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Dissolution and vaginal absorption characteristics of metronidazole and ornidazole

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The aim of this study was to investigate and compare the dissolution and vaginal absorption characteristics of metronidazole (MTZ) and ornidazole (ONZ) vaginal suppositories. The formulations were prepared by a simple fusion method using Witepsol H15. The solubility, partitioning and dissolution characteristics of these drugs were investigated in phosphate (pH 7) and lactate buffer (pH 4.5) solutions. MTZ and ONZ were labeled with Technethium-99m (^{99m}Tc) and their suppositories were applied to carry out the vaginal absorption and biodistribution studies in rabbits. Scintigraphic images were collected using Sophy DST and DSX gamma cameras. The dissolution of ONZ from the vaginal suppository was slower than that of MTZ at phosphate buffer and similar in lactate buffer. 49% of the administered ONZ dose remained in the rabbit's vagina after 2 h, while this value was calculated as 38% for MTZ. Total activity calculated in uterus and urinary bladder was found as 16% and 22% for MTZ and ONZ, respectively. The biodistribution studies showed that the radioactivity of MTZ in urine and blood was higher than ONZ. The radioactivity of ONZ detected in all organs, especially in uterus, kidneys and urinary bladder, was greater than MTZ. This study determined that the two labeled 5-nitroimidazole derivatives had a high absorbability performance in vagina. MTZ to a large extent transferred to blood and ONZ gathered in lipoid tissues, due to their partition characteristics.

1. Introduction

Intravaginal administration of drugs especially for treatment of *Trichomonas* and *Candida* infections, may lead to a systemic effect due to remarkable absorption of some drugs from the vaginal wall (Slovin and Robinson 1996). Prostaglandins, estrogens are hormones which are rapidly and extensively absorbed through the vaginal epithelium because of its large surface area. This route bypasses first pass metabolism, and reduces or eliminates the gastro-intestinal and hepatic side effects (Bourin et al. 1983; Slovin and Robinson 1996; Vermani and Garg 2000). The vagina offers certain unique features that can be exploited in order to achieve desirable therapeutic effects (Vermani and Garg 2000).

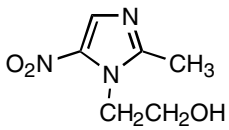
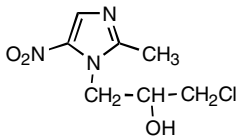
Metronidazole, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (MTZ), the prototype antimicrobial nitroimidazole, was originally introduced to treat *Trichomonas vaginalis* infections (Lopez-Nigro et al. 2003). Common adverse effects of metronidazole involve the gastro-intestinal tract and the neurological system, especially in high doses (Martindale 1996). Ornidazole, 1-(3-chloro-2-hydroxy) propyl-2-methyl-5-nitroimidazole (ONZ) is the chloromethyl analog of metronidazole. It has the same antimicrobial spectrum as MTZ. Thus, it is used similarly in the treatment of susceptible protozoal infections and prophylaxis of anaerobic bacterial infections. ONZ is also well absorbed and distributed after oral administration and its only distinctive feature is its prolonged half life compared with MTZ (Lamp et al. 1999). The chemical features of MTZ and ONZ are given in Table 1.

Radioisotopes have been extensively used in the testing of biodistribution of various pharmaceutical dosage forms (Meseguer et al. 1994; Liu et al. 1997). Nearly 80% of all radiopharmaceuticals used in nuclear medicine are Technethium-99m (^{99m}Tc) labeled compounds. Its half-life is long enough to allow an investigator to carry out radiopharmaceutical synthesis and to collect useful images (Liu et al. 1997).

Hard surface of the tablets and the number of ingredients may lead to irritation of the vaginal epithelium in intravaginal application. The slippery and smooth surface of suppositories may facilitate application, and thus the irritation and burning in the vulvo-vaginal region will be less. Therefore suppository preparations are more suitable dosage forms for vaginal administration.

In this study, the vaginal suppositories of MTZ and ONZ were prepared with Witepsol H15 to compare their *in vitro* drug release properties. They were labeled with ^{99m}Tc in order to investigate the vaginal absorption and biodistribution in rabbits.

