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Original Article

DEVELOPMENT AND *IN VIVO* EVALUATION OF ABACAVIR SULPHATE MUCOADHESIVE BUCCAL FILMS

MADHURI P.*, RADHA G. V.

Department of Pharmaceutics, GITAM School of Pharmacy, Rishikonda, Visakhapatnam-530045, Andhra Pradesh, India
***Corresponding author: Madhuri P.; *Email: madhuri.vanam1@gmail.com**

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ABSTRACT

Objective: The objective of the study was to evaluate the pharmacokinetic parameters of abacavir sulphate mucoadhesive buccal films *in vivo*.

Methods: Abacavir sulphate mucoadhesive buccal films were developed using the solvent casting method and the prepared buccal films were evaluated for qualitative and quantitative parameters. Pharmacokinetic parameters (maximum plasma concentration [C_{max}], maximum plasma concentration [T_{max}], area under the curve [AUC], and biological half-life [$t_{1/2}$]) were evaluated *in vivo* using healthy albino white rabbits. The blood samples were collected evaluated and the results were compared with Ziagen a reference standard. The Modern Version 6 software and the pharmacokinetic function (Microsoft Excel add-in) applications were used to conduct the statistical study.

Results: The abacavir sulphate mucoadhesive buccal films were prepared successfully and the evaluated qualitative and quantitative parameters were within in the acceptable range. The results of the study stated that C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\alpha}$, and $t_{1/2}$ of abacavir sulphate mucoadhesive buccal film were found to be 93.86 ng/ml, 8 h, 1652.21 ng/ml×h, 2939.76 ng/ml×h, and 17.96 h, respectively. These results were comparable with the reference standard.

Conclusion: The overall absorption of abacavir sulphate was more in the test formulation with respect to the reference product at the same dose. Hence the study concludes that abacavir sulphate mucoadhesive buccal films achieved prolonged muchoadhesion and improved bioavailability compared to the conventional formulation.

Keywords: Abacavir sulphate, Mucoadhesion, Buccal films, Prolonged release, Pharmacokinetics

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INTRODUCTION

In terms of flexibility and comfort, mucoadhesive buccal films may be preferable over adhesive tablets, and it was not easy to wash or remove by saliva as oral gels [1]. Because of the abundant vascularization of the oral mucosa and its drug permeability, this route offers a desirable alternative to the oral or parenteral route for systemic delivery of drugs [2]. Over the last two decades, researchers have been drawn to the term mucoadhesion because of its potential to optimize localized drug delivery by keeping a preparation at the site of action or systemic administration by retaining a formulation in close contact with the absorption site (in the buccal cavity) [3]. Oral transmucosal medication administration avoids pre-systemic elimination in the gastrointestinal tract and liver [4]. These features combine to make the oral mucosa an appealing and viable target for systemic medication administration [5]. Buccal film is a thin matrix modified release dosage form that doesn't dissolve and is made up of one or more polymer films or layers that contain the drug and/or other excipients [6]. To enhance bioavailability, buccal medication administration is a highly effective method; this is due to the buccal mucosa's abundant blood supply, which makes it possible for the medicine to enter the systemic circulation directly [7]. Additionally, buccal dosage forms make it possible to quickly stop drug absorption in the event of a negative reaction [8]. Tablets, gels, and patches are all types of buccal dosage forms, with patches being the most flexible and comfortable [9]. Additionally, they can avoid oral gels' relatively brief length of residence on the mucosa, which is readily washed away and eliminated by saliva [10]. The novel feature of this study was to develop buccal films of abacavir sulphate to improve its bioavailability, reduce gastric irritation, and reduce the number of doses administered.

The most effective antiviral medication for herpes simplex infection is abacavir sulphate. Abacavir sulphate is a human immunodeficiency virus type I (HIV-I) nucleoside reverse transcriptase inhibitor (NRTI). It acts as a chain terminator of DNA synthesis and inhibits HIV reverse transcriptase. It has a short biological half-life of one hour and a dose-dependent, extremely variable oral absorption. Gastric irritability, high first-pass metabolism, and short biological half-life are this drug's key downsides. However, the medication is well tolerated when taken orally [11]. In order to eliminate the need for repeated dosing and boost systemic transport, bypass first-pass metabolism, and better bioavailability, an oral delivery method for abacavir sulphate must be developed [12].

