Bioavailability Of Metronidazole From *In Situ* Gelling And Mucoadhesive Suppositories Formulated With Carbopol ETD 2020

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Abstract

Metronidazole containing granules were formulated with different quantities of Carbopol ETD 2020. The granules were sized and used for rectal suppository formulation using theobroma oil as the base. The suppositories were formulated by pour moulding and some *in vitro* properties such as weight uniformity, liquefaction time, absolute drug content, appearance, mucoadhesive strength and release characteristics were evaluated. The *in vivo* release of metronidazole from the suppositories was studied in rabbits. Results obtained indicated that this polymer could be used to achieve sustained release effect. The plasma concentration time plots also indicated that absorption of metronidazole from the mucoadhesive suppositories actually occurred in the rectum and that sustained effect was achieved.

Key words: Bioavailability, metronidazole, *in situ* gelling, mucoadhesive strength, and suppositories.

Introduction

Bioadhesion is the attachment of synthetic or biological macromolecules to a biological membrane or tissue. A mucoadhesive system adheres and presumably interacts with the mucus layer (Junginger, 1990). When the mucus layer is involved, it is more appropriately called mucoadhesion. This has the ability to increase the contact time of the drug with biological tissue and may be employed in targeted drug delivery or sustained release action (Mortazavi and Smart, 1984). Polymers utilised for this must have good adhesion and must be biocompatible and non-toxic. Such formulations have been used to target genetically engineered molecules (Mikos and Peppas, 1993) and also in the management of glaucoma, insulin delivery, cancer chemotherapy and immunisation (Marcotte *et al.*, 1990). In addition to mucoadhesive action, Carbopols form gels on exposure to aqueous medium. The network of cross-linked aggregates moderates diffusion of the drug giving sustained release action. As the Carbopol swells, the linear distance between cross-links increases allowing drugs to diffuse out. Carbopol ETD 2020 has unique dispersion performance, which allows it to wet quickly, yet hydrate slowly. This behaviour minimises lumping and facilitates drug incorporation during wet granulation.

The study was aimed at evaluating the suitability of Carbopol ETD 2020 granules containing metronidazole as a means of prolonging the release of

metronidazole in theobroma oil suppositories.

Materials and Methods

Theobroma oil (BP grade), metronidazole (M&B) and Carbopol ETD 2020 (B.F. Goodrich) were used as procured from their suppliers without further purification. Other solvents and reagents were of analytical grade and were used as such. Distilled water was obtained from a glass still.

Preparation of suppositories: Five batches of suppositories were prepared employing appropriate quantities of metronidazole and Carbopol ETD 2020, as shown in Table 1. Metronidazole was embedded in the Carbopol ETD 2020 by wet granulation using water as granulating fluid. The damp mass was passed through a sieve of 1.70 mm aperture and dried at 50 °C for 1 h. The dried granules were passed through number 20 mesh (1.0 mm) and were incorporated into the theobroma oil as shown in Table 1. The mixture in each case was formulated into suppository by pour moulding, using a 1 g mould.

Evaluation of the suppositories: The following parameters were employed for the evaluation of the various batches of suppositories.

Appearance: Visual examination of the suppositories was carried out by means of a hand lens to check for the presence or absence of air bubbles, uniformity of mixing, presence of contraction holes and brittle failure. Two suppositories from each batch were examined both externally and internally. In the latter case, the suppositories were cut open longitudinally and examined with a hand lens.

Weight uniformity: From each batch, twenty suppositories were randomly selected and weighed individually and also collectively using an analytical balance (Sauter, KGD 7470 W. Germany). The average weights and coefficients of variation were calculated.

Liquefaction time: The method of Setnikar and Fantelli (1962) was used as follows: One suppository from each batch was wrapped in a transparent polyethylene and the open end tied with thread. The wrapped suppository was introduced into a round bottom flask containing phosphate buffer pH 7.2. This assembly was maintained at 37 ± 1°C by means of a thermo-regulated hot plate. The other end of the thread was attached to a retort stand and by means of this, the suppository was withdrawn for examination. The time taken for the suppositories to melt completely at that temperature was recorded. This determination was repeated several times and the average of the ten determinations taken as the liquefaction time for each batch.

