EFFECT OF ALUMINUM HYDROXIDE ON PERMEATION OF ACECLOFENAC IN ABSENCE AND PRESENCE OF HYDROXYPROPYL-β-CYCLODEXTRIN THROUGH GOAT INTESTINE: EVALUATION OF THERMODYNAMIC PARAMETERS OF PERMEABILITY

BRAJA B PANDA¹, TAPAN PATEL², SUBRATA MALLICK¹,2*¹

¹Department of Pharmaceutics, ²Department of Pharmaceutical Technology, School of Pharmaceutical Sciences, Siksha ‘O’ Anusandhan University, Kalinganagar, Bhubaneswar-751003, Orissa, India. Email: s_mallickin@yahoo.com; profsmallick@gmail.com

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ABSTRACT

Doctor generally advises to take antacid like aluminium hydroxide to combat gastrointestinal complications during long term oral use of aceclofenac. The purpose of this study was to examine the intestinal permeability potential of aceclofenac upon concomitant use with aluminium hydroxide. Effect of aluminium hydroxide on intestinal permeation of aceclofenac in different ratios in absence and presence of hydroxypropyl-β-cyclodextrin (HBC) has been evaluated. The time-dependent permeation of the drug from aluminium hydroxide dispersion was measured across the isolated goat intestine using organ bath. Thermodynamic parameters such as activation energy (Ea), enthalpy (ΔH), entropy (ΔS) and free energy (ΔG) of activation for the intestinal transport of aceclofenac were evaluated. Permeation of aceclofenac from drug-aluminium hydroxide dispersion increased exponentially with increasing temperature. Permeability of aceclofenac from aluminium hydroxide dispersion in 1:4 ratio has been significantly affected and the same value was also decreased after 1h incubation of the dispersion at 37°C. HBC remarkably improved permeation by protecting the drug through inclusion complexation.

Keywords: Permeability, Drug transport, Aluminium hydroxide, Hydroxypropyl-β-cyclodextrin, Thermodynamic parameters.

INTRODUCTION

Aceclofenac, a phenyl acetic acid derivative non-steroidal anti-inflammatory drug displays good efficacy and tolerability not only in the topical treatment of inflammation in periodontitis but also in the systemic therapy for rheumatic disorders. But the long term oral use leads to gastrointestinal complications like ulceration, perforation and obstruction. To combat these adverse effects of aceclofenac, doctor generally advises to take antacid like aluminium hydroxide.

Aluminium hydroxide interacts with the carboxy and carboxyl groups of quinolone irreversibly and reduces bioavailability of the drug significantly. This is an important factor affecting the intestinal absorption and bioavailability of gatifloxacin upon concomitant use of aluminium hydroxide with gatifloxacin. It is understood from the report that permeation of gatifloxacin was significantly affected in presence of aluminium hydroxide.

An effort has been made here to examine the intestinal permeability potential of concomitant use of carboxyl group containing aceclofenac and aluminium hydroxide which has not been studied earlier. The pharmaceutical industry requires rapid and accurate methods for intestinal permeability potential in the early stages of drug discovery and its formulation development. In this report, we examined the effect of aluminium hydroxide on permeability of aceclofenac in their different ratios through goat intestine ex vivo. Thermodynamic behavior for intestinal permeability was determined to reveal mechanisms of intestinal transport of drug molecules. Mechanisms of carmal drug penetration have been studied by several researchers using thermodynamic approach. The time-dependent permeation of the drug from aluminium hydroxide dispersion was measured across the isolated goat intestine using organ bath at different temperatures. Considerable interest has been generated in the use of cyclodextrins for improvement of chemical stability and bioavailability and also reducing the side effects and toxicity of the drug.

As concomitant use of aluminium hydroxide with aceclofenac supposed to affect drug transport, permeability of the drug from aluminium hydroxide dispersion in presence of hydroxypropyl-β-cyclodextrin (HBC) was also determined to examine any improvement. The activation energy and the enthalpy, entropy and free energy of activation for the movement of aceclofenac have been evaluated.

MATERIALS AND METHODS

Aceclofenac was obtained as a gift sample from Aristo Pharma Pvt. Ltd., Mandideep, India. Aluminium hydroxide was procured from CDH, New Delhi, India (minimum assay 47% (Al₂O₃); pH of solution not more than 1.0; maximum limits of impurities chloride 0.5%, sulfate 0.25%, arsenic 0.0005%).