The current research aims for *in vivo* evaluation of mucoadhesive buccal films acting as transmucosal drug delivery systems containing the abacavir sulphate to increase bioavailability.

MATERIALS AND METHODS

Materials

Abacavir sulphate sample was gifted by Aurobindo Pharma Ltd, India. Colorcon Pvt. Ltd. Verna, Goa provided free samples of HPMC K4M, HPMC K15M, HPMC K100M, and ethyl cellulose, sodium CMC, and HPC. Rest of the chemicals and reagents were analytical and pharmacopeial grade. White rabbits were procured from Vab Bioscience, Hyderabad.

Methods

Preparation of mucoadhesive buccal films of abacavir sulphate

Solvent casting method was used to generate the films containing abacavir sulphate. Different formulations containing hydroxypropyl methylcellulose (HPMC) K4M, HPMC K15M, and HPMC K100M as film-forming agents were developed by considering hydroxypropyl cellulose (HPC) and sodium carboxymethylcellulose as mucoadhesive release rate retarding polymers. Propylene glycol was chosen as a plasticizer. To prevent blistering on dried films, the plasticized ethyl cellulose solution was mounted as a backing membrane. The backing membrane was prepared by dissolving ethyl cellulose (5%) in acetone and isopropyl alcohol (65:35) with

20% dry weight of dibutyl phthalate polymer. To eliminate the air bubbles, the drug polymer solution was sonicated in a bath sonicator (ENUP 750, REmi India). After being poured into a mould with a backing membrane, the plasticized polymeric solution was dried in a vacuum oven (BTI-51, Biotechnics, India) for 24 h at 50 °C. The dried bilayer films were divided into squares with sides of 2 cm that each contained 300 mg of abacavir sulphate. These buccal films then wrapped in aluminium foil, placed in a desiccator, and utilized for additional research [13].

Bioanalytical method development of abacavir sulphate

Chromatographic conditions

A Deuterium lamp with a maximum wavelength of 303 nm was used in the Elico double beam SL 210 UV-visible spectrophotometer to perform the reverse phase HPLC (RP-HPLC) study. Agilent 1260 infinity DAD detector, Eclipse XDB C18 column with 5 µm particle size and dimensions of 4.6 X 250 mm column, 1260 infinity quaternary pump, Ezchrome software, flow rate of 1 ml/min, and run-time pressure of 2140 psi were used for HPLC analysis. By maintaining 303 nm and flowrate of 0.7 ml per minute with a phase mono basic phosphate buffer and acetonitrile in the ration 40:60 effluents were examined. This RP-HPLC method was employed to find an internal standard of abacavir sulphate in specimen (rabbit) plasma. The injection had a 20 µl of volume. Each sample's running time was 10 min. Until the end of process, the ambient temperature was maintained [14].

Standard solutions

100 µl of recently withdrawn serum were mixed with 400 µl of acetonitrile solution to produce the sample. The clear supernatant liquid was poured in another micro tube and then dried on evaporating after vortexing for 1 minute and centrifuged at 4500 x g for 30 min. 20 µl of the solution from reconstituting the residue with 100 L of mobile phase were used for HPLC analysis [15].

Extraction

For the purpose of developing the calibration standard, rabbit blood that had been anticoagulated with heparin. By adding the appropriate aliquots of working standard solutions to 0.5 ml of plasma, calibration standard solution samples were freshly made in rabbits' plasma to produce concentrations of 25, 50, 100, 150, and 200 µg/ml. Samples were combined with 5 ml of 0.1M-bis-(2 ethyl hexyl) phosphate in chloroform after 2 min of agitation, and then centrifuged at room temperature for 10 min at 2000 rpm. The supernatant liquid was then placed into a second tube with a volume of 2.5 ml, and 1 ml of 0.5N HCl was then added. The aqueous layer was separated after 5 min of centrifuging, and 20 L was then fed into the HPLC. The chromatogram was recorded and response of major peaks was measured [16].

$$\text{Amountofdrugin } \% = \frac{AS}{AT} \times \frac{WS}{100} \times \frac{5}{50} \times \frac{100}{WT} \times \frac{50}{5} \times \frac{P}{100} \times AV \times 100$$

Where, AS = average area of drug peak for standard, AT = average area of drug peak for test sample, WS = weight of drug taken for standard (in gm), WT = weight of drug taken for test sample (in gm), P = percentage purity of standard, AV = average weight in gm.

