

**PERMEATION STUDIES ON TRANEXAMIC ACID USING ISOLATED
INTESTINAL SEGMENTS OF RAT****Shweta Agarwal***

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Author****Shweta Agarwal**L.R Institute of Pharmacy,
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India.**ABSTRACT**

Permeation studies on Tranexamic acid were conducted as permeation characteristics of the drug were not known. The studies were conducted on different isolated intestinal segments of adult Wistar rats i.e on the duodenum, jejunum and ileum of rats. In a freshly sacrificed rat, the intestinal segments were identified and equal length of each segment was isolated, washed and stabilized in phosphate buffer pH 6.8. Equal length of each segment was ligated at one end and the same volume of drug solution in distilled water having a concentration of 40 mg/ml was filled in the ligated segments and the segments were kept in the aerated inner tube of organ bath having 20 ml phosphate buffer pH

6.8 as receptor medium. Samples were withdrawn from the receptor medium at 15 min time interval for 75 min or till an almost constant value was obtained, and analyzed. Results showed maximum permeation through duodenum and jejunum.

KEYWORDS: tranexamic acid, duodenum, jejunum, ileum, permeation studies.**INTRODUCTION**

Chemically Tranexamic acid is trans-4-(aminomethyl) cyclohexane carboxylic acid. It is a hydrophilic drug and is used as haemostatic and anti-fibrinolytic agent.^[1] It is official in British Pharmacopoeia and European Pharmacopoeia. Tranexamic acid, despite being a hydrophilic drug and having a low molecular weight has a bioavailability of only 30-35% in the dose range of 0.5-2 g.^[1,2] Permeation studies on tranexamic acid were conducted to determine the segmental permeation pattern of the drug and to find out the probable cause of its low bioavailability.

Normally permeation studies are carried out using the everted intestinal sac method, but the disadvantage of this method is that it allows for only one drug measurement per intestinal segment.^[3] So this method was modified by using the uneverted intestinal segments, allowing for repetitive sampling of serosal fluid.

MATERIALS AND METHODS

Tranexamic acid was obtained as gift sample from M/s Mercury Laboratories Pvt. Ltd., Baroda. Shimadzu UV/Vis spectrophotometer (model 1601) with 1 cm matched silica cells was used for analysis and estimation of drug in solution. All the other chemicals were of analytical grade. Phosphate buffer pH 6.8 was prepared as per I.P '96.

Small intestine of a freshly sacrificed adult Wistar rat was removed and its different segments identified. The first 7-8 cm part attached to the stomach was identified as duodenum; next to it was jejunum, which had a slightly thicker wall than duodenum. Ileum constituted the last portion of small intestine after jejunum. All the segments were removed and cleaned. Cleaning was done by passing distilled water with force through the lumen of the intestine to remove all digested and undigested material present. These segments were then kept in phosphate buffer pH 6.8 I.P '96 for 30 min before use for stabilization.

Drug solution of concentration 40 mg/ml was prepared by dissolving 200 mg of drug in 5 ml of phosphate buffer pH 6.8. Then 6 cm measured length of each intestinal segment was taken and ligated at one end with thread. 0.5 ml of the prepared drug solution was filled in each segment using a pipette and then the other end was also ligated. The length of segment between ligations was kept same (i.e 5 cm) for each segment to prevent variation in surface area. These intestinal segments were then suspended in the inner tube of organ bath having 20 ml of phosphate buffer pH 6.8 as the receptor medium and aeration was started. Temperature was maintained at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. 2 ml samples were withdrawn from the receptor medium of each segment at 15 min time interval and the volume withdrawn was replaced with equal volume of fresh buffer to mimic physiological conditions.

The withdrawn samples were filtered through a grade I Whatman filter paper and analyzed for the amount of drug present by a sensitive, colorimetric method developed and validated by Agarwal *et.al.*^[4] The colorimetric analysis method involved the addition of 3 ml of 1,2-naphthoquinone-4-sulphonic acid sodium salt dye solution to a beaker containing 1 ml of the filtered sample to be analyzed and 20 ml of borate buffer pH 9.5 I.P'96. This was heated on a

water bath at $70^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 45 min to develop a orangish-yellow drug-dye complex. The contents were then diluted to 50 ml with 0.1N hydrochloric acid in 50 ml volumetric flask. The absorbance of this solution was taken at 460 nm against reagent blank. From the absorbance obtained the amount of drug present in each sample and in the receptor compartment at different intervals of time was calculated using the calibration curve. The above procedure was repeated thrice for each intestinal segment obtained from 3 different rats. All the data obtained for each segment was expressed as mean \pm SEM.

RESULTS AND DISCUSSION

The results obtained from permeation studies in the form of percent cumulative drug permeated at various time intervals through different segments of the small intestine of rat have been presented in Table 1.

