Preparation of bisoprolol fumarate nasal spray and its nasal delivery in rats

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Abstract: The aim of the present work was to prepare a nasal spray of bisoprolol fumarate (BF). The Pharmacokinetics and relative bioavailability of the BF nasal formulation were evaluated in Wistar rats. The BF nasal spray after administration exhibited very fast absorption and higher plasma drug concentration. The maximum plasma concentration (C_{max}) and the time to reach it (T_{max}) were 409.5ng/ml and 3.6 min for the BF nasal spray, 39.4ng/ml and 26.7min for the drug solution, respectively. The bioavailability of the BF nasal spray was greater than 1500.0%. Meantime, the effect of the BF nasal spray on nasal mucociliary movement was also studied with a toad palate model. The BF nasal preparation showed minor ciliotoxicity, but the adverse effect was temporary and reversible.

Keywords: β_1-adrenoceptor blocker; bisoprolol fumarate; nasal spray; pharmacokinetics; ciliotoxicity

INTRODUCTION

Bisoprolol fumarate (BF) is a synthetic selective β_1-adrenoceptor blocker and usually used as the fumarate salt in drug formulations (Shaikha *et al*., 2008; Modamio *et al*., 1998). It can reduce the heart rate and the force of contraction of heart and thus drug has been widely used in the management of hypertension, myocardial infarction, angina pectoris and tachyarrhythmia (Ding *et al*., 2007; Zhou *et al*., 2007). Presently, the marked dosage forms of BF are mainly tablets and capsules. Following oral administration of the above these preparations, the drug is slowly absorbed into the systemic circulation and furthermore undergoes the extensive first-pass metabolism, resulting in slow onset of action and relative low bioavailability. T_{max} and absolute bioavailability for these BF oral preparations were reported to be 2-4h and 60-80% (Kirch *et al*., 1978; Liu *et al*., 2000), respectively. Thus, oral administration of BF is not an ideal dosing route in the relief and treatment of some acute cardiovascular diseases such as angina pectoris, myocardial infarction and tachyarrhythmia.

In recent decades, intranasal administration of drug has been thought as an attractive route to attain systemic effects. Known major advantages of this route are avoidance of the hepatic and gastro enteric first-pass effect compared to oral delivery and a convenient dosing routine (Haschke *et al*., 2010). The human nasal cavity also has a relatively large surface area (150cm^2) because of the presence of a large number of microvilli, a highly vascularized epithelium, which promote absorption. Another important feather is the rapid onset of action compared to oral dosing (Pontiroli *et al*., 1989; Behl *et al.*, 1998; Costantino *et al.*, 2007).

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Propranolol, a nonselective *β*-adrenoceptor antagonist, is widely applied in the treatment of several cardiovascular diseases. Previous study has proved that the drug after nasal administration shows a very rapid absorption and a high bioavailability, approximately 100% (Duchateau *et al*., 1986; Hussain *et al*., 1980). The intranasal administration of propranolol appears, however, less suitable for chronic treatment because of the local side effects on the nasal epithelium and the ciliary epithelial function (Donk *et al.*, 1982; Ducehatau *et al*., 1986; Jiang *et al*., 1999). Since BF is one of propranolol congeners and also possesses high pharmacological activity, whose therapeutic effects can be produced at low doses (5-20 mg daily) (Cardillo *et al*., 1992), the drug is very likely to be readily absorbed from nasal cavity and suitable for nasal administration to obtain systemic effects. Thus, the aim of present research was to prepare a BF nasal spray and to further investigate the Pharmacokinetics and relative bioavailability of the drug in rats. Additionally, a limitation for nasal dosing is that drug and excipients should not damage the normal functions of the nasal ciliary epithelium, so the effect of the BF nasal formulation on nasal mucociliary movement was also evaluated in this study.

