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Note

The effect of a viscosity and an absorption enhancer on the intra nasal absorption of metoprolol in rats

Nadine Kilian, Douw G. Müller *

Department of Pharmaceutics, Potchefstroom University for CHE, Potchefstroom, 2520, South Africa

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Abstract

During this investigation metoprolol was used as a model drug to investigate the effect of formulation variables on the nasal absorption of a low dose of metoprolol. This was done firstly, to compare the bioavailability of metoprolol after peroral administration with the bioavailability found after intra nasal administration and secondly, to determine the influence of the incorporation of methyl cellulose as a viscosity enhancing agent and polysorbate-80 as an absorption enhancer into various formulations, on the bioavailability of metoprolol. In order to achieve the objectives the following metoprolol formulations were prepared: formulations containing metoprolol in a sodium chloride solution (0.9% (w/v)) for nasal administration, as well as for per oral administration, a formulation containing metoprolol in methyl cellulose (2% (w/v)) only, and two formulations containing metoprolol in methyl cellulose at two different concentrations (1% and 2% (w/v)) respectively, with the addition of polysorbate-80 (0.1% (w/v)) to both. A bioavailability study was conducted in rats by determining the metoprolol plasma concentrations at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, and 4 h after administration of the respective formulations. A HPLC method was used to determine the various plasma concentrations. The AUC values, used as an indication of bioavailability, showed that the bioavailability of metoprolol was significantly improved after nasal administration (4.1715 μg/ml per h) compared to per oral administration (1.5648 μg/ml per h). The inclusion of 2% methyl cellulose into the formula resulted in a further increase (AUC = 5.7930 μg/ml per h) in the AUC compared to both the previous mentioned formulas, probably due to an increase in the contact time of the formulation with the nasal mucosa. The inclusion of 0.1% polysorbate-80 concomitantly with the methylcellulose (2%) showed no statistically significant difference in the AUC values. In fact a decrease in the bioavailability was observed (AUC = 3.3087 μg/ml per h) possibly due to drug entrapment in the presence of polysorbate. The bioavailability of the formulation containing methyl cellulose (1%) and polysorbate-80 (0.1%) was equivalent to the formulation containing only 2% methyl cellulose. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Nasal absorption; Metoprolol; Bioavailability; Formulation; Methyl Cellulose; Polysorbate-80

* Corresponding author.

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1. Introduction

The nasal route of drug administration has many advantages for the delivery of drugs into the systemic circulation. This route of drug delivery bypasses the first pass effect through the liver, metabolism in the gastro-intestinal mucosa as well as degradation in the gastro-intestinal tract. The nasal mucosa is richly supplied with blood vessels and is highly permeable for most drugs. The rate and extent of drug absorption through the nose can be compared to intravenous administration (Chien et al., 1989).

A high dose of metoprolol can be used for the treatment of hypertension and *angina pectoris* (Hoffmann and Lefkowitz, 1991). Metoprolol however, shows poor bioavailability, only 40% (Regardh et al., 1974) of a dose reaches the systemic circulation after per oral administration due to the first pass effect. Sometimes it is necessary to add excipients to formulations in order to enhance the absorption of drugs (De Boer et al., 1990). This project was undertaken in order to determine the effectiveness of the nasal route for the administration and absorption of metoprolol in rats and to determine if manipulation of the formulation would have an influence on the bioavailability of the drug.

2. Materials and methods

2.1. Materials

The metoprolol tartrate was kindly donated by Ciba–Geigy, Isando, South Africa. Methyl cellulose, sodium chloride and polysorbate-80 was obtained from Saarchem, Muldersdrift, South Africa. Procaine hydrochloride was obtained from Sigma (St. Louis, USA). All other reagents and solvents were of analytical grade and double distilled water was used throughout the study. HPLC-grade acetonitril (HiPersolve, BGH, Poole, UK) and *n*-butylchloride (NT, Johannesburg, South Africa) was used.

2.2. Preparation of formulations of oral and nasal solutions

Five formulations (Table 1) were prepared. Formulations A_1 (nasal control) contained 1 mg drug per 500 μl dose and A_2 (oral control) contained 1 mg metoprolol per 50 μl dose. Formulations B and C_1 contained 2% (m/v) methyl cellulose with 1 mg metoprolol respectively with the addition of 0.1% (m/v) polysorbate-80 to formulation C_1. Formulation C_2 contained only 1% (m/v) methyl cellulose with 0.1% (m/v) polysorbate-80 and 1 mg metoprolol. The solutions were prepared in 0.9% (m/v) sodium chloride to obtain isotonic solutions in order reduce nasal irritation as much as possible. Methyl cellulose formulations were prepared by stirring the substance in 0.9% (m/v) sodium chloride solution for 24 h before adding the metoprolol. Methyl cellulose and polysorbate-80 containing formulations were also both stirred for 24 h before the addition of metoprolol.