Table 1: Chemical features and n-octanol/aqueous buffer partition coefficients of MTZ and ONZ

Compd.	Empirical formulas Molecular weight	Solubility (mg/ml)	* P_o
		Lactate buffer Phosphate buffer Water	Lactate buffer ± SD Phosphate buffer ± SD Water ± SD
Metronidazole	 $(C_6H_9N_3O_3)$ 171.15	13.4 ± 7.6 11.3 ± 5.3 12.3 ± 5.1	0.7 ± 0.1 0.5 ± 0.2 0.6 ± 0.1
Ornidazole	 $(C_7H_{10}ClN_3O_3)$ 219.63	22.8 ± 1.6 21.9 ± 7.8 22.5 ± 5.6	0.8 ± 0.4 0.7 ± 0.1 1.7 ± 0.1

* Partititon coefficient (P_o) studies were carried out with six samples for each of the drug substance in water, lactate (pH 4.5) and phosphate buffer (pH 7) solutions in a concentration at about 10^{-2} M. *n*-octanol: buffer solution ratio was kept as 1 for all P_o studies. Values are means ± standard deviation (SD)

2. Investigations, results and discussion

Solubility plays a prime role in the dissolution of a drug substance from a solid dosage form. In this paper, solubility studies were performed to determine the maximum amount of the dissolved ONZ and MTZ, in water and the buffer solutions. The results are given in Table 1. According to these results, ONZ were found to be better soluble than MTZ in water, lactate and phosphate buffer solutions. Our findings were concordant with the study of Schwartz and Jeunet (1976).

Lipophilicity is a very useful physicochemical parameter reflecting the transfer properties of a compound. This can be described by the partition coefficient (P_o), which is defined as the ratio of concentrations of a compound in all its forms between an aqueous phase (buffer) and an oil phase. In particular, the *n*-octanol/buffer partition coefficient is commonly used to reflect the lipophilicity of a potential drug compound (Comer and Tam 2001). Our results indicated that ONZ had more affinity to *n*-octanol as lipophilic media, when compared to MTZ in all solutions (Table 1). The investigations of Martin et al. (1990) also demonstrated that ONZ was the most lipophilic derivative among nitroimidazole type antimicrobials. On the other hand, the P_o of ONZ was noticeably increased in water compared to pH 4.5 lactate and pH 7 phosphate buffers.

The drug release from suppositories and subsequent absorption involves several stages, starting from suppository melting or softening at body temperature, followed by drug migration through the suppository mass and its transfer from suppository surface to the environment, and finally drug solubilization in biological fluids and drug permeation across membranes. The excipient properties can affect not only the rate, but also the extent of absorption (Chicco et al. 1999). In many studies, Witepsol H15 was found suitable for the drug release from suppository formulations (Dimitrova et al. 2000; Takatori et al. 2004). It is a standard suppository base that is commonly used as a lipophilic base with a melting range between 33.5–35.5 °C and a hydroxyl value of 5–15, possessing several advantages (Berko et al. 2002). It is designed to minimize the sedimentation of active materials but rapid cooling should be avoided. Therefore in this study, Witepsol H15 was used. The unlabeled suppositories had the average weights of 3.02 ± 0.01 g for MTZ and 3.011 ± 0.02 g for ONZ. The labeled suppositories prepared for *in vivo* biodistribution studies had the average weights of 0.65 ± 0.02 g for MTZ and 0.64 ± 0.01 g for ONZ. The unlabeled and labeled formulations had the average heights of 2.50 ± 0.01 cm and 1.5 ± 0.02 cm; and thickness of 1.5 ± 0.02 cm and 0.65 ± 0.02 cm, respectively.