MATERIALS

Chemicals

BF with a purity of 99.8% was purchased from Hainan Wante Pharmceutical Co., Ltd (Haikou, China); metoprolol tartrate was supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); dimethy-β-cyclodextrin (DM*β*CD) with a purity of 98.3% was kindly supplied by Binzhou Zhiyuan Biological Technology Co., Ltd (Shandong, China); chlorobutanol with a purity of 99.8 % was purchased from Sinopharm Chemical Reagent Co. Ltd

(Beijing, China); acetonitrile was of HPLC grade from Fisher Scientific (New Jersey, USA).

Apparatus

For the analysis of BF plasma samples was used a HPLC system with fluorimetric detection. The system consisted of a Shimadzu LC-10AT pupm, a SIL-10AXL auto sampler with 100µl injection loop, a SCL-10A fluorometer (Shimadzu Inc, Japan) and a C18 column (Wondasil, 5µm, 4.6mm×250mm, GL Sciences Inc, Japan). The mobile phase was a mixture of acetonitrile and 0.01M ammonium dibasic phosphate buffer in a volume ratio of 35/65 (v/v). The flow rate was 1.0ml/min and the excitation wavelength of the fluorometer was set at 275nm.

Animals

Male Wistar rats weighing 250-300g were purchased from Inner Mongolia University experimental animal center (Hohhot, China) and used in pharmacokinetic study; toads weighing 30-50g were kindly supplied by Inner Mongolia medical University experimental animal center (Hohhot, China) and their upper palate mucosa were applied to evaluate the ciliotoxicity of BF nasal formulation. All animal experiments were approved by the animal care and ethics committee of Inner Mongolia Medical University.

METHODS

Preparation of BF nasal spray

The doses used in this study were calculated according to oral doses in humans and compensated from body weight. Accurately weighted BF was dissolved in small volume of distilled water, while both required amount of DMβCD together with chlorobutanol dissolved in another appropriate volume of distilled water under continuous stirring in 40 degree water bath, after which, the these two solutions were mixed together to obtain a homogeneous solution. Then, the remaining water was added to the mixture solution to reach the specified volume of the BF nasal formulation. Finally, the pH of BF nasal spray solution was adjusted to be approximately 6.0 using 0.1M hydrochloric acid solution or sodium hydroxide solution, followed by filtration through a 0.45-µm membrane filter.

Animal experiments

Wistar rats were fasted for 12h prior to the experiments with free access to water. Twelve rats were randomly divided into two groups of six animals each. Nasal administration studies were performed as previously reported method (Gu *et al*., 2005; Bjerre *et al*., 1996). Briefly, the animals were first anesthetized with 1.2g/kg of urethane injected intraperitoneally. They were then placed on their backs and a polyethylene tube was inserted into the trachea to prevent nasal respiration, after which, the oesophagus was tied to this cannula to prevent peroal absorption. BF nasal spray solution was given at a dose of 112.5 µg/kg (10µl/100g body weight) unilaterally through the nasal cavity with PVC tubing connected to a microliter syringe. Blood samples of 0.5ml each time were withdrawn into heparinized PE tubes from the retroorbital plexus at 1, 3, 5, 10, 20, 30, 60, 90, 120, 180, 240, and 360 min after administration of the formulation. For oral dosing, BF solution prepared with 1% (w/v) carboxy methylcellulose was administered to the anesthetized rats via gastric gavage at a dose of 450µg/kg. Blood samples were collected at 5, 10, 20, 30, 45, 60, 75, 90, 120, 180, 240 and 360 min post-dosing. All the plasma samples were obtained by centrifuging the blood samples at 3000 rpm for 10 min and were stored at -60 degree until assayed.