2.3. Determination of pH

As nasal absorption can be dependant on the ionized species present (pK_a of metoprolol is 9.5) at the site of absorption, the pH of each formulation was determined at room temperature using a Jenway pH meter (Labotec, Johannesburg, South Africa).

2.4. Determination of viscosity

As viscosity may have an influence on the extent and rate of absorption the various degrees of viscosity of the methyl cellulose solutions were determined at room temperature with a Brookfield DV-II + viscosity meter (Brookfield, Stoughton, USA). Readings were taken at rotation speeds of 6, 10, 12, 20, 30, 50, 60 and 100 rpm A period of 5 min was allowed between each reading in order for the measurements to stabilize. Readings were also taken in the same manner with the same speeds in decreasing order. Viscosity was determined with a spindle number LV2 in a sample size of 80 ml at room temperature.

Table 1
Composition of the various formulations (per 10 ml solution)

Ingredient	Formula				
	A_1 (mg)	A_2 (mg)	B (mg)	C_1 (mg)	C_2 (mg)
Metoprolol tartrate	200	20	200	200	200
Methyl cellulose	—	—	200	200	100
Polysorbate-80	—	—	—	0.0108 g[a]	0.0108 g[a]
Sodium chloride	90	90	90	90	90
Route of administration	nasal	oral	nasal	nasal	nasal
Dosage volume (μl)	50	500	50	50	50

[a] 1 ml polysorbate-80 is equivalent to 1.08 g polysorbate-80.

2.5. HPLC

The assay for metoprolol in the various solutions as well as the determination of the metoprolol plasma concentrations were done using a validated HPLC method. The system consisted of a Novapak C8, 150 × 3.9 mm steal column with a particle size of 4 μm (Waters, Milford, MA). A LiChroCart, 4 × 4 mm (Merck, Germany) guard column was used. These columns were fitted to a Spectra-Physics model SP 8800 pump, a Spectra-Physics model FL2000 fluorescence detector and a Spectra-Physics model Chromjet integrator. A Spectra-Physics model AS3000 automatic sampler was used. The emission wavelengths for procaine and metoprolol were 224 and 274 nm while the excitation wavelengths were 354 and 300 nm respectively. The mobile phase consisted of acetonitril/0.005 M dodecan-1-sulfonic acid buffer (35:65), at pH 3.5. The pH was regulated with the addition of 5% (v/v) phosphoric acid. The flow rate were 1.8 ml/min and the sample size was 25 μl.

2.6. Sample preparation

To 900 μl plasma sample was added 100 μl of a standard solution, 0.5 ml (10 μg/ml) procaine solution as the internal standard, as well as 0.1 ml (2 M) NaOH and 3.0 ml *n*-butyl chloride. The mixture was slowly rotated (50 rpm) for 10 min and centrifuged (Hettich EBA 3S Centrifuge, Labotec, Johannesburg, South Africa) for 2 min at 3000 rpm. The aqueous phase was frozen with dry ice, and the top organic layer was carried over to a clean conical glass tube. To this was added 0.1 ml (0.05 M) H_2SO_4. This mixture was shaken for 2 minutes with a Heidolph REAX vortex mixer (Labotec, Johannesburg, South Africa) and then centrifuged at 3000 rpm. The aqueous phase was again frozen with dry ice and the organic layer was removed with a Pasteur pipette. The aqueous layer was thawed and carried over to a 250 μl sample container and sealed. 25 μl of each sample was injected onto the HPLC column to be analyzed.

2.7. Standard curve

The same procedure as described above was used to compile a standard curve. To 900 μl of plasma was added 0.5 ml of the internal standard solution as well as 100 μl of standard solutions with various concentrations (0.1, 0.125, 0.25, 0.5, 1.0, 10.0 and 25.0 μg/ml respectively). These samples were prepared for HPLC analysis as previously described.

