Development of thiomer based buccal films for the enhancement of bioavailability: An in-vivo analysis

Muhammad Zaman¹, Muhammad Hanif²*, Muhammad Wahab Amjad³, Asif Mahmood¹, Shahid Shah⁴, Maria Abdul Ghafoor Raja³, Shahid Rasool⁵ and Rai Muhammad Sarfraz⁵

¹Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan
²Department of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan
³Faculty of Pharmacy, Northern Border University, Arar Saudi Arabia
⁴Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad, Pakistan
⁵College of Pharmacy, University of Sargodha, Sargodha, Pakistan

Abstract: Present work was conducted to improve the bioavailability of Tizanidine HCl (TZN) by formulating mucoadhesive buccal films (MBFs) using novel thiolated arabinoxylan (TAX) as film former. MBF’s were prepared by solvent casting technique followed by their evaluation for surface morphology and folding endurance. Moreover, pharmacokinetic parameters including $C_{\text{max}}$, $t_{\text{max}}$, $t_{1/2}$ and AUC were determined after administering standard oral solution (SOS) and MBFs of TZN at a dose of 1mg/kg. Successful thiolation was confirmed by the presence of 4.98 to 7.04 mmol of thiol content per gram of the polymer. Results of in-vivo pharmacokinetics have signified ($p=0.0089$) the suitability of MBFs as a carrier of drug through buccal route. Results have explored that, $t_{1/2}$ was increased from 2.51hrs (SOS) to 10 hrs, $C_{\text{max}}$ from 42.3 ng/ml (SOS) to 105ng/ml and $t_{\text{max}}$ from 2hrs (SOS) to 6h. Conclusively, TAX has exhibited the potential to form MBFs thereby offering sustained release of TZN with improved pharmacokinetic profile.

Keywords: Mucoadhesive buccal films, in-vivo pharmacokinetics, thiolated arabinoxylan, tizanidine HCl, statistical analysis.

INTRODUCTION

Nature has always been a remarkable source of various active as well inactive pharmaceutical ingredients. Arabinoxylan (AX), which is a natural polymer and can be extracted from variety of plants, where, Plantago ovata is one of its major source. Alkaline extraction is the commonly used technique, which has been extensively employed for the extraction of AX at an alkaline pH ranging from 12-13. Literature is the evident of its safe and effective biomedical application and has been applied successfully as suspending agent, thickening agent and sustained release mucoadhesive film former. It possesses mucoadhesive properties which can be further enhanced by incorporating thiol group in its backbone. Integration of thiol group laid to the formation of a newer generation of the polymer, called as thiomer (Skaria et al., 2014). Thiomers offer better mucoadhesion than non-thiolated polymer and it can be enhanced up to 140 folds. Due to their mucoadhesive nature, these are ideal for the development of buccal drug delivery systems. TZN, a muscle relaxant has poor bioavailability i.e. 30-40%, owing to its extensive first pass effect. Due to poor bioavailability, it required to administer repeatedly, at a dose of 2-4mg three times a day. Its average plasma half-life is about 2.5 hrs. Poor oral bioavailability, short half-life and repeated doses, make TZN a suitable candidate for sustained release mucoadhesive drug delivery system.

Current study was design with an aim to utilize newly modified natural polymer (TAX) to fabricate mucoadhesive buccal films for improved bioavailability of TZN.

MATERIALS AND METHODS

Materials

Pharmedic Laboratories Lahore Pakistan has generously gifted TZN to complete this research project. Orthophosphoric acid and 1-ethyl-3-(3-dimethylamino propyl) carbodiimide (EDAC) were purchased from Sigma-Aldrich Germany. Thiol glyclicic acid (TGA) from Barcelona Spain, while other chemicals including Hydroxypropyl methyl cellulose (HPMC K15), diethyl ether, Methanol, Sodium Hydroxide Dihydrogen potassium phosphate were obtained from Merck Germany. The used chemical and reagents were of analytical grades.

Experimental animals

Selected animals (Albino rabbits) weighing 2.5 to 3kg were provided 12h light-dark cycles under suitable environmental conditions of freely available water and air with standard chow diet (Patel and Amin, 2011). The studies were accomplished under the guideline of the “instructional animal research ethic committee” of the Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan.