The physiologic parameters of the specific region of interest should be taken into consideration in experimental design. The vaginal pH of a healthy woman of reproductive age is acidic (pH = 4–5); this value is maintained by *Lactobacilli* that convert glycogen from exfoliated epithelial cell into lactic acid (Richardson and Illum 1992; Castle et al. 2001). However, factors such as stages of menstrual cycle, aging and sexual activity can affect vaginal pH, which can also be elevated up to 7 during bacterial vaginosis and vaginal infections (Milani et al. 2000). Therefore, the dissolution studies were conducted in lactate (Parrot 1988) and phosphate (Nobilis et al. 2001) buffers to simulate the healthy and infected states.

Suppository drug release is influenced by factors such as drug-vehicle interactions, solubility, particle size of drug and drug concentration in the vehicle (Roseman 1981). The hardness of the vaginal suppositories also plays an important role in the release of drugs. The hardness values were determined as 2.23 and 2.26 kg for MTZ and ONZ unlabeled vaginal suppositories, respectively. As can be seen from hardness values, there was no significant discrepancy between the two formulations. The dissolution data of MTZ and ONZ vaginal suppositories in pH 7 phosphate and pH 4.5 lactate buffers using USP 24 basket method are seen in Figs. 1 and 2. Our studies showed that the release profiles of MTZ and ONZ were similar in lactate buffer. However the released amount of MTZ in phosphate buffer was higher than ONZ. This condition could be explained by the P_o obtained (Table 1). The P_o results of MTZ and ONZ were evaluated with one way ANOVA

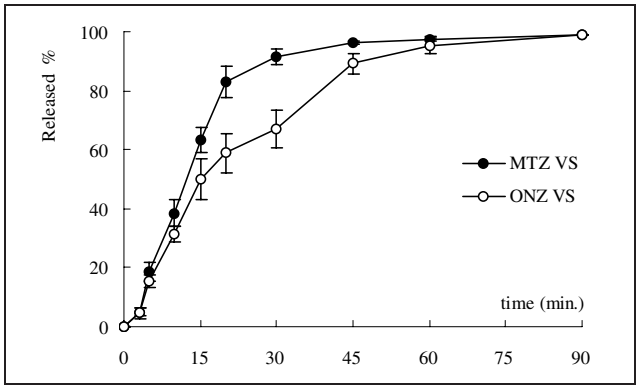


Fig. 1: Dissolution profiles of 500 mg metronidazole and ornidazole released from vaginal suppository formulations using USP 24 basket method, at 900 ml, pH 7 phosphate buffer at 37 ± 0.5 °C. The rotation speed is 100 rpm. All samples were run in six times. Values are means ± SD, in some cases the error bars are smaller than the symbols. (MTZ VS: Metronidazole vaginal suppository; ONZ VS: Ornidazole vaginal suppository)

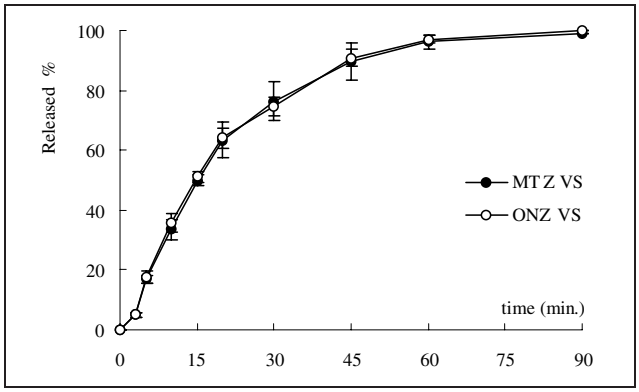


Fig. 2: Dissolution profiles of 500 mg metronidazole and ornidazole released from vaginal suppository formulations using USP 24 basket method, at 900 ml, pH 4.5 lactate buffer at 37 ± 0.5 °C. The rotation speed is 100 rpm. All samples were run in six times. Values are means ± SD, in some cases the error bars are smaller than the symbols. (MTZ VS: Metronidazole vaginal suppository; ONZ VS: Ornidazole vaginal suppository)

and it was found that the P_o of MTZ and ONZ was significantly different in water and pH 7 phosphate buffer. However, no difference was obtained in pH 4.5 lactate buffer (Table 2). The dissolution profile did not change for ONZ in either buffer, but a significant increment was determined for MTZ release in phosphate buffer. As a result of comparatively low P_o value of MTZ in phosphate buffer than lactate buffer, a faster drug release was obtained from vaginal suppositories (Fig. 1). The dissolution results of MTZ and ONZ were evaluated with independent-t test and a significant difference was noticed in phosphate buffer solution when compared statistically ($P < 0.05$). All the results of dissolution data are listed in Tables 3 and 4.