Krebs Ringer buffer solution

Krebs Ringer buffer solution of pH 7.4 was prepared fresh in distilled water using Analytical Grade Chemicals (containing in mmol/l: 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 2H₂O, 1.2 MgSO₄, 7H₂O, 1.2 KH₂PO₄, 25 NaHCO₃ and 8.3 D-glucose). Calcium chloride was added last in the form of solution in order to prevent the precipitation of bicarbonate. Long time survival of isolated tissue in cloudy physiological solution may be in question and may give an erratic response with drugs.

Tissue preparation

Isolated tissue of the duodenal part of small intestine of goat was collected from slaughter house not later than 1 h and immediately immersed in standard Krebs Ringer buffer solution. The tissue was washed gently with Krebs Ringer solution to remove the mucous and lumen contents and oxygenated continuously (95% O₂ - 5% CO₂) to maintain (at 37°C) the homeostasis of the intestine cells. The tissue was cut into two 8 cm pieces and used for permeation study (one blank without drug and other test with drug).

Ex vivo intestinal permeability studies

The methods employed were modified from experimental procedures as described in the literature. Accurately weighed amount of aceclofenac was dissolved in Krebs solution. The resultant drug solution sample (4 mL of 1 mg/mL) was injected into the lumen of the duodenum (6 cm length exposed for permeation) using a syringe, and the two sides of the intestine were tightly closed. Then the tissue was placed in a chamber of organ bath having accurate temperature regulator and aeration was continued. The receiver compartment was filled with 40 mL of Krebs solution. Samples were withdrawn at regular time interval from the receiver compartment and filtered through a 0.45 µm membrane filter (Whatman Puradisc 25 Nylon, India). Absorbance data were recorded at 273 nm using UV–vis spectrophotometer (JASCO V-630 spectrophotometer, Software: Spectra Manager). Same amount of fresh Ringer solution was replaced to the donor compartment after each sampling. The mean of at least three determinations was used to calculate the cumulative amount of drug permeated using standard calibration curve and the error expressed as standard deviation (mean ± sd, n =
Analysis of experimental data

Calculation of the Apparent Permeability Coefficients

Apparent permeability coefficients \( (P_{app}) \) were calculated according to Eq. 1:

\[
P_{app} = \frac{dQ}{dt} \times \frac{1}{A C_0} \quad \ldots \ldots (1)
\]

Where \( P_{app} \) (cm/s) is the apparent permeability coefficient, \( dQ/Adt \) the amount of drug permeated per unit surface area and per unit of time calculated from the regression line of time points of sampling, \( A \) (cm²) the surface area available for permeation, and \( C_0 \) (µg/ml) the initial drug concentration in the donor compartment.

Activation energy \( (E_a) \) was determined from the regression line of Arrhenius equation:

\[
P_{app} = A \exp \left( \frac{E_a}{RT} \right) \quad \ldots \ldots \ldots (2)
\]

\[
\ln P_{app} = \ln A - \frac{E_a}{RT} \quad \ldots \ldots \ldots (3)
\]

Arrhenius plot of \( \ln P_{app} \) versus \( 1/T \) was constructed where \( T \) is absolute temperature.

Other useful thermodynamic relationships are:

\[
E_a = \Delta H + RT \quad \ldots \ldots \ldots (4)
\]

\[
\Delta G = \Delta H - T\Delta S \quad \ldots \ldots \ldots (5)
\]

\[
\Delta G = -RT \ln P_{app} \quad \ldots \ldots \ldots (6)
\]

Where, \( \Delta H \) and \( \Delta S \) are the enthalpy and entropy of activation, respectively. \( \Delta G \) is the free energy of activation. Using above equations all the parameters was determined.

Statistical analysis

The data are presented as mean ± standard deviation of the mean. The statistical significance was determined by the paired t-test. Significant differences were judged at the \( p < 0.05 \) level.

RESULTS

Intestinal permeability

The cumulative amount of aceclofenac permeated as a function of time in presence of aluminum hydroxide in different ratios (1:1, 1:2 and 1:4 w/w) at 37°C with zero time incubation before permeation for the transport through goat intestine [Ac:AH(1:1); Ac:AH(1:2); Ac:AH(1:4)] has been depicted in Fig. 1. The profiles of aceclofenac alone [Aceclo] and drug-aluminum hydroxide dispersion in presence of hydroxypropyl β cyclodextrin [Ac:AH(1:4)HBC] have been included in the figure to examine the effect of inhibition and recovery in permeation if any. The linear appearance rate \( dQ/dt \) (µg.cm⁻².s⁻¹) of aceclofenac in the receiver side was determined from slope of this plot and used to calculate the \( P_{app} \) (mean ± sd, n = 3). The permeation of aceclofenac has been decreased with the increased amount of aluminium hydroxide. The plot shows a remarkably inhibited effect of permeation in Ac:AH(1:4) and recovery has been noticed in presence of HBC [Ac:AH(1:4)HBC].