BF analysis in plasma

For the assay of BF, rat plasma sample was pretreated as reported earlier (Cai *et al*., 2008). Briefly, in 200µl plasma sample, 10µl of 1.05 µg/ml metoprolol tartrate methanol solution (internal standard) and 50µl sodium hydroxide solution (1.0 M) were added, after which, the samples were vortexed with 0.75ml tert-butyl methyl ether for 30sec and centrifuged at 3000rpm for 5min. An aliquot of the organic layer was transferred to a clean PE tube and evaporated to dryness under a nitrogen steam in 60 degree water bath. The residue was reconstituted in appropriate volume of methanol, and after centrifugation, 60µl of the supernatant was injected for HPLC analysis.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed using the 3P97 Pharmacokinetic program (issued by the State Food and Drug Administration of China for pharmacokinetic studies). C_{max} and T_{max} were observed as raw data. The area under the concentration-time curve (AUC) was calculated using the linear trapezoidal method. The analysis and comparison of the pharmacokinetic parameters between nasal and oral administrations of BF were performed using the SPSS statistical software (version 11.0, SPSS Inc.). A *P* value less than 0.05 was thought statistically significant.

Nasal ciliotoxicity studies

The 2.5% BF nasal spray solution was freshly prepared prior to the experiments. To explore the effect of the absorption enhancer as well as the preservative alone used in the formulation on nasal ciliary movement, 3% DM βCD solution and 0.5% chlorobutanol solution were also prepared as test solutions. Meanwhile, 0.9% normal saline (NS) and 1% sodium deoxycholate were used as a negative and positive control, respectively. Nasal ciliotoxicity experiments were performed using toad palate model according to the previously reported method (Puchelle and Tournier, 1983; Jiang *et al*., 1995). The upper palate mucosa was first separated from toads and cut into the small pieces of the same size (3mm×3 mm),

then rinsed with 0.9% NS, after which, the mucosa samples were spread on the glass slide and immediately treated with 0.2 ml of test solutions. Lastly, they were observed under an optical microscope and the lasting time of ciliary movement (LTCM) of the toad palate was recorded. If the ciliary movement of the mucosa sample was found to stop, the sample was immediately rinsed with saline, its LTCM would continue to be recorded. The total LTCM for the sample would be the sum of the two recorded LTCMs.

RESULTS

The quantification of BF plasma concentration was based on the peak area ratio of drug to internal standard ($R=A_{BF}/A_{IS}$). The linearity was observed over the concentration range of 4.0-1200.0ng/ml with correlation coefficient of over 0.99. A typical calibration curve was as follows: R=0.0105C-0.0238 (r=0.9968, n=6). The lowest limit of quantification (LLOQ) for the determination of BF in rat plasma was found to be 2.0ng/ml. Accuracy of the determination of BF in rat plasma (n=9) is 99.05±5.18%. Within-day and between-day precision were all below 10.0%. Extraction recovery of BF in rat plasma (n=9) was all above 85.0%.

As shown in fig. 1 and table 1, a very rapid absorption of the BF nasal spray after nasal administration was observed in rats and T_{max} was only less than 4.0min. Conversely, the maximal plasma concentrations (C_{max}) were reached at about 30min after the drug solution was orally given to the rats. Furthermore, the obtained C_{max} for nasal route at a dose of 112.5μg/kg was found to be approximately 10 times greater than that for oral dosing at dose of 450μg/kg. The relative bioavailability of the BF nasal spray was calculated to be approximately 1500.0% compared to oral route.

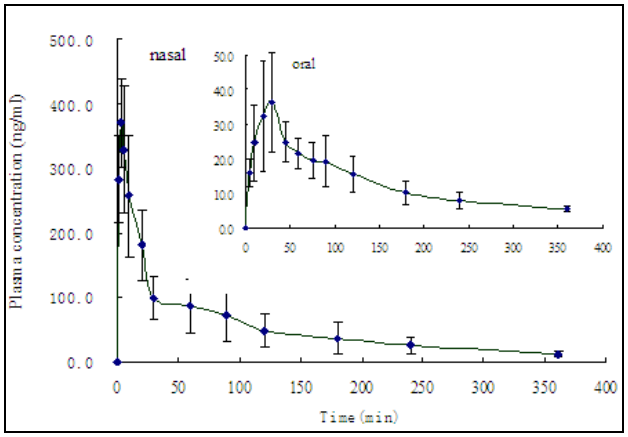

Fig. 1: Mean plasma concentration-time profiles after nasal administration of BF nasal spray at a dose of 112.5μg/kg and oral dosing of BF solution at a dose of 450μg/kg to rats, respectively. (n=6)