The imaging studies give the opportunity to view the *in vivo* release characteristics of the formulations (Aşıkoglu et al. 1995; Özyazıcı et al. 2003). Rabbits are suitable models for the investigation of vaginal drug delivery because, in the absence of male contact, the histology of the vaginal epithelium should be quite constant, resulting in expected minimal variability in membrane permeability behavior (Acartürk and Robinson 1996). Therefore we decided to carry out imaging studies in rabbits with labeled active substances to investigate the *in vivo* absorption and biodistribution characteristics for vaginal suppository formulations. Vaginal absorption and bioavailability of MTZ from different dosage forms were studied by some authors (Buttar and Siddiqui 1976; Bourin et al.

Table 2: One way ANOVA test results of partition data obtained from MTZ and ONZ in water, pH 4.5 lactate and pH 7 phosphate buffer solutions ($P < 0.05$)

Medium	Group	Mean	Std. deviation	P
Water	MTZ	0.689	9.82×10^{-4}	0.000*
	ONZ	1.719	2.21×10^{-2}	
Lactate	MTZ	0.788	2.00×10^{-3}	0.198
buffer	ONZ	0.879	8.25×10^{-2}	
Phosphate	MTZ	0.560	2.04×10^{-2}	0.000*
buffer	ONZ	0.751	7.37×10^{-3}	

P = Symp. Sig. (2-tailed)
Symbol (*) indicates a significant difference: $P < 0.05$

Table 3: Independent t-test results of dissolution data obtained from MTZ and ONZ formulations in pH 4.5 lactate buffer solution ($P < 0.05$)

Time	Group	Mean	Std. deviation	P
3 min	MTZ	4.935	0.640	0.808
	ONZ	5.043	0.845	
5 min	MTZ	16.851	1.413	0.466
	ONZ	17.651	2.167	
10 min	MTZ	33.502	3.305	0.267
	ONZ	35.725	3.239	
15 min	MTZ	49.862	1.733	0.252
	ONZ	51.141	1.910	
20 min	MTZ	63.403	5.959	0.780
	ONZ	64.207	3.372	
30 min	MTZ	76.328	6.570	0.535
	ONZ	74.422	3.100	
45 min	MTZ	89.522	6.344	0.648
	ONZ	90.869	2.963	
60 min	MTZ	96.238	2.238	0.435
	ONZ	97.096	1.291	
90 min	MTZ	100	0.00	1.000
	ONZ	100	0.00	

P = Symp. Sig. (2-tailed)

Table 4: Independent t-test results of dissolution data obtained from MTZ and ONZ formulations in pH 7 phosphate buffer solution ($P < 0.05$)

Time	Group	Mean	Std. deviation	P
3 min	MTZ	4.625	1.760	0.742
	ONZ	4.937	1.423	
5 min	MTZ	18.546	3.339	0.066
	ONZ	15.221	2.107	
10 min	MTZ	38.448	4.650	0.009*
	ONZ	31.426	2.502	
15 min	MTZ	63.102	4.315	0.003*
	ONZ	49.988	6.920	
20 min	MTZ	82.907	5.138	0.000*
	ONZ	58.816	6.526	
30 min	MTZ	91.524	2.505	0.000*
	ONZ	67.198	6.317	
45 min	MTZ	96.251	0.530	0.004*
	ONZ	89.184	3.562	
60 min	MTZ	97.450	0.877	0.101
	ONZ	95.298	2.786	
90 min	MTZ	100	0.00	1.000
	ONZ	100	0.00	

P = Symp. Sig. (2-tailed)
Symbol (*) indicates a significant difference: $P < 0.05$

1983; Buttar 1985; Fredricsson et al. 2001; Özyazıcı et al. 2003). However, Witepsol H15 suppositories containing labeled MTZ and ONZ were investigated comparatively for the first time in this study.