Amount of aceclofenac permeated versus time in presence of aluminum hydroxide in different ratios after 1h incubation at 37°C has been presented in Fig. 2.

The profiles of aceclofenac alone and drug-aluminum hydroxide dispersion in presence of hydroxypropyl β cyclodextrin (HBC) have also presented to get a comparative view [Aceclo; Ac:AH(1:1); Ac:AH(1:2)1h; Ac:AH(1:4)1h; Ac:AH(1:4)HBC1h]. The \( dQ/dt \) (µg.cm⁻².s⁻¹) was determined from slope of this plot and used to calculate the \( P_{app} \) (mean ± sd, n = 3) calculated. The plot shows a remarkably inhibited effect (\( p<0.05 \)) of permeation in Ac:AH(1:4)1h and a remarkable effect of recovery (\( p<0.05 \)) in presence of HBC [Ac:AH(1:4)HBC1h].
Fig. 1: Plot of the cumulative amount of aceclofenac permeated versus time in presence of aluminum hydroxide in different ratios for the transport through goat intestine *ex vivo* without incubation before permeation. The profiles of aceclofenac alone and drug-aluminum hydroxide dispersion in presence of hydroxypropyl-β-cyclodextrin (HBC) have presented to get a comparative view (Ac; Ac:AH(1:1); Ac:AH(1:2); Ac:AH(1:4) and Ac:AH(1:4)HBC). The linear appearance rate \( \frac{dQ}{A}.dt (\mu g. cm^{-2}.s^{-1}) \) of aceclofenac on the receiver side was determined from slope of this plot and used to calculate the \( P_{app} \) (mean ± sd, n = 3). The plot shows a remarkable inhibition effect of permeation in Ac:AH(1:4) and a remarkable recovery found also in presence of HBC (Ac:AH(1:4)HBC).

Fig. 2: Plot of the cumulative amount of aceclofenac permeated versus time in presence of aluminum hydroxide in different ratios for the transport through goat intestine *ex vivo* with 1 h incubation before permeation. The profiles of aceclofenac alone and drug-aluminum hydroxide dispersion in presence of hydroxypropyl-β-cyclodextrin (HBC) have presented to get a comparative view (Ac; Ac:AH(1:1); Ac:AH(1:2); Ac:AH(1:4) and Ac:AH(1:4)HBC). The \( \frac{dQ}{A}.dt (\mu g. cm^{-2}.s^{-1}) \) of aceclofenac was determined from slope of this plot and used to calculate the \( P_{app} \) (mean ± sd, n = 3). The plot shows a remarkable inhibition effect of permeation in Ac:AH(1:4) and remarkable effect of recovery also in presence of HBC (Ac:AH(1:4)HBC).
Fig. 3: The time-dependent permeation of aceclofenac at temperatures 23, 30, 37 and 45°C from drug-aluminium hydroxide dispersion (1:4 ratio) after 1 h incubation at 37°C in absence of hydroxypropyl-β-cyclodextrin. The amount of permeation of aceclofenac was enhanced gradually by the rise of temperature.

The time-dependent permeation of aceclofenac from drug-aluminum hydroxide dispersion (1:4 ratio) with 1 h incubation before permeation in absence and presence of hydroxypropyl-β-cyclodextrin (1:1 molar ratio with drug) at temperatures 23, 30, 37 and 45°C is presented in Fig. 3 and Fig. 4 respectively.

Fig. 4: The time-dependent permeation of aceclofenac at temperatures 23, 30, 37 and 45°C from drug-aluminium hydroxide dispersion (1:4 ratio) after 1 h incubation at 37°C in presence of hydroxypropyl-β-cyclodextrin (1:1 molar ratio with drug). Here also the amount of permeation of aceclofenac was enhanced gradually by the rise of temperature.

In both the cases the permeation of aceclofenac was enhanced gradually by the rise of temperature.
Fig. 5: Permeability of aceclofenac through goat intestine from drug-aluminium hydroxide dispersion at different ratios before and after 1 h of incubation at 37° C. Significantly decreased permeability has been observed in presence of maximal amount of aluminium hydroxide [shown bracketed between Ac and Ac:AH(1:4); p<0.05] and significant improvement was also possible in presence of HBC in Ac:AH(1:4)HBC when compared with Ac:AH(1:4) [shown bracketed; p<0.05]. Permeability in presence of HBC after 1h of incubation improved significantly (between Ac:AH(1:4)1h and Ac:AH(1:4)HBC37; p<0.05) and the same has been decreased only slightly in comparison to drug alone (no significant difference between Ac and Ac:AH(1:4)HBC37; p<0.05).