Determination of ex vivo mucoadhesive strength: The mucoadhesive strengths of the suppositories were determined using a tensiometer (A. Kruss model Nr.3124, Germany), adapted to measure mucoadhesive strength. Freshly excised rabbit's rectum was rinsed with normal saline and a portion (2 cm x 5 cm) pinned to the platform of a tensiometer. A 7 min contact time was allowed between glass plate and the mucosa of the rectum. The reading on a microform balance in degrees established the force required to detach the glass plate from the mucosa. The weight of glass beads required to re-zero the tensiometer was noted. A suppository from each

batch was glued to the glass plate using an adhesive and the suppository placed in contact with the mucosa for 7 min to allow for mucoadhesive interaction to take place. A force applied by means of a screw required to detach the suppository from the tissues was determined in degrees and converted to tension using a modified equation of Harkins and Jordan (1930). This was repeated ten times for each batch of suppositories.

$$T = \frac{MgF}{2S} \qquad \text{Eqn. 1}$$

where T is the tension equivalent to mucoadhesive strength, *M* is the mass required to zero the lever *F* is the instrument constant, *S* is the surface area of suppository in contact with the mucous membrane and *g* is the acceleration due to gravity.

S was calculated from the modified equation of Puffer and Crowel (1973)

$$A = \frac{\pi D^2}{2} + \pi DL \qquad \text{Eqn. 2}$$

Where *A*, *D* and *L* are the surface area, diameter and length of suppository respectively. By geometrical calculation, one fifth of the suppository surface area adhered to the mucous membrane. Thus, *S* is given as:

$$S = \frac{1}{5}A \qquad \text{Eqn. 3}$$

Therefore,

$$S = \frac{1}{5}\left(\frac{\pi D^2}{2} + \pi DL\right) \qquad \text{Eqn. 4}$$

Substituting for *S* in Eqn. 1, the tension becomes:

$$T = \frac{M.g.F}{\frac{2}{5}\left(\frac{\pi D^2}{2} + \pi DL\right)} \qquad \text{Eqn. 5}$$

$$T = \frac{5M.g.F}{\pi D^2 + 2\pi DL} \qquad \text{Eqn. 6}$$

Eqn. 6 was used to calculate the tensions equivalent to the mucoadhesive strengths of the suppositories.

Absolute drug content: Twenty suppositories were selected from each batch and weighed together. The average weight was calculated. They were crushed together and an amount equal to the average weight was weighed out from the mass. This quantity was allowed to melt and hydrate in 70 ml of distilled of water at 37 °C for 24 h. The content was made up to 100 ml with the distilled water, filtered and the solution was analysed at 277 nm using a spectrophotometer (SP 6–450 UV/Vis Pye Unicam). The absorbance readings were converted to concentration by reference to the Beer's law plot for metronidazole. This determination was repeated five times for each batch.

In vitro bioavailability studies: The dissolution profile of metronidazole from the suppositories was assessed using the static basket-magnetic stirrer assembly. The dissolution medium consisted of 300 ml of phosphate buffer (pH 7.2) maintained at 37 ± 1 °C. In each case, one suppository from each batch was placed inside the fluid in a basket immersed halfway into the dissolution medium and the magnetic stirrer operated at 100 rev./min. Five millilitre samples were withdrawn at predetermined intervals and diluted appropriately. For each 5 ml withdrawn, 5 ml of phosphate buffer was reintroduced into the dissolution medium. The diluted samples were thereafter analysed spectrophotometrically and the result presented graphically.

In vivo bioavailability studies: Six healthy rabbits of varying sexes weighing between 2.8 kg and 3.5 kg

Table 1: Quantities of ingredients used in suppository formulation

Batch	Theobroma oil (g)	Carbopol ETD 2020 (g)	Metronidazole (g)
A	12.18	1.0	1.4
B	11.68	1.5	1.4
C	10.68	2.5	1.4
D	9.68	3.5	1.4
E	8.18	5.6	1.4
F	12.18	-	1.4