*Corresponding author: e-mail: muhammadhanif14@yahoo.com
Synthesis of TAX
TAX was synthesized by the esterification reaction using TGA as –SH group precursor and HCl as catalyst. For this purpose, 1gm of AX was soaked in distilled water and subjected to continuous stirring followed by addition of 0.125 mill-molar solution of EDAC. TAG was added in the solution in sufficient quantity to obtain 1:1 of AX and TGA. pH of this mixture was adjusted to 5 by the addition of 1M solution of sodium hydroxide. After pH adjustment, the reaction mixture was kept in dark for 4h and under continuous stirring at room temperature. TAX was separated from the mixture by dialysis technique, which was performed thrice. First, against 5mM HCl in tubing of cellulose membrane having molecular weight 12 kDa for consecutive 3 days, second using the same medium but with the addition of 1% NaCl and third again by using 5mM HCl for 2days. Separated TAX was lyophilized under the conditions -50°C temperature and 0.013mbar pressure to obtained dried samples of the polymer.

Determination of thiol substituents
Thiol constituents attached with the polymer have been determined by employing Ellman’s reagent technique. 0.5% dispersions of AX was prepared as control for comparison with the same strength of prepared polymer e.g. TAX, followed by their dilution to the ultimate concentration of 0.15% (w/v) using 5M phosphate buffer of pH 8.0. Equal volume of samples and Ellman’s reagent (0.3% w/v) were allowed to react for 2h at room temperature. Furthermore, the reaction mixture was subjected to UV-visible spectrophotometric analysis at 450nm. Serial dilutions of TGA with Ellman’s reagent were prepared to construct a calibration curve, used for the determination of Thiol contents.

Fabrication of MBFs
Solvent evaporation technique was used for the preparation of sustained release MBFs according to the formulation composition described in table 1. Accuracy in the measurements was achieved by preparing aqueous solutions/dispersion of the ingredients such as 3% solution and dispersion of TZN and polymer respectively. Two percent (2%) aqueous solutions of HPMC K15 and 2% of polysucralse, and 6% of glycerol were prepared separately. Required volume of polymeric dispersion was poured in beaker of 100ml, taken on hotplate magnetic stirrer, followed by addition of glycerol solution. Solutions of HPMC K15 and drug were mixed one by one in a separate beaker and after uniform mixing, they were added in the mixture of polymer and plasticizer. Finley, sweetener and few drops of few drops of flavoring agent were added in the later under continuous stirring, until a homogeneous mixture was achieved. The ultimate mixture was casted into a glass Petri dish and subjected to drying in hot air oven at 40 °C to get dried films.

In vitro analysis of MBFS
MBFs were evaluated for different in-vitro properties like thickness of the film, weight variation, pH of the film surface, drug assay, folding fortitude and surface morphological analysis.

Determination of Ex-vivo mucoadhesiveness
Mucoadhesiveness of the prepared films was determined by using modified physical balance consisted of two pans. A glass slide was attached to the bottom of one the pans, and other slide beneath of that pan. The second pan was balanced by attachment of another glass slide with the second pan of the balance. Pieces of buccal mucosa was taken, and attached to both slides and a film was sandwiched between them. Slides were stuck to each other by applying 50gm pre-load force for few minutes. With the help of lever, balance was lifted and second pan was added with the weight in gradual manner, until the slides were separated from each other. Weight (g) which was required to separate the slides was recorded.

Study design
Study was conducted by following cross over study design provided with washout period of two weeks. Animals were divided into three groups (n=6) medicated in such a way that in the first period of the study, animal 3 and 5 were administered with standard oral solution (SOS), 2 and 6 with buccal films. In the second period, SOS and films were administered to 2 and 4, and 1 and 5 respectively. In the final period, SOS was given to 1 and 6, while 3 and 4 have been treated with films, respectively (table 2).

Dose and dose frequency
All the animals have received single dose of 1mg/kg TZN in the form of oral solution and buccal films.

Collection of blood samples
Blood samples of 1ml each from the marginal ear vein were taken at pre-decided time intervals (0.5, 1, 2, 4, 6, 8, 12 and 24h) and collected into heparinized tubes.

Drug extraction from the collected blood samples
Drug was extracted from plasma by liquid-liquid extraction technique using methanol and di-ethyl ether as protein precipitant. Samples were subjected to extraction twice, first by adding methanol in blood (3:1) and then by diethyl ether in the same ratios. Each time, the mixture was centrifuged for 10 min at 5000 rpm and then vortexed for 2 min. Supernatant was transferred into another test tube and are dried by using light stream of nitrogen at 40°C. Dried sample was reconstituted in 200μl of mobile phase, and 20μl of the ultimate sample was injected into the HPLC system for analysis.

Chromatographic conditions
RP-HPLC method was applied for the analysis of the drugs. Mobile phase was composed of methanol and
water in the ratio of 80:20, respectively and the pH 3.0 was adjusted by orthophosphoric acid. Sample was run for 10 min at a flow rate of 0.8ml/min, ambient temperature and average column pressure was maintained at 1400 psig.