Fig. 5 shows the permeability of drug through goat intestine from Aceclo, Ac:AH(1:1), Ac:AH(1:2), Ac:AH(1:4), Ac:AH(1:4)HBC. In set I run of experiment, permeability value (cm/sec × 10^-6) of Aceclo [1.22 ± 0.021] has been decreased in presence of aluminium hydroxide with zero time of incubation before permeation [1.156 ± 0.036, 1.085 ± 0.045 and 0.818 ± 0.022 in Ac:AH(1:1), Ac:AH(1:2) and Ac:AH(1:4) respectively]. Significantly decreased permeability has been observed in presence of maximal amount of aluminium hydroxide [between Aceclo and Ac:AH(1:4); p<0.05] and significant improvement was also possible in presence of HBC [1.023 ± 0.041 in Ac:AH(1:4)HBC; when compared with Ac:AH(1:4) p<0.05]. In set II run of experiment after 1h of incubation at 37° C permeation value (cm/sec × 10^-6) decreased to 1.090 ± 0.033, 1.050 ± 0.052, and 1.063 ± 0.012 [in Ac:AH(1:1)1h, Ac:AH(1:2)1h and Ac:AH(1:4)1h respectively]. Permeability (cm/sec × 10^-6) in presence of HBC after 1h of incubation [1.123 ± 0.045] improved significantly (between Ac:AH(1:4)1h and Ac:AH(1:4)HBC37; p<0.05] and the same has been decreased only slightly in comparison to drug alone (no significant difference between Aceclo and Ac:AH(1:4)HBC37; p<0.05). The permeability (cm/sec × 10^-6) of aceclofenac from drug-aluminium hydroxide dispersion at temperatures 23, 30, 37 and 45°C in absence and presence of hydroxypropyl-β-cyclodextrin with 1 h incubation at 37° C before permeation [(0.558 ± 0.022) to (0.628 ± 0.018) and (0.992 ± 0.031) to (1.135 ± 0.039) respectively] is presented in Fig. 6. It has been noticed that permeability of aceclofenac was significantly enhanced (p<0.05) in presence of HBC at all the temperatures.

Fig. 6: The permeability (cm/sec × 10^-6) of aceclofenac from drug-aluminium hydroxide dispersion of Ac:AH(1:4) at temperatures 23, 30, 37 and 45° C in absence and presence of hydroxypropyl-β-cyclodextrin with 1 h incubation at 37° C before permeation. It has been noticed that permeability of aceclofenac was significantly enhanced (*; p<0.05) in presence of HBC at all the temperatures.
Evaluation of thermodynamic parameters

Fig. 7 shows Arrhenius plot for the permeability against the inverse of temperature. The permeability increased exponentially with increasing the temperature \(^{11,22,23}\) and the activation energy \( (E_a, \text{kJ} \cdot \text{mol}^{-1}) \) determined for AcAH(1:4)HBC has been increased significantly in comparison to AcAH(1:4) after 1h of incubation (4.96 ± 0.18 and 4.18 ± 0.21 respectively). That means increasing degree of permeability of drug for AcAH(1:4)HBC was significantly more than that for AcAH(1:4) with increasing the temperature.

Entropy is related to the dispersal of a system’s energy and the freedom of motion of its particles at a specific temperature. The increase in entropy of activation \( (\Delta S_a \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}) \) for permeation is the value for amount permeated minus the value for the amount to be permeated at a specific temperature. We found entropy of activation \( (\Delta S_a \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}) \) a negative value of -114 for AcAH(1:4) and -106 for AcAH(1:4)HBC with 1 h incubation before permeation. A negative entropy change contributes to making the free energy change positive for the forward process of permeation and thereby tends to make that process occur spontaneously. No major change in \( \Delta S_a \) was observed with the elevation of temperature. The free energy change \( (\Delta G_a \text{ kJ} \cdot \text{mol}^{-1}) \) for AcAH(1:4) and AcAH(1:4)HBC with 1 h incubation before permeation was positive (35.43 to 37.75 and 34.02 to 36.19 respectively). That means increased permeability for AcAH(1:4)HBC at that temperature (35.43 to 37.75 for AcAH(1:4) and 34.02 to 36.19 for AcAH(1:4)HBC) with 1 h incubation before permeation. It is also temperature dependant. As understood from Eq.(4) \( \Delta G \) was increased with increase of temperature 23, 30, 37 to 45°C.