Table 2: Physical properties of the suppositories

Batch	Mean weight (g ± CV)	Drug content (mg ± SD)	Mucoadhesive strength (NM^{-2} ± SD)	Liquefaction time (min ± SD)
A	1.20 ± 1.63	101.8 ± 3.4	36.55 ± 8.13	7.24 ± 2.12
B	1.20 ± 2.55	99.1 ± 1.9	45.92 ± 9.04	7.20 ± 3.11
C	1.22 ± 1.70	101.5 ± 3.2	64.65 ± 10.07	7.30 ± 1.08
D	1.24 ± 1.36	102.4 ± 4.1	55.29 ± 11.11	7.42 ± 2.12
E	1.26 ± 1.40	102.9 ± 2.7	82.47 ± 9.09	8.74 ± 3.24
F	1.22 ± 1.75	103.2 ± 2.1	1.44 ± 10.01	5.01 ± 3.17

Table 3: Result of some pharmacokinetic parameters

Batch	C_{pmax} (ng/ml ± SD)	T_{max} (h)	AUC (ng/ml.h)
A	74.7±11.8	5	130.48
B	85.9±13.2	5	146.00
C	62.0±10.5	4	73.72
D	104.5±14.1	3	149.08
E	33.6±8.9	4	52.60
F	23.9±9.5	4	39.16

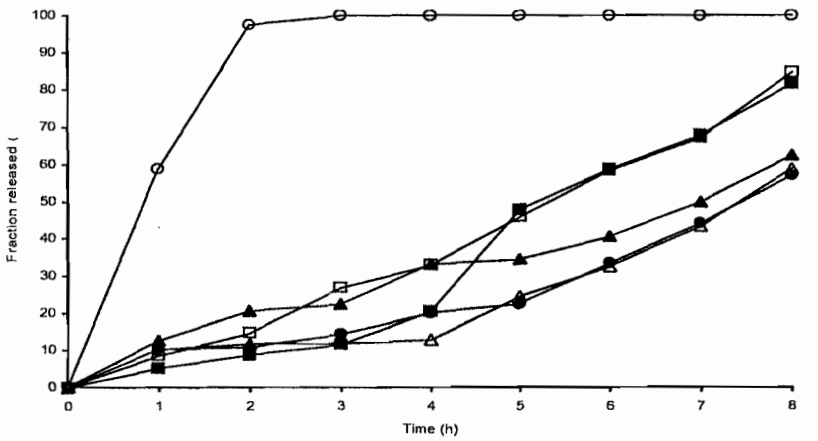

Fig. 1. *In vitro* release profile of metronidazole from the suppositories.

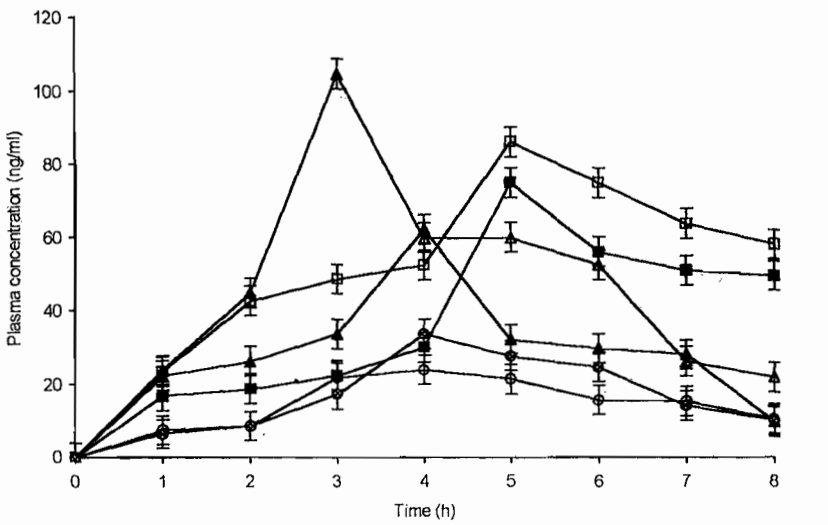

Fig. 2. Plot of average plasma concentration versus time *(n=5)*

were used. Prior to test, the animals were fasted for 15 h but allowed free access to water. This was to allow for total evacuation of rectal content, which would interfere with the *in vivo* mucoadhesion of the suppositories. One suppository was inserted into the rectum of each of the rabbits. The anus was sealed with adhesive tape to prevent the suppository from being expelled. At hourly intervals, blood samples (0.05 ml) were collected from each rabbit's ear vein using sterile syringes; mixed with 0.1 ml of 0.1 M EDTA solution in a test tube and the mixture diluted to 1 ml with distilled water. The blood samples were centrifuged using a centrifuge (Gallenkamp) at 2000 rev/min. The serum was appropriately diluted with distilled water and the drug content analysed spectrophoto-metrically. This experiment was repeated five times for each batch, each time allowing a wash out period of one week.