TABLE 1: Percent cumulative drug permeated at various time intervals \pm SEM.

| Time (min) | % cumulative permeation (duodenum segment) | % cumulative permeation (jejunum segment) | % cumulative permeation (ileum segment) |
|------------|--|---|---|
| 15 | 96.9 ± 0.30 | 84.8 ± 0.27 | 66.2 ± 0.24 |
| 30 | 96.5 ± 0.37 | 97.6 ± 0.37 | 76.7 ± 0.26 |
| 45 | $96.8 \pm 0.28^*$ | $97.5 \pm 0.40^*$ | 77.5 ± 0.36 |
| 60 | 96.4 ± 0.39 | 97.6 ± 0.42 | 77.4 ± 0.34 |
| 75 | 96.8 ± 0.40 | 97.5 ± 0.38 | 77.4 ± 0.38 |
| 90 | 96.8 ± 0.32 | 97.2 ± 0.31 | 77.2 ± 0.39 |
| 105 | 96.7 ± 0.27 | 97.3 ± 0.26 | 77.3 ± 0.37 |
| 120 | 96.5 ± 0.35 | 97.1 ± 0.36 | 77.5 ± 0.33 |

Each value is mean \pm SEM ($n=3$). One way ANOVA followed by Turkey test for data at time interval 45 min.*Denotes significant difference when compared to data for ileum at 95% significance level.

The analyzed data obtained from permeation studies of drug solution through different segments of small intestine shows that most rapid permeation occurs through duodenum, although the extent of permeation is less than that in jejunum. In duodenum the percent cumulative of drug permeated is 96.9% at 15 min and then it becomes constant showing that most of the drug has permeated in the first 15 min. In jejunum maximum percent cumulative permeation of drug obtained was 97.6% at 30 min, after which the percent cumulative release becomes constant showing that the rate of permeation is slightly less than that in duodenum but the extent of permeation is greater than in duodenum. In ileum segment maximum

percent cumulative permeation obtained is 77.5% at 45 min and after that it becomes constant, showing that the rate as well as extent of permeation is very less as compared to duodenum and jejunum. All the data are expressed as mean \pm SEM and analyzed by one way ANOVA followed by Turkey's test ($n=3$) using the software Graph Pad Prism version 6.

The results obtained from the study indicate that most of the drug absorption occurs from the proximal part of small intestine i.e from duodenum and jejunum, more so because by the time the drug reaches the ileum segment most of it would have been absorbed in the duodenum and jejunum. The data for duodenum indicate that the percent cumulative drug permeated at time intervals after 15 min. is statistically not significantly different from the amount of drug permeated at 15 min., analyzed by the unpaired t test ($p>0.05$) at 95% significance level. Similar statistical analysis were also carried out for jejunum and ileum showing that the percent cumulative drug permeated after 30 min for jejunum and after 45 min for ileum are not significantly different than the drug permeated at 30 min and 45 min for jejunum and ileum respectively. The data indicate that there is a significant difference in the extent of absorption of the drug at 45 min. for duodenum and jejunum when compared with ileum ($p<0.05$) at 95% significance level. There is no significant difference in the extent of absorption of drug between duodenum and jejunum ($p>0.05$) at 95% significance level, at time interval 45 min. Time point 45 min was chosen for the comparison of data because at this time point the extent of absorption of drug is maximum for all the three intestinal segments (Table 1).

CONCLUSION

From the permeation studies of tranexamic acid carried out on different segments(i.e duodenum, jejunum and ileum) of small intestine of rats, it can be concluded that drug permeates rapidly and to a greater extent through the duodenal and jejunal segments of the small intestine as compared to ileum. Thus it can be concluded that the segmental permeation pattern of the drug is jejunum > duodenum > ileum. The cause of low bioavailability of tranexamic acid, among other factors, could be attributed to its low permeation rate through ileum.

REFERENCES

1. British Pharmacopoeia, Vol.II, London; Her Majesty's Stationery Office: 2005; 1997.
2. Pilbrant A, Schannang M, Vessman J. Pharmacokinetics and bioavailability of tranexamic acid. Eur J Clin Pharmacol., 1981; 20(1): 65-72.

3. Salomon A, Stavchansky M, James MW. Bioavailability in tablet technology. In: Lachman L, Lieberman HA, Schwartz JB (eds.). Pharmaceutical dosage forms-Tablets Vol 2, New York; Marcell Dekker Inc.: 1989; 543-5.
4. Agarwal S, Murthy RSR. A novel and sensitive colorimetric method for estimation of Tranexamic acid in bulk and in pharmaceutical dosage form. Int J Res Pharm Biomed Sci., 2012; 3(2): 947-950.