2.8. Churgical procedure

Three male BD IX rats (weighing between 200 and 250 g) were used for each time interval. Each rat was sedated with 0.02% (v/v) halothane–air mixture in a glass container (4 l). After sedation each rat was coupled to an anaesthesia apparatus filled with halothane (Fluothane, Zeneca, Wood-

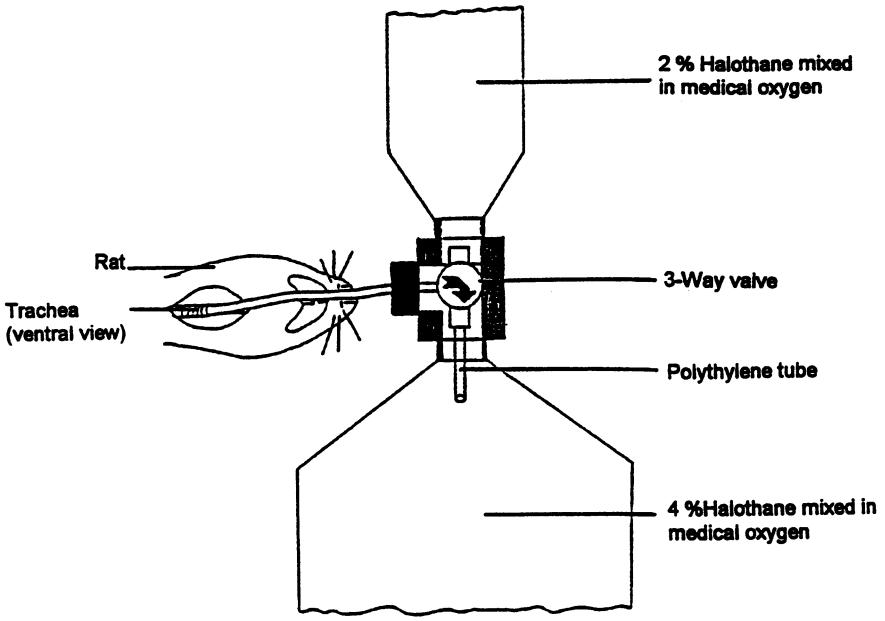

Fig. 1. Schematic representation of the anaethetised animal during a nasal absorption study.

mead, South Africa) and medical oxygen (Fedgas, Alrode, Johannesburg, South Africa) in order to induce anaesthesia for the duration of the experiment. The ventral area of the neck was shaven and an incision was made to expose the trachea. An incision was made in the trachea and a polyethylene tube was inserted to ensure anaesthesia. The nasopalatine channel of the rat was sealed with Supergel® glue (Henkel, Alrode, Johannesburg, South Africa). A schematic representation of the rat under anaesthesia is shown in Fig. 1. A micropipet was used to administer the various formulations directly through the left nasal opening into the nasal cavity of the rat.

2.9. Euthanasia and sample collection

The depth of anaesthesia was determined by monitoring the rate of breathing as well as the pedal reflex of the rat. Euthanasia was applied by the intraperitoneal injection of Eutha-naze (0.8 ml Eutha-naze solution, Premier, Bryanston, South Africa) 5 min before sampling. A 5 ml blood sample was taken by cardiac puncture at the following time intervals after drug administration: 0 (blank), 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3 and 4 h. Three determinations ($N = 3$) were done per time interval. The blood samples (4.5 ml) were placed in Venoject tubes (Becton Dickenson, UK) containing heparin.

2.10. Oral administration

The per oral solution (500 μl) was administered directly into the duodenum by means of an aluminium tube.

3. Results

3.1. pH

The pH values observed for the various formulations varied from 6.45 for formulation A_2 to 6.74 for formulation C_1. The assumption thus was made that the ionization state of the drug in the various formulations were practically similar and that the state of ionization as a factor to consider in the comparison of the bioavailabilities found after administration of the various formulations, was negligible.

Table 2
Mean metoprolol plasma concentrations ($n = 3$) as found after administration of the various formulations ($\alpha = 0.05$)

Theoretical time (h)	Plasma concentration (μg/ml) (mean ± S.D.)				
	A_1 (nasal)	A_2 (oral)	B (nasal)	C_1 (nasal)	C_2 (nasal)
0.00	0.00 ± 0.0000	0.00 ± 0.0000	0.00 ± 0.0000	0.00 ± 0.0000	0.00 ± 0.0000
0.25	1.83 ± 0.2836	0.72 ± 0.0739	1.88 ± 0.3859	1.83 ± 0.1309	1.41 ± 0.7872
0.50	3.34 ± 1.3552	0.68 ± 0.1161	3.22 ± 0.2144	1.39 ± 0.6915	3.40 ± 0.4607
0.75	1.61 ± 0.1567	0.82 ± 0.0544	1.39 ± 0.4471	1.42 ± 0.3526	2.09 ± 1.2696
1.00	0.88 ± 0.0093	0.32 ± 0.1173	2.29 ± 0.5350	1.66 ± 0.3215	1.88 ± 0.1916
1.50	1.36 ± 0.0006	0.72 ± 0.0464	1.83 ± 0.7641	0.82 ± 0.8363	1.57 ± 0.7474
2.00	0.95 ± 0.1310	0.24 ± 0.1628	1.40 ± 0.2442	1.06 ± 0.1853	1.58 ± 1.1099
3.00	0.70 ± 0.3463	0.22 ± 0.0315	1.12 ± 0.0580	0.36 ± 0.3013	0.95 ± 0.3089
4.00	0.57 ± 0.3697	0.42 ± 0.1011	0.89 ± 0.0231	0.37 ± 0.2624	0.48 ± 0.0817