**In-vivo pharmacokinetic studies**

Prepared formulations were evaluated for different in vitro parameters and after satisfactory results, they were subjected to in-vivo analysis. TZN at the dose of 1mg/kg was administered in the form of standard oral solution (SOS) and buccal film. PK Solver® software was used following non-compartmental model to determine the pharmacokinetic parameters. Maximum plasma concentration (C\text{max}) and time (t\text{max}) required to achieve C\text{max} were calculated from the visual inspection of plasma concentration-time curves. Area under the curve (AUC) was calculated by the application of ‘Trapezoidal rule’. Both AUC from 0 to 24 (AUC\text{0–24}) and as well as from 0 to infinity (AUC\text{0–}\infty, ng h/ml) were determined. The AUC\text{0–}\infty was calculated with the help of AUC\text{0–24} using following mathematical expression.

\[
AUC_{0–\infty} = AUC_{0–24} + C*/k
\]

Where C* = last measured concentration, k is the elimination rate constant.

Half-life (t½) of the drug was calculated as:

\[
t_{1/2} = 0.693/k
\]

**STATISTICAL ANALYSIS**

Graphpad prism ver 7.0 was used to analyze statistically by employing analysis of variances (ANOVA) followed by Bonferroni’s Multiple Comparison Test, where confidence of interval was set to 95% having n=6.

**RESULTS**

Successful thiolation of the polymer was confirmed through Ellman’s reagent technique, which has shown 4.98 to 7.04mmol/gm of thiol contents. Thiolated polymer was utilized in different trials, executed to develop a suitable formulation of MBFs followed by their evaluation to find out a formulation, suitable for administration of TZN through buccal route.

Selected formulations were subjected to various characterizations and the results have revealed that films were of uniform thickness showing standard deviation of ±0.83, variations in the weight of the films were also negligible (S.D = ±1.9). Surface pH (6.59) of the films was much closer to the pH of buccal cavity, signifying their potential as suitable carrier of the drug for its buccal administration. Drug contents were more than 95% thus conforming to USP recommendations, whereas mucoadhesion strength was 10.37±0.18 N indicating its ability to remain attached with buccal mucosa for longer time period. SEM micrographs of the blank as well as TZN loaded MBFs were scanned at different magnification powers. Both blank and TZN loaded MBF’s exhibited smooth and uniform texture with absence of definite pores. Moreover, uniform distribution was also reflected in fig. 1B of fabricated buccal films.

RP-HPLC method was applied for the in-vivo quantification of TZN. Chromatographic method was found suitable, accurate and precise with %RSD less than 1%. Detected retention time was found to be 2.612min with suitable resolutions of 2.1. LLOD and LLOQ were in the range of 1-2ng/ml and showed good linearity among selected concentrations (10-50ng/ml) with coefficient of correlation (R²) 0.9985. Typical chromatograph of TZN along with internal standard and its linearity graph are shown in fig. 2.

**In-Vivo pharmacokinetic evaluation**

Results of mean plasma concentration have been calculated and analyzed by ANOVA, which was applied using Graph Pad Prism version 7. Half-life (2.51 h), which was calculated after the administration of SOS oral administration was extended greatly to 10.43h, when the drug was delivered through MBF (Singh et al., 2017, El Mahrouk et al., 2014).

Graphical illustration of plasma profiles of TZN released from TAX based films were illustrated in fig. 3, where it can be observed that greater average AUC\text{0–24} (table 3) and AUC\text{0–}\infty (fig. 3B) were 1133.41 and 1298.99 ng h/ml, respectively indicating better amount of drug obtained, leading to better bioavailability achievement. Appreciable increase (2.5 folds) in average C\text{max} was noticed as it was increased from 42.3ng/ml, achieved as after administration of SOS to 104 - 105ng/ml, which were obtained after the delivery of drug using MBF (table 3). Similarly, time for drug to reach its maximum blood concentration (t\text{max}) was also momentarily improved (6h) indicating slow release of the drug from MBFs signifying drug retarding ability of TAX.