The free energy change \( (\Delta G \text{ kJ} \cdot \text{mol}^{-1}) \) indicated that the heat is absorbed by the system (endothermic) and it is temperature dependant.

As understood from Eq.(4) \( \Delta H \) was decreased with elevation of temperature 23, 30, 37 to 45°C \([1.72 to 1.53 for \text{AcAH}(1:4) \text{ and } 2.50 \text{ to } 2.32 \text{ for } \text{AcAH}(1:4)\text{HBC} \] with 1 h incubation before permeation. At particular temperature more heat absorption for AcAH(1:4)HBC compared to AcAH(1:4) indicated accelerated permeation for AcAH(1:4)HBC at that temperature. The lower the transfer free energy \( (\Delta G; \text{kJ} \cdot \text{mol}^{-1}) \), higher will be the permeability coefficient and vice versa\(^{25}\). At particular temperature \( \Delta G (\text{kJ} \cdot \text{mol}^{-1}) \) has been decreased for AcAH(1:4)HBC compared to AcAH(1:4) which indicated increased permeability for AcAH(1:4)HBC at that temperature (35.43 to 37.75 for AcAH(1:4) and 34.02 to 36.19 for AcAH(1:4)HBC) with 1 h incubation before permeation.

Aluminum hydroxide has an isoelectric point of 11.4\(^{10,11}\) and will show an alkaline pH not more than 10 in distilled water. The surface of the aluminum hydroxide will be positively charged at particular temperature for AcAH(1:4)HBC indicated more heat absorption for AcAH(1:4)HBC compared to AcAH(1:4) after 1h incubation. Aluminum hydroxide might have interacted to form complex through carboxyl and carbonyl groups of aceclofenac\(^{5}\). The possible assumption is also suggested by some literatures that the interaction between antacids containing polyvalent cations and fluoroquinolones is due to chelation of the ions\(^{27-29}\) and aluminum in particular, forms a very stable complex which is not easily soluble\(^{32}\). Inclusion complexation with beta cyclodextrins can improve chemical stability, bioavailability and also reduce side effects and toxicity of the drugs\(^{3-17}\). Similarly, presence of HBC remarkably improved permeation due to protection of drug by inclusion complexation. Drug-aluminum hydroxide interaction became rate limited compared to permeation rate in presence of HBC.

In this study, we applied a thermodynamic approach by determining individual parameters like activation energy, entropy, and free energy of activation of permeability. Significant increase of \( E_a \) for AcAH(1:4)HBC proved significant improvement of drug permeability facilitated by HBC in comparison to AcAH(1:4) after 1h of incubation. Significant increase of positive values of \( \Delta H \) at a particular temperature for AcAH(1:4)HBC indicated more heat was absorption and accelerated permeation compared to AcAH(1:4). The lower transfer free energy \( (\Delta G) \) proved higher permeability containing polyvalent cations is thought to be due to chelation of antibiotics by the ions\(^{7,27-29}\). In the present study permeation of aceclofenac has been gradually decreased as the aluminum hydroxide amount increased and the permeation has still been remarkably inhibited after 1h incubation at 37°C.

At particular temperature more heat absorption for AcAH(1:4)HBC compared to AcAH(1:4) which indicated increased permeability for AcAH(1:4)HBC at that temperature (35.43 to 37.75 for AcAH(1:4) and 34.02 to 36.19 for AcAH(1:4)HBC) with 1 h incubation before permeation.

DISCUSSIONS

Poor bioavailability of drug may result because of erratic gastrointestinal absorption due to drug-drug interaction. The reduced enteral absorption of quinolones in presence of antacids
The increasing temperature. Presence of HBC remarkably improved and drug-aluminum hydroxide interaction became rate limited. Permeation by protecting the drug through inclusion complexation permeation. The time-dependent permeation of aceclofenac from drug-aluminum hydroxide dispersion increased exponentially with increasing the temperature. Presence of HBC remarkably improved permeation by protecting the drug through inclusion complexation and drug-aluminum hydroxide interaction became rate limited. Thermodynamic parameters such as activation energy, enthalpy, entropy and free energy of activation of permeability have been evaluated to understand mechanisms of intestinal transport of drug molecules when it was particularly affected by aluminum hydroxide.

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