Procedure

Animal model

White rabbits (Vab Bioscience, Hyderabad) were used as animal models to find the bioavailability and pharmacokinetic characteristics of the abacavir sulphate buccal films. Rabbits were utilized because their buccal membrane's permeability and structure are very similar to those of humans. The subjects weighing 2.5 kg were employed in this experiment. The study was carried out in accordance with the recommendations made 97 by the Institutional Animal Ethical Committee (approval number: ASPEN/12/2019) and was overseen by a licensed veterinarian. Animals were placed in standard cages in a light-controlled environment with a temperature of 25±2 °C and 50±5% RH for environment familiarization. Ten days before to the experiment, rabbits were sent out and given an adjustment period. During the period of acclimatization, animals were kept on a regular pellet diet with unlimited access to water. Animals were kept on a fast for 6 h before the trial actually began [17].

Dosing

Random research design was in use, where the animals were randomly split into 2 groups, each with 6 animals. The animals in the first group were given 5 ml of aqueous abacavir sulphate solutions and the second group was given a dosage of mucoadhesive buccal film formulations abacavir sulphate. Prior to the experiment, rabbits were given an intramuscular injection of a Xyalzine (1.5 mg/kg) and Ketamine (9.0 mg/kg) to make them unconscious. One-third of the first dose of Xyalzine and Ketamine was injected intramuscularly to maintain the light plane of anesthesia. The rabbit lips were opened using a specifically made mouth restrainer 10 min after the anaesthesia. The mucoadhesive buccal films containing abacavir sulphate were put to the buccal part of the oral cavity with the film side down, moistened with 30 ml of simulated saliva of pH 6.8, and kept firmly in place with a finger above the lip for 30 seconds to assure adherence [18].

Blood sample collection and processing

Up to 10 h, blood samples were collected at regular intervals. Using a 21 G needle, 1 ml of blood was taken from the animals' marginal ear vein in each study at 0.5, 1.0, 2.0, 3.0, 5.0, 8.0, and 10.0 h after dosing. Prior to treatment, blood samples were taken from each rabbit. Blood was collected and spun at 4000 rpm for 4 min at 4 °C in 2 ml centrifuge tubes containing 100 µl of ethylene diamine tetra acetic acid solution (1.0 mg/ml). The obtained supernatant of plasma was stored at-20 °C until the further studies [19].

Sample analysis

Frozen plasma samples were defrosted by leaving the sealed tubes at a temperature of (25±2 °C) for at least 60 min. Acetonitrile was used to precipitate the plasma sample's protein content. 300 µl of ethylene diamine tetra acetic acid solution (1.0 mg/ml). To the obtained supernatant of plasma samples 1.5 ml of acetonitrile was added, and the mixture was vortexed. Next, the mixture was centrifuged (Micro III, Remi India) at 4 °C for 20 min at 13000 rpm. The supernatant was carefully taken and evaporated to dryness using vacuum evaporator. The dried residue was then further reconstituted using a solvent system that contained methanol and phosphate buffer (pH 6.8) at a ratio of 1:4 (% v/v). The samples were then examined using the RP-HPLC analytical technique. The plasma drug concentration was determined at various research time points. By interpolating the peak area of the best formulation on the calibration curve spiked the blank plasma over the range measured, drug concentration was estimated [20].

Pharmacokinetic analysis

Various pharmacokinetic parameters like maximum plasma concentration (C_{max}), maximum plasma concentration (T_{max}), area under the curve (AUC), and biological half-life ($t_{1/2}$) were calculated. C_{max} and T_{max} were directly derived from the plasma concentration-time data. The linear trapezoidal rule was used to calculate the area under the plasma concentration time curve up to the last time (t) displaying a detectable concentration of the analyte ($AUC_{0\text{-}t}$). The $AUC_{0\text{-}\infty}$ values were determined by adding the quotient of *Ct and the appropriate Kel to the corresponding AUC0-t.

$$AUC0 - \infty = \frac{AUC0 - t + * Ct}{Kel}$$

Where *Ct is the last detectable plasma drug concentration.

