A pumps, Waters M490 variable-wavelength UV absorbance detector, Waters 710B autosampler, and EZChrome software. A Microsorb-MVTM C4 column (5 μm, 4.6 mm ID × 25 cm) was used and ISIS 3082 was eluted with 6.25 mM Tris buffer

(pH = 9). The flow rate of the mobile phase was 0.5 ml/min from 1 to 10 min and 1 ml/min from 10 to 19 min. ISIS 3082 was detected at 260 nm. ISIS 3082 had a retention time of 3.6 min under the conditions described above. Standard curve was established by linear regression of peak area and concentration of the standards, and ISIS 3082 in the samples was quantified accordingly. A calibration curve was established in the range of 0.2–10 μg/ml and the minimum detection limit was 0.1 μg/ml. A typical standard curve for ISIS 3082 is shown in Fig. 1. Sodium glycocholate investigated as a permeation enhancer in this study did not interfere with ISIS quantitation (data not shown).

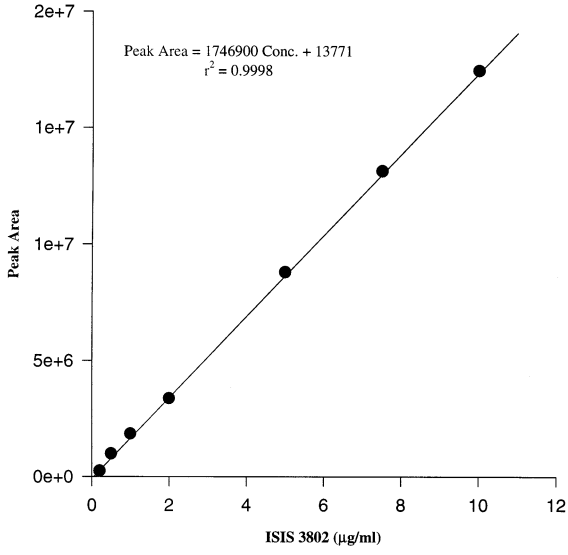

Fig. 1. A typical ISIS 3082 standard curve.

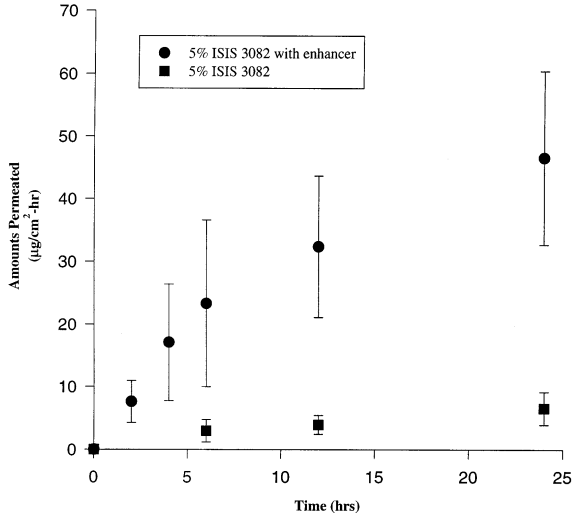

Fig. 2. Buccal permeation of ISIS 3082.

Table 1
Steady state flux and permeability of ISIS 3082 ($n = 3$)

Donor Conc. (%)	teady State Flux (μg/cm^2 h)	Permeability Coefficients ($\times 10^8$ cm/s)
5	0.19 ± 0.16	0.11 ± 0.09
5[a]	4.13 ± 5.55	3.25 ± 2.42
3[a]	0.87 ± 0.29	0.81 ± 0.27
1[a]	0.51 ± 0.33	1.43 ± 0.93

[a] with 100 mM Sodium glycocholate.

2.5. Effect of sodium glycocholate on the permeation of ISIS 3082

The influence of the enhancer, sodium glycocholate (100 mM), on the permeation of ISIS 3082 was investigated by in vitro permeation studies as described above and evaluated by the enhancement ratio, which is defined as the ratio of permeability coefficient with enhancer to permeability coefficient without enhancer.

3. Results and discussion

The buccal permeability of the model AON, ISIS 3082, across porcine buccal mucosa was determined in the permeation study using 5% (w/v) AON. The influence of 100 mM sodium glycocholate on permeability was studied using 1, 3, and 5% of ISIS 3082. A typical cumulative permeation profile of the model AON from 5% of ISIS 3082 with and without 100 mM enhancer is shown in Fig. 2. The steady state flux and apparent permeability coefficients of ISIS 3082 at different concentrations is given in Table 1. The steady state flux of ISIS 3082 was found to increase with an increase in concentration in the donor solutions. The average permeability coefficients of

ISIS 3082 across the porcine buccal mucosa from donor solutions without enhancer and with 100 mM enhancer were 1.05×10^{-9} and 1.83×10^{-8} cm/s, respectively. Sodium glycocholate 100 mM concentration in donor solution enhanced the permeation of ISIS 3082. The permeability of the AON in this study is higher than that of transdermal delivery of AON reported by Wang et al. (1996). Results of the permeation studies through buccal mucosa have shown that transbuccal route has the potential for the delivery of AON.

In a separate study conducted in our laboratory, the permeability of mononucleotide derivative, acyclovir with molecular weight 225 was determined (Shojaei et al., 1998). The permeability coefficient of acyclovir without enhancer was found to be 6.65×10^{-6} cm/s. Sodium glycocholate enhanced acyclovir flux 9 times when present at a concentration of 100 mM in the donor formulation. It has been reported that a non-selective 'wide-open' porous pathway was found to be responsible for the permeation of a polar compound, acyclovir (Shojaei et al., 1998). The co-application of sodium glycocholate to the buccal epithelium was found to provoke lipid solubilization, both in the intercellular domains and from the cell membranes (Hoogstraate et al., 1996b; Gibaldi and Feldman, 1970). The solubilization of lipids in the intercellular space may increase the diffusivity of hydrophilic compounds such as ISIS 3082 and acyclovir in the buccal mucosa and thus enhance their overall transport rate. The independence of pH on enhancement ratio for multicharged species such as acyclovir indicated a single mechanism of enhancement through the same route of transport as the permeant, i.e. the paracellular route (Shojaei et al., 1998). Since ISIS 3082 is also a multiple charged species, a similar transport mechanism can explain the enhancement effect of sodium glycocholate.