Ligand exchange method was used to label MTZ and ONZ with ^{99m}Tc. Unbound $^{99m}TcO_4$ stayed at the origin. The bounding efficiency was found to be approximately 90% for both of the drugs. The scintigraphic images of rabbits were collected by the SOPHY DSX gamma camera. The images taken at predetermined time intervals are seen in Figs. 3 and 4.

Scintigraphic images showed that, ^{99m}Tc labeled MTZ and ONZ were stable in the matrix until the end of 20th min (Figs. 3 and 4). Both of the drugs showed a slight distribution after 30 min. It was thought that the lag time of the drug distribution might depend on the melting period of Witepsol H15 (suppository base) in the vaginal site, at 37 °C. Beginning with the 60th min, the labeled MTZ and ONZ compounds passed through vagina and gathered in uterus. The systemic effect of ^{99m}Tc labeled drugs could be observed due to the visualization of the kidneys. From this viewpoint, it could be appeared that the systemic effect of MTZ started faster than that of ONZ (Fig. 3). The migration of MTZ to kidneys and urinary bladder was observed earlier than for ONZ. The migration of drugs was completed after 90 min and from 90th min to 120th min the radioactivity was fixed in the urinary bladder. It was also noticed that the liver uptake was less than the kidneys for both of the labeled drugs. It could be assumed that labeling MTZ with ^{99m}Tc formed a complex that has the solubility and molecule size still enough to diffuse through the vascular structures. The labeled ONZ appeared to be larger in molecule and less soluble when compared to MTZ.

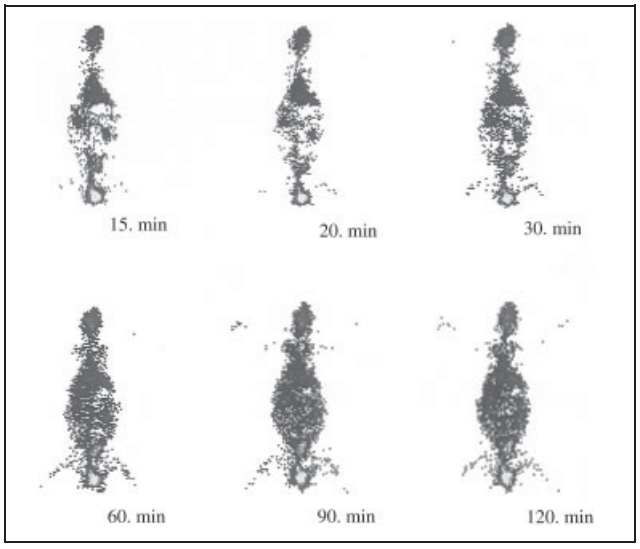


Fig. 3: Scintigraphic images of ^{99m}Tc labeled metronidazole collected at 15, 20, 30, 60, 90 and 120th min. from female, New Zealand rabbit weighing approximately 2.5–3 kg

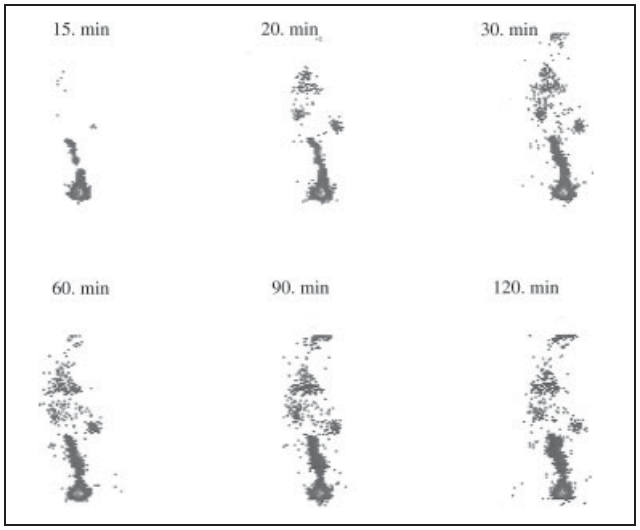


Fig. 4: Scintigraphic images of ^{99m}Tc labeled ornidazole collected at 15, 20, 30, 60, 90 and 120th min. from female, New Zealand rabbit weighing approximately 2.5–3 kg