Also as can be seen in table 2, the LTCM of toad palate treated with was 2.5% BF nasal formulation was found to be 45min, which was far less than the duration for 0.9 % saline. However, while the toad palate was rinsed with 0.9% saline immediately after its mucociliary movement ceased, the mucociliary movement of the sample soon restored and still last about 4h. Total LTCM for the above palate samples was 280min, which was nearly close to the duration for 0.9% NS. The cilliary movement of the sample treated with 1% sodium deoxyycholate solution nearly promptly stopped. Additionally, among the additives utilized in the BF nasal formulation, the preservative 0.5% chlorobutanol solution showed moderate ciliotoxicty, but the adverse effect was also reversible. Meantime, the effect of the enhancer 3% DMβCD solution on the mucociliary movement of the toad palate was found to be similar to those observed from the BF nasal formulation.

DISCUSSION

Based on the usual doses of the marketed BF oral preparations, and the volume of drug solution applied in the nasal cavity of an adult, which is restricted to 0.05-0.15ml (Iium, 2002), the concentration of the BF nasal spray solution was established to be 2.5%. As 2-5% DMβCD is commonly utilized in nasal formulations as mucosa penetration enhancer, and 0.5% chlorobutanol is also used as a preservative in injections and ophthalmic preparations, the concentrations of DMβCD and chlorobutanol in the BF nasal preparation were selected to be 3% and 0.5%, respectively. Additionally, our previous research has confirmed that BF showed a high oil-water partition coefficient (lipophilicity) and good chemical stability in approximately pH6.0 aqueous solution (Hao and Gu, 2013). Under the same pH condition, chlorobutanol solution possessed a good chemical stability and bacteriostatic effect. Moreover, the ideal pH of a nasal formulation should be within 4.5-6.5 so as to avoid nasal mucosa irritation. Thus, it is reasonable that the pH of BF nasal spray was finally adjusted to be about 6.0 because under such pH conditions, majority of the drug existed as unionized species, which are absorbed better compared with ionized species (Arora *et al*., 2002). Finally, the osmolarity of the BF nasal preparation was not adjusted to be isotonic since very small volume of drug solution was applied in nasal cavity.

As for the Pharmacokinetics behavior of the BF nasal spray, it is very obvious that BF was more easily absorbed following nasal administration than after oral route in rats. The relative bioavailability of the BF nasal spray was calculated to be up to 1500.0%, implying that nasal dosing resulted in about 15-fold higher than oral route in the absorption extent of the drug. The oral bioavailability of bisoprolol in rats was reported to be approximately 10%, showing very low absorption extent of the drug

Table 1: The Pharmacokinetic parameters of BF nasal spray in rats using oral dosing as reference

Administration route	Dose (μg/kg)	C_{max} (ng/ml)[a)]	T_{max} (min)[a)]	AUC_{0-6} (ng·h/ml)	$AUC_{0-\infty}$ (ng·h·ml)	Relative bioavailability (%)[b)]
Nasal	112.5	409.5±46.4	3.6±1.0	344.9±130.5	383.0±127.6	1547.6±515.8
Oral	450	39.4±16.6	26.7±5.2	79.9±18.8	98.8±15.1	100

a) The data are expressed as mean ±S.D. (n=6); b) Relative to the oral route

Table 2: Effect of BF nasal spray on the toad palate cilia movement

Solution (w/v)	LTCM (min)	LTCM after rinsing (min)	Total LTCM (min)	Relative %[a)]
0.9% NS	296.6±7.6		296.6±7.5	100
2.5% BF nasal spray	45.3±1.5	234.3±16.9	280.5±12.3*	94.6
1%Sodium deoxyycholate	4.3±0.6	0	4.3±0.8**	1.5
0.5%Chlorobutanol solution	35.3±1.6	125.6±5.1	165.2±4.8**	55.6
3% DM*β*CD solution	54.2±4.0	253.3±13.5	302.2±11.9*	101.8

a) Relative to the NS group; * No significantly different from NS group, $P > 0.05$. ** significantly different from NS group, $P < 0.01$