The apparent elimination half-life ($t_{1/2}$) of drug in plasma was calculated by using the following equation.

$$t_{1/2} = \frac{\ln 2}{Kel}$$

Statistical data analysis

Standard deviation (SD) and the mean were used to express all values. The paired t test was used to compare the pharmacokinetic

parameters after giving the reference standard and the abacavir sulphate mucoadhesive film formulations to healthy rabbits in a single dosage. The paired t test with a probability of P<0.05 was considered significant. The Modern Version 6 software and the PK function (Microsoft Excel add-in) applications were used to conduct the bioavailability test. Microsoft Excel's study Tool Pak add-in was used to conduct the statistical study [21].

RESULTS AND DISCUSSION

An ideal mucoadhesive buccal film should be soft, flexible, compact, mechanically strong, and possess adequate mucoadhesive strength. A combination of HPMC K100M, HPC, ethanol, water and propylene glycol were selected to obtain an optimized, firm, compact and thin mucoadhesive buccal film. The optimized mucoadhesive buccal films broadcasted acceptable qualitative and quantitative parameters [22].

In vivo studies were performed to analyze the pharmacokinetic parameters using white rabbits. During the study, it was observed that all patches remained intact and adhered well to the buccal mucosa of the rabbit. There were also no noticeable signs of any irritation or redness at the sites of application [23].

The HPLC method used for the measurement of the concentrations of abacavir sulphate from plasma was sufficiently sensitive and suitable for the analysis. HPLC chromatograph depicts the retention time of abacavir sulphate and internal standard as 5.68 min and 4 min, respectively (fig. 1).

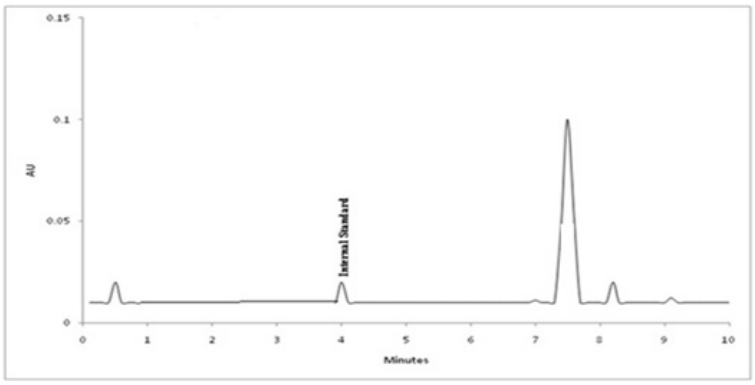

Fig. 1: HPLC chromatogram of abacavir sulphate and internal standard

From the calibration curve, the plasma drug concentrations were determined for each rabbit and the mean plasma drug concentrations were calculated, with a standard deviation for each treatment group, and the drug concentration-time profiles were plotted in fig. 2.

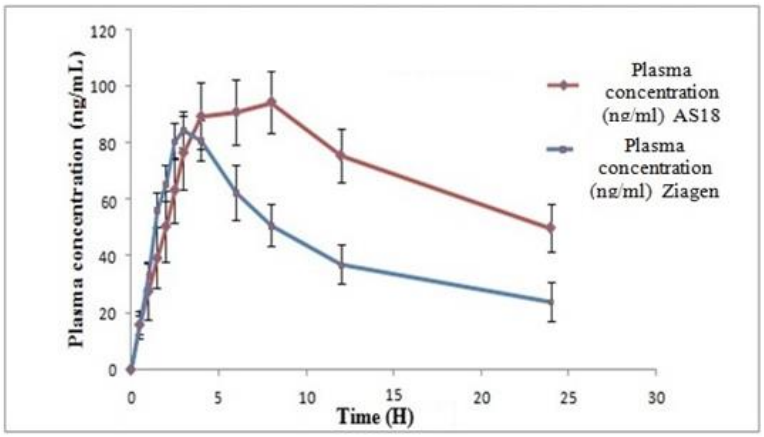

Fig. 2: Mean plasma concentration-time profile of abacavir sulphate buccal films and reference standard (Ziagen tablets)
Note: All the values were expressed in (n=3) mean±SD

The mean plasma concentration, the time profiles following the application of abacavir sulphate buccal films and oral administration of the reference standard (Ziagen tablets) in each group of rabbits, and the pharmacokinetic parameters determined are summarized in table 1.