Cicinelli et al. (1998) supposed that a radiotracer is absorbed through the vaginal mucosa, raising its concentration in the paravaginal spaces, lymph and vaginal venous vessels and reaching ultimately the systemic circulation. In another study, in which ^{99m}Tc-pertechnetate was used, extra pelvic uptake did not occur until at least 30 min later and uterine activity was detectable approximately 2 h after vaginal administration (Cicinelli et al. 2001). As a result of our imaging studies, it could be assumed that the vaginal route of administration created a distribution to uterus and urinary bladder for MTZ and ONZ (Figs. 3 and 4). Theoretically, four different mechanisms could explain this phenomenon: 1 – direct (passive) diffusion through tissues, 2 – passage through the cervical lumen from the vagina to the uterus, 3 – venous or lymphatic circulatory systems, 4 – countercurrent vascular exchange with diffusion between utero-vaginal veins and/or lymph vessels and arteries (Cicinelli and Ziegler 1999).

In addition to scintigraphic imaging studies, biodistribution studies were conducted to clarify the *in vivo* fate of the drugs. The calculations in our study were made by 'Region of Interest Process' (ROI), considering the initial count rate of one suppository as 100%. The other count rates were compared with the initial values. The obtained values were evaluated to estimate biodistribution rate.

49% of the administered ONZ dose remained in the rabbit's vagina after 2 h. This value was calculated as 38% for MTZ, according to the activity detected from suppositories and surrounding tissues. MTZ is faster absorbed from vaginal epithelium than ONZ. A greater penetration of drug is expected when there is less affinity of the drug for the base of the suppository formulation. If there is little affinity between the drug and the base, the drug will liberate and the permeation rate will be high (Özyazıcı et al. 2003). Therefore, it was thought that the lower absorption of ONZ detected from the vaginal site might be due to the stronger affinity between the base (Witepsol H15) and ONZ, compared to the affinity between the base and MTZ.

Labeled ONZ and MTZ showed high radioactivity in urine at the end of 2 h (Figs. 3 and 4). The amount of radioactivity for 1 g of the urinary bladder was multiplied with the whole organ weight when the urinary bladder was empty. There was approximately 10 g urine in the bladder. MTZ had faster accumulation in urine and showed high drug levels in blood and urine, compared to ONZ. Biodistribution data for ^{99m}Tc labeled MTZ and ONZ following administration are shown in Fig. 5.

It is known that free ^{99m}Tc which is a little molecule gathers in the gastric and thyroid region by the systemic circulation due to high penetration activity (Cicinelli et al. 2001). These data showed that labeled drugs were slightly metabolized and the unmetabolized labeled drugs gathered particularly in the urine. MTZ is metabolized in the liver

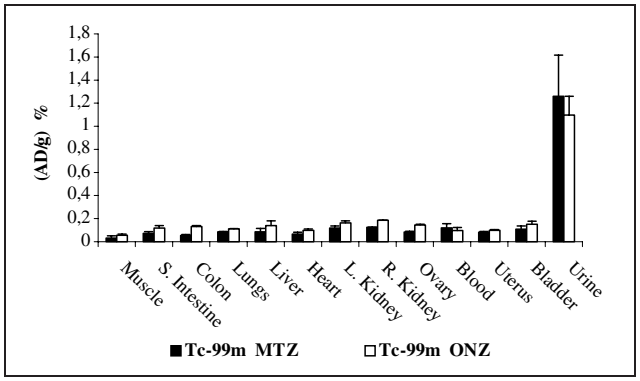


Fig. 5: The biodistribution of ~1mCi ^{99m}Tc labeled, 100 µg MTZ and ONZ administered to rabbits by vaginal route. The data represents the ratio of the organs activity to the total radioactivity of the vaginal suppository by ROI process. The average of the% applied dose (AD%) of ^{99m}Tc labeled MTZ and ONZ per 1 g of organ according to organs in rabbits after 120 minutes was shown. The data points with error bars are means of mean applied dose% ± SD, with n = 6, (in some cases the error bars are smaller than the symbols)