Results and Discussion

The suppositories were torpedo-shaped with a pointed apex and a blunt base. This is in accordance with earlier recommendations (Black, 1995). This shape would enable forward propulsion of the suppository once the blurred end is inserted. The suppositories were cream coloured, with a smooth exterior. Internal examination on longitudinal cutting showed absence of air bubbles and contraction holes. The suppositories showed fairly uniform weights as shown in Table 2. They had low coefficients of variation (CV). No suppository varied from the mean by more than 5 %. This indicates that the mixture of the granules and the suppository base was very

homogenous. This result fell within the specification required for suppositories (BP, 1998). The absolute drug contents are shown in Table 2. Variations in the drug contents may be due to drug losses on granulation and on partial sedimentation of the granules before pouring, which may also have accounted for the observed variation in weight. Batch F had the highest drug content probably because it did not involve granulation.

Mucoadhesive strength studies showed that the batch containing only theobroma gave lower mucoadhesion than the polymer containing batches. In general, higher Carbopol ETD 2020 quantity in the suppository produced higher mucoadhesive strength. However, batch C gave higher mucoadhesive strength than batch D. This may be due to inadequate hydration of batch D suppositories since it contained more Carbopol ETD 2020. Strong mucoadhesion did not occur since hydration precedes mucoadhesion. The rectum contains little quantity of fluid and this exists together with mucus. As the dosage form melts, the Carbopol granules containing the drug hydrate, and attaches to the mucous surface and as *in situ* gelation occurs, the embedded drug is released. Mucoadhesion also prevents upward displacement of suppository, thus avoiding drug release and absorption into the superior haemorrhoidal vein that would lead to hepatic inactivation of the incorporated drug. With conventional suppositories, there is possibility of the dosage form moving up high in the rectum, hence reducing overall bioavailability. Mucoadhesion also inimizes suppository ingression *in recto*. The liquefaction time of the suppositories fell within the limits specified in European Pharmacopoeia (Ph. Eur., 2001). In the cases of batches A to E, the suppositories melt and the granulations are exposed, and the granules absorb water, gel *in situ* and then release the incorporated drug. For batch F, the drug is released as the base melts. Liquefaction time is analogous to disintegration time of tablets. A formulation that does not liquefy easily may be expelled before drug release occurs and may also exert mechanical irritant action on rectal ampulla (Setnikar and Fantelli, 1962). The theobroma oil batch showed shortest liquefaction time, while batch E with highest content of Carbopol showed highest melting time. This is attributed to the heat resistance of Carbopol, which thus modifies the liquefaction of theobroma oil. Figure 1 shows the release profile of metronidazole from the suppositories. The release of metronidazole was prolonged in all the batches except batch F containing no Carbopol.

This was expected since polymers have been shown to retard the release of incorporated drug. Generally, the higher the concentration of the polymer, the slower the rate of drug release from such a matrix. The *in vivo* bioavailability result is presented in Fig. 2 while Table 3 shows the pharmacokinetic parameters of the bioavailability studies result. The table shows that the batches attained variable concentrations in plasma. Batches C – F showed earlier peaks than those of A and B. Batch F had low bioavailability attaining a maximum plasma concentration of 23.9 ng/ml after 4 h. This low bioavailability of batch F could be due to probable first pass effect due to migration of the suppository to upper parts of the rectum since this batch did not contain any mucoadhesive material or leakage from the rectum. Whereas other batches showed high area under curve (AUC) batches E and F had very small AUCs (Table 3). The poor

bioavailability of batch E suppositories could be attributed to high liquefaction time.

Conclusion

The studies above demonstrated that Carbopol ETD 2020 can be successfully used to modify the pharmacokinetics of metronidazole in rectal suppositories. This mucoadhesive granules exposed by melted suppository would adhere to the mucosa of the rectum and prevent drug absorption into the superior rectal vein. The bioavailability of metronidazole was sufficiently altered to the extent that a sustained release effect was observed.

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