Mean, mean metoprolol plasma concentrations.
A_1 (nasal), 1 mg metoprolol tartrate in 0.9% (m/v) sodium chloride solution.
A_2 (per oral), 1 mg metoprolol tartrate in 0.9% (m/v) sodium chloride solution.
B (nasal), 1 mg metoprolol tartrate in 2% (m/v) methyl cellulose solution.
C_1 (nasal), 1 mg metoprolol tartrate in methyl cellulose solution (2% m/v) with polysorbate-80 (0.1% m/v).
C_2 (nasal), 1 mg metoprolol tartrate in methyl cellulose solution (1% m/v) with polysorbate-80 (0.1% m/v).

3.2. Viscosity

The viscosities of formulations A_1 and A_2 could not be determined because of their aqueous nature. The viscosities of formulations B, C_1 and C_2 were determined at various spindle speeds as described previously. Little differences in the viscosity of formulations B (545 centiPoise) and C_1 (533 centiPoise) at spindle speeds of 50 rpm respectively, was observed. This was to be expected because both contained the same concentration (2%) of methyl cellulose. Formula C_2 showed a lower viscosity (127 centiPoise at 50 rpm) due to a lower concentration (1%) of methyl cellulose. A decrease in concentration of methylcellulose cause a factor four decrease in viscosity of this formulation. The inclusion of polysorbate-80 showed no influence on the viscosity of the formulations.

3.3. Bioavailability

The mean metoprolol concentrations as well as the mean standard deviations as found as a function of time for each formulation ($N = 3$) are shown in Table 2. Table 3 shows the pharmacokinetic parameters calculated for metoprolol after administration of the various formulations. The AUC values were calculated using a method described by Jawien (1992). Statistical differences (Miller, 1981) were shown by making use of 95% confidence intervals. An alpha-value of 0.05 showed statistical significant differences between the various formulations. If the alpha-value was not included by the two intervals, it meant that the two particular formulations differed statistically significantly ($p < 0.05$) from each other. Table 4 shows the confidence intervals calculated for the various formulations.

4. Conclusion

From Table 2 it is evident that dramatic differences in the plasma levels were found after administration of the various formulations. From this data it is obvious that the nasal route is far more superior to the oral route of administration. Because the AUC is indicative of the amount of drug absorbed, it was used to quantify the differences in bioavailability between the various formulations. The AUC found for metoprolol (Table 3) from formulation A_2 (per oral route) was 1.5648 μg/ml per h which was only 37.5% of the AUC (4.1715 μg/ml per h) observed with the

Table 3
Calculated pharmacokinetic parameters for the metoprolol in plasma

Formula	Route of administration	AUC (μg/ml per h)	C_{max} (μg/ml)	T_{max} (h)
A_1	nasal	4.1715	3.34	0.50
A_2	per oral	1.5648	0.82	0.75
B	nasal	5.7930	3.22	0.50
C_1	nasal	3.3087	1.83	0.25
C_2	nasal	5.5011	3.40	0.50

nasal route of administration. This observation compare well with the data obtained by Hoffmann and Lefkowitz (1991), which showed a 40% relative bioavailability for metoprolol after per oral administration. The T_{max} (0.50 h) observed for nasal administration was shorter compared to a T_{max} of 0.75 h found for the peroral route. The nasal C_{max} was 3.34 μg/ml compared to the 0.82 μg/ml for the peroral route of administration.

Inclusion of 2% methyl cellulose (formula B) into the formula cause a further increase (38.9%) in the relative bioavailability (AUC of 5.7930 versus 4.1715 μg/ml per h) of metoprolol compared to the standard nasal formula (formula A_1). This difference in the AUC's was statistical significant ($p < 0.05$). These results prove that an increase in the viscosity enhances the nasal absorption of certain drugs, in this case metroprolol, probably due to increased contact time of the drug with the absorption surface.