Table 1: Mean pharmacokinetic parameters of abacavir sulphate buccal films and test reference standard (Ziagen tablets) in rabbits

Pharmacokinetic parameter	Unit	Reference	Test
C_{max}	ng/ml	84.21±0.59	93.86±0.71
t_{max}	h	3±0.12	8±0.9
AUC_{0-t}	ng/ml×h	1023.01±0.87	1652.21±0.67
$AUC_{0-\alpha}$	ng/ml×h	1548.60±0.34	2939.76±0.98
$t_{1/2}$	h	15.38±0.8	17.96±013

All the values were expressed in (n=3) mean±SD

It is evident from fig. 2 that the abacavir sulphate mucoadhesive buccal films plasma level was significantly high ($P<0.05$) upon buccal application throughout the study period (up to 24 h) as compared to the reference standard. In both cases, the absorption was rapid, as evidenced by high plasma drug levels detected (abacavir sulphate buccal films: 84.21±0.59 ng/ml; reference standard: 93.86±0.71 ng/ml), though statistically significant ($P<0.05$). Buccal administration exhibited an increased C_{max} value (93.86±0.71 ng/ml), which was higher than the reference standard (84.21±0.59 ng/ml). These findings suggest that a higher drug concentration was attained when abacavir sulphate is administered via a buccal route. A rapid decline in drug plasma level was noticed in both treatments after the C_{max}, most likely due to the short half-life (1.5 h) of abacavir sulphate. Being a BCS class III drug, the intrinsic permeability of abacavir sulphate was likely to be low, which was evidenced by the low AUC values in both treatments (table 1). However, the observed AUC_{0-t} in abacavir sulphate buccal film was higher than the reference standard administration. The increased abacavir sulphate level in buccal therapy indicated sufficient permeability of the drug via the buccal mucosa [4]. However, oral therapy of abacavir sulphate generally undergoes extensive first-pass metabolism in the liver, thus causing a reduced drug plasma level compared to the buccal route [6]. On the other hand, the T_{max} value was tripled in abacavir sulphate mucoadhesive buccal films when compared to oral standard. These results indicate the ability of the developed film to achieve a higher drug concentration in a prolonged time, and hence, was supposedly a better delivery system to treat vital infections without having multiple doses [7, 24].

These findings suggest the amount of abacavir sulphate reaching the systemic circulation following buccal administration is significantly higher than via the oral route. Furthermore, the buccal route has been shown to prolong the delivery of abacavir sulphate, suggesting these films are able to maintain drug levels in the plasma for a longer period of time and may be useful for prolonging the duration of antiviral therapy [8, 9]. Indeed, the findings of the study were encouraging and substantiate the primary objective of designing a mucoadhesive buccal films-impregnated drug delivery system for the delivery of abacavir sulphate across the buccal mucosa. Mucoadhesive drug delivery of abacavir sulphate gives rapid absorption and good bioavailability due to its considerable surface area and high blood flow [19]. Abacavir sulphate delivery across the mucosa bypasses the first-pass hepatic metabolism and avoiding the degradation of gastrointestinal enzymes [14-17].

The results of the current study also substantiate that the improved bioavailability of mucoadhesive films via buccal application could be due to the transmucosal transport of abacavir sulphate directly into the systemic circulation, compared to the oral route, which shows a relatively lower bioavailability.

CONCLUSION

The numerous experiments could lead to the following inference. The oral bioavailability of the optimized abacavir sulphate mucoadhesive buccal films was observed from the results to be significantly higher when compared to the marketed formulations. The *in vivo* pharmacokinetic investigation was carried out in healthy albino rabbits. The prolonged duration of the dosage form's mucoadhesion mechanism in the buccal area may be the cause of the higher bioavailability.

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Nil

AUTHORS CONTRIBUTIONS

MP carried out the study by collecting data and drafted the manuscript after performing the necessary statistical analysis and in the preparation of the manuscript. RGV aided in the conception of the topic, designing the study and supervision of the study, correction and final approval of the manuscript.

CONFLICTS OF INTERESTS

Declared none

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