(Bühring *et al*, 1986). The reasons for the fast absorption and high bioavailability of BF after nasal administration are probably as follows. Firstly, BF is one of propranolol congeners, they have similar chemical structures and moreover all belong to the "amino-functional" drugs, which are amenable to being delivered transdermally and often contain one or more primary amine, secondary amine and tertiary amine radicals (Kanios and Mantelle, 2007). Another possible reason is that BF and propranolol possess analogous physiochemical properties. For example, the molecular weight of the two drugs are both below 1000 Da and the oil -water partition coefficients (log *P*) and dissociation constants (p*K*a) for BF are 2.20 and 9.31, respectively (Caudron *et al.*, 2004), 2.65 and 9.03 for propranolol, respectively (Balon *et al*., 1999; Vogelpoel *et al*., 2004). They both have relatively high lipophilicity. Lastly, the good bioavailability of BF after nasal dosing might also be attributed to the avoidance of hepatic first pass metabolism and the increased permeability of the nasal mucosa by DM*β*CD (Behl *et al*., 1998; Bshara *et al*., 2014). Consequently, the Pharmacokinetic profiles of the BF nasal spray was very similar to those obtained for nasally administered propranolol, both showing very fast absorption and high bioavailability.

Lastly, in the toad palate model experiments we observed that the adverse effect of the BF nasal spray on nasal mucociliary movement was transient and reversible. Thus, it is quite possible that the cilotoxicity of BF itself was far less than that of propranolol, since the cilotoxicity of the latter has been proved to very severe and moreover irreversible (Zhang and Jiang, 2001). Additionally, as the preservative 0.5% chlorobutanol and the enhancer 3% DM*β*CD only exhibited mild and reversible nasal ciliotoxcity, it might be reasonable that these two additives were used in the BF nasal formulation. One of reasons for minor nasal ciliotoxicity of the BF nasal preparation might be the existence of complexation of chlorobutanol with DM*β*CD in BF nasal spray solution, which resulted in the lower-concentration preservative contacting nasal mucosa (Marttin *et al.,* 1998; Kim *et al*., 2009).

CONCLUSIONS

BF nasal spray was first prepared and its formulation mainly consisted of the drug, DM*β*CD and chlorobutanol. The Pharmacokinetics and relative bioavailability of the BF nasal formulation were investigated in rats. Nasal absorption studies proved that BF was very rapidly absorbed through the nasal mucosa of Wistar rats and the relative bioavailability of BF was up to approximately 1500.0% relative to oral route. Furthermore, the effect of the BF nasal formulation as well as its additives on the ciliary movement of the toad palate was also studied. Chlorobutanol exhibited moderate ciliotoxicity and effect of DM*β*CD on the nasal ciliary movement appeared to be mild. The whole BF nasal formulation showed mild cilitoxicity and moreover the adverse effect was proved to be transient and reversible.

In conclusion, the nasal administration of BF may be an alternative to intravenous route and the BF nasal formulation will also be a very hopeful preparation for the clinical treatment of some acutely cardiovascular diseases with the obvious advantages such as rapid absorption and onset of action, high bioavailability, ease of use, and self-medication. A further study is necessary to investigate the pharmacokinetics and bioavailability of the BF nasal formulation in humans. The in-vivo effect of the BF nasal spray on the human nasal mucociliary movement and nasal epithelial function also remained to accomplish.

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