**Table 1**: Composition of prepared MBFs

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Polymer</th>
<th>Plasticizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>179</td>
<td>36</td>
</tr>
<tr>
<td>8</td>
<td>320</td>
<td>64</td>
</tr>
<tr>
<td>9</td>
<td>250</td>
<td>32</td>
</tr>
</tbody>
</table>

Quantities of polymer and plasticizer were used in ‘mg’
Development of thiomer based buccal films for the enhancement of bioavailability

Table 2: Latin-Square Crossover Design for in vivo pharmacokinetics of TZN HCl in rabbits

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Drug Products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period 1</td>
</tr>
<tr>
<td>1</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>MBFs</td>
</tr>
<tr>
<td>3</td>
<td>SOS</td>
</tr>
<tr>
<td>4</td>
<td>--</td>
</tr>
<tr>
<td>5</td>
<td>SOS</td>
</tr>
<tr>
<td>6</td>
<td>MBFs</td>
</tr>
</tbody>
</table>

Table 3: Pharmacokinetics of TZN after administration of MBFs and SOS solution (1mg/kg)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MBFs</th>
<th>SOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>10.43</td>
<td>2.51</td>
</tr>
<tr>
<td>$t_{max}$ (h)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>105</td>
<td>42.3</td>
</tr>
<tr>
<td>AUC $0-t$ (ng/ml*h)</td>
<td>1133.41</td>
<td>149.1</td>
</tr>
</tbody>
</table>

DISCUSSION

As from results, it was evident that pharmacokinetic parameters i.e. $t_{1/2}$, $C_{max}$, AUC and $t_{max}$ of the TZN were markedly increased when formulated into MBF’s. Results of the half-life were in accordance to the findings of Fatima et al., who have developed TZN loaded in-situ gels for its rectal delivery. In their studies, they also have claimed of prolonged half-life, achieved due to sustained and prolonged release of TZN.

Similarly, El-Mahrouk et al., have prepared chitosan lactate wafers for buccal delivery of TZN with higher value of $t_{max}$ (Moawad et al., 2017, El-Mahrouk et al., 2014).

Thus highlighting the importance of this route for sustained delivery of the drugs. Likewise, higher value of $C_{max}$ may be accredited due to the reason that thiolated polymers have the ability to augment the permeation, as they possess greater mucoadhesive strength and prolong contact time of the dosage form with the mucosal surface (Iqbalet al., 2012, Deepak et al., 2012, Bernkop-Schnürch, 2005, Yadav et al., 2014). Additionally, they can provide sustained effect without disturbing the viscosity of the solution (Bhatia and Ahuja, 2013). Hence, thiolated polymers possess unique ability of bioavailability enhancement (Deepak et al., 2012, Bernkop-Schnürch).

Statistical evaluation

Statistical analysis was performed for the comparative analysis of results obtained after the administration of SOS and MBFs. It was observed that results of all the studied aspects of TAX based MBFs were significantly different from those of SOS. It was the confirmation that TAX is a suitable polymer, which can be used not only to

Table 4: Statistical data table for pharmacokinetic parameters of TZN

<table>
<thead>
<tr>
<th>Bonferroni's Multiple Comparison Test</th>
<th>Parameters</th>
<th>Mean Diff.</th>
<th>T</th>
<th>Significant</th>
<th>$P$ value</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal Films vs SOS</td>
<td>$C_{max}$</td>
<td>-32.02</td>
<td>3.587</td>
<td>Yes (*)</td>
<td>0.0089</td>
<td>0.9291</td>
</tr>
<tr>
<td>Buccal Films vs SOS</td>
<td>$t_{1/2}$</td>
<td>7.373</td>
<td>73.52</td>
<td>Yes (***), 0.0001</td>
<td>0.9983</td>
<td></td>
</tr>
<tr>
<td>Buccal Films vs SOS</td>
<td>AUC</td>
<td>881.2</td>
<td>114.9</td>
<td>Yes (***), 0.0009</td>
<td>0.9986</td>
<td></td>
</tr>
<tr>
<td>Buccal Films vs SOS</td>
<td>$t_{max}$</td>
<td>3.960</td>
<td>121.7</td>
<td>Yes (**)</td>
<td>0.0001</td>
<td>0.9993</td>
</tr>
</tbody>
</table>

Fig. 1: SEM micrographs of A) Blank B) TZN loaded MBFs
modify the release of the drug but also to enhance its pharmacokinetic profile (table 4).

CONCLUSION

Studies were accomplished successfully as prepared MBFs have shown better pharmacokinetic profile as compared to oral solution. Half-life as well as improvement in the bioavailability of the drug had necessitated the effectiveness of TAX as sustained release mucoadhesive film former.

REFERENCES


Moawad FA, Ali AA and Saleem HF (2017). Nanotransfersomes-loaded thermosensitive in situ gel as a rectal delivery system of tizanidine HCl:

Fig. 2: Description of typical chromatogram of TZN and Internal Standard and linearity curve of TZN in the concentration range of 10-50ng/ml

Fig. 3: Plasma concentration of TZN after administration of SOS (A) and buccal film (B) to the rabbits
development of thiomer based buccal films for the enhancement of bioavailability