The inclusion of an absorption enhancer (0.1% polysorbate-80) together with a viscosity enhancer (formula C_1) unexpectedly caused a decrease in the bioavailability of metoprolol when compared with both the standard formula (A_1) as well as formula B containing the viscosity enhancer. The AUC observed for metoprolol from formulation C_1 (3.3087 μg/ml per h) was only 79.3% compared to that of formula A_1 (control) (4.1715 μg/ml per h). Although this decrease in bioavailability was not statistically significant ($p < 0.05$), it was surprising to find that the addition of polysorbate-80 to the formula resulted in a nearly 20% decrease in the relative bioavailability. This decrease can probably be attributed to drug entrapment by the surface active polysorbate-80. If compared to formulation B the decrease in the observed AUC was 42.9%, which is even more dramatic. The addition of polysorbate-80 to formula (formula C_1) containing 2% methyl cellulose, thus caused the AUC of metoprolol to decrease by nearly 43%. The addition of polysorbate-80 caused no change in the viscosity of formulation C_1 and therefore cannot be the reason for the unexpected lower bioavailability observed. However, it was found that metoprolol showed faster absorption ($T_{max} =$ 0.25 h) from formula C_1 compared to A_1 ($T_{max} =$ 0.50 h). The incorporation of an absorption enhancer concommitantly with a viscosity enhancer, in this case, thus showed no advantages in the transnasal delivery of metoprolol, but instead, decreased the bioavailability.

Formulation (C2) with a lower viscosity (1% methyl cellulose) together with 0.1% polysorbate-80 showed an increase in the relative bioavailability (5.5011 μg/ml per h) of 31.9% compared to the control formula (4.1715 μg/ml per h). This increase in bioavailability was statistical significant ($p < 0.05$). Although the concentration of methyl cellulose was decreased by 50% the bioavalibility

Table 4
Confidence intervals ($\alpha = 0.05$) for the statistical comparison of the various formulations

Formulations	Confidence intervals
A_1 vs A_2	[3.6119; 1.6014]
A_1 vs B	[−2.6268; −0.6162]
A_1 vs C_1	[−0.1425; 1.8681]
A_1 vs C_2	[−2.3349; −0.3243]
B vs A_2	[−5.2335; −3.2230]
B vs C_1	[1.4790; 3.4896]
B vs C_2	[−0.7134; 1.2972]
C_1 vs A_2	[−2492; −0.7386]
C_1 vs C_2	[−3.1977; −1.1871]

was similar to the formulation B which contained 2% methyl cellulose. The viscosity of formulation C_2 was four times less than that of formulation C_1, but similar bioavailabilities were observed. In this formulation the presence of the absorption enhancer polysorbate-80 played a definite role in enhancing the absorption of metoprolol. If the change in absorption was solely influenced by viscosity, as was shown by formula B, the bioavailability of formulation C_2 should have decreased by 50%. It would seem that the potential absorption enhancing effect of polysorbate-80 is influenced by the degree of the viscosity of the formula. It is possible that viscosity may influence the type and size of micelles which may be responsible for possible dose entrapment. In this case drug entrapment may be less, resulting in a higher rate of release from the dosage form. Together with this it seems that the influence of polysorbate-80 on the mucosal membrane to enhanced the absorption of the drug is experienced to an greater extent in an environment with a lower viscosity.

References

Chien, Y.W., Su, K.S.E., Chang, S.F., 1989. Nasal Systemic Drug Delivery. Marcel Dekker, New York, pp. 1–310.

De Boer, A.G., van Hoogdalem, E.J., Breimer, D.D., 1990. Improvement of drug absorption through enhancers. Eur. J. Drug Metab. Pharmackinet. 15, 155–157.

Hoffmann, B.B., Lefkowitz, R.J., 1991. Catecholamines and sympatomimetic drugs. In: Gilman, A.G., Rall, T.W., Nies, A.S., Talyor, P. (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics. 8th edn. vol. 1. McGraw-Hill, New York, pp. 221–243.

Jawien, W., 1992. A method for statistical comparison of bioavailability when only one concentration–time point per individual is available. Int. J. Clin. Pharmacol. Ther. Toxicol. 30, 484.

Miller, R.G., 1981. Simultaneous Statistical Inference, 2nd edn. Springer-Verlag, New York, pp. 1–299.

Regardh, C.-G., Borg, K.O., Johansson, R., Johnsson, G., Palmer, L., 1974. Pharmacokinetic studies on the selective β_1 receptor antagonist metoprolol in man. J. Pharmackinet. Biopharmaceut. 2, 347–364.

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