The permeability of ISIS 3082 across porcine buccal mucosa is much lower (more than 5000 times) than that of acyclovir. However, the enhancement effects of sodium glycocholate at the concentration of 100 mM on the permeability of ISIS 3082 is twice of that of acyclovir. The greater enhancement of ISIS 3082 of sodium glycocholate could possibly be explained by the differences in the molecular weights of the compounds, the molecular weight of ISIS 3082 is 28 times higher than the molecular weight of acyclovir. In the absence of sodium glycocholate, ISIS 3082 has lower diffusivity in buccal mucosa due to higher molecular weight compared to acyclovir. Consequently, the permeability of acyclovir is much higher. However, when the cell membranes are fluidized or paracellular pathways are opened, the resistance reduction to the large molecule is much more significant than to the small molecule. As a result, in the presence of sodium glycocholate the enhancement was much higher for ISIS 3082.

4. Conclusions

The fundamental parameter for designing a transbuccal delivery system, permeability of AON through buccal mucosa membrane, was determined. The feasibility of the delivery of oligonucleotides through buccal route has been shown in the presence of enhancer, sodium glycocholate. Sodium glycocholate enhanced the buccal permeability of phosphorothioate AON, ISIS 3082, by effectively decreasing the resistance of paracellular pathway.

Acknowledgements

The authors would like to thank Dr Bret Berner for his valuable suggestions in preparing this manuscript and Long Ranch, Inc., Ripon, CA for providing porcine buccal tissue. This work is partially supported by the Scholarly/Artistic Activity Grant of the University of the Pacific.

References

Agrawal, S., 1996. Antisense oligonucleotides: towards clinical trials. Trends Biotechnol. 14, 376–387.

Agrawal, S., Temsamani, J., Galbraith, W., Tang, J., 1995. Pharmacokinetics of antisense oligonucleotides. Clin. Pharmacokinetics. 28, 7–16.

Bennett, C.F., 1998. Antisense Oligonucleotides: Is the glass half full or half empty? Biochem. Pharmacol. 55, 9–19.

Brand, R., Iversen, P., 1996. Iontophoretic delivery of a telomeric oligonucleotide. Pharm. Res. 13, 851–854.

Brand, R., Wahl, A., PL, I, 1998. Effects of size and sequence on the iontophoretic delivery of oligonucleotides. J. Pharm. Sci. 87, 49–52.

Crooke, S.T., 1997. Antisense Oligonucleotides, Eur. Bio-Pharm. Rev. 42–50.

Ebert, C.D., Heiber, S.J., Dave, S.C., Kim, S.W., Mix, D., 1994. Mucosal delivery of macromolecules. J. Control. Rel. 28, 37–44.

Gibaldi, M., Feldman, S., 1970. Mechanisms of surfactant effects on drug absorption. J. Pharm. Sci. 59, 579–589.

Hoogstraate, A.J., Coos Verhoef, J., Pijpers, A., van Leengoed, L.A., Verheijden, J.H., Junginger, H.E., Bodde, H.E., 1996a. In vivo buccal delivery of the peptide drug buserelin with glycodeoxycholate as an absorption enhancer in pigs. Pharm. Res. 13, 1233–1237.

Hoogstraate, A.J., Senel, S., Cullander, C., Verhoef, J., Junginger, H.E., Bodde, H.E., 1996b. Effects of bile salts on transport rates and routes of FTIC-labelled compounds across porcine buccal epithelium in vitro. J. Control. Rel. 40, 211–221.

Hoogstraate, A.J., Verhoef, J.C., Tuk, B., Pijpers, A., van Leengoed, L.A., Verheijden, J.H., Junginger, H.E., Bodde, H.E., 1996c. In-vivo buccal delivery of fluorescein isothiocyanate-dextran 4400 with glycodeoxycholate as an absorption enhancer in pigs. J. Pharm. Sci. 85, 457–460.

Iversen, P.L., Mata, J., Tracewell, W.G., Zon, G., 1994. Pharmacokinetics of an antisense phosphorothioate oligodeoxynucleotide against rev from human immunodeficiency virus type 1 in the adult male rat following single injections and continuous infusion. Antisense Res. Develop. 4, 43–52.

Sakai, R.R., He, P.F., Yang, X.D., Ma, L.Y., Guo, Y.F., Reilly, J.J., Moga, C.N., Fluharty, S.J., 1994. Intracerebroventricular administration of AT1 receptor antisense oligonucleotides inhibits the behavioral actions of angiotensin II. J. Neurochemistry 62, 2053–2056.

Shojaei, A.H., Berner, B., Li, X., 1998. Transbuccal delivery of acyclovir: I. In vitro determination of routes of buccal transport. Pharm. Res. 15, 1182–1188.

Vlassov, V.V., Karamyshev, V.N., Yakubov, L.A., 1993. Penetration of oligonucleotides into mouse organism through mucosa and skin. Febs Letters 327, 271–274.

Wang, L., Ghanem, A.-H., Higuchi, W.I., Ruffner, D.E., 1996. Transdermal Delivery of Antisense Oligodeoxynucleotides against Herpes Simplex Virus. Pharm. Res. 13, S-383.