mainly by oxidation and the parent compound is recovered from urine (Jessa et al. 1996). More recently, urinary excretion of MTZ metabolites in human was found to be considerably lower after vaginal administration than after intravenous or oral administration which is an evidence for the fact that less drug passed into the blood circulation after vaginal administration (Fredricsson et al. 2001).

The radioactivity of labeled ONZ was higher than that of labeled MTZ when all organs were calculated (Fig. 5). Especially ONZ radioactivity obtained from kidneys, ovaries, liver, column and small intestine were significantly higher. Total activity calculated with the ROI process, in uterus and urinary bladder was found as 16% and 22% for MTZ and ONZ, respectively. In the study of Yang et al. (1999), ^{99m}Tc labeled MTZ was administered by i.v. route and high activity (5.84%) was detected in kidneys 2 h later. In the study of Aşıkoglu et al. (2000) 1, 3, 5 and 24 h after i.v. injection of ^{131}I-ONZ to rats, the accumulated radioactivity was counted for kidneys as 2.6, 4.4, 3.1 and 0.8% ID/g, respectively. In our study, in which the drug is applied vaginally, the average of the % applied dose of, ^{99m}Tc labeled MTZ and ONZ per 1 g of kidneys was detected as 0.242 and 0.348, respectively.

In conclusion, the findings of our biodistribution studies revealed that MTZ had affinity not only to lipophilic media like organs, but also to hydrophilic media like urine and blood. The affinity of drugs to hydrophilic media could increase their elimination rate. The elimination process begins via kidneys and liver, when drug passes to blood and urine. This characteristic might lead the drug to show a fast elimination. Moreover, the wide distribution of ONZ observed especially in organs (like liver, kidneys, colon etc.) might be caused by the high affinity of the drug. The results gained from previous studies showed that the tissue elimination of ONZ is a slow process due to its lipophilicity and this is in compliance with our dissolution and partition studies (Martin et al. 1990; Condomines et al. 1988).

MTZ and ONZ suppositories prepared with Witepsol H15 used for treatment of local vaginal infections could also be effective in surrounding tissues and organs. The use of ONZ suppositories could be more suitable at pH 7 when slow absorption is required. Targeting and dosing the drugs might be controlled by the development of novel formulations.

3. Experimental

3.1. Materials

MTZ was obtained from İ.E. Ulagay İlaç San. ve Tic. A.Ş. (Turkey). ONZ was gifted from Fako Pharmaceutical Company. Witepsol H15 was obtained from Deva Ilac San. ve Tic. A.Ş., (Turkey). A PharmaTest PTWII dissolution apparatus was used for dissolution studies. The spectrophotometer was Shimadzu UV-1208. ^{99m}Tc pertechnetate, was eluded from molybdene-99/technetium-99m generator (CIS bio-international, France). Pyrophosphate was obtained from SIGMA (Germany). ITLC and PC (Instant Thin Layer and Paper Chromatography) strips were obtained from Gelman Sci. ITLC-SG, Ann Arbor, MI; Whatman 31ET (Maidstone, UK), respectively. Gamma cameras were Sophy DST and DSX gamma camera (Sopha Medical) and Velp Scientifica magnetic stirrer was used. All the other materials were of analytical grade.

3.2. Solubility studies

The solubility of ONZ and MTZ was determined in water, pH 4.5 lactate (Parrot 1988; Gjellan and Graffner 1994) and pH 7 phosphate buffer solutions (Nobilis et al. 2001). Excess amount of active substances and 10 ml of media were placed in glass vials maintained at 37 ± 0.5 °C on a magnetic stirrer at 400 rpm (Variomag-EC) for 24 h. At appropriate time intervals, aliquots were taken from buffer solutions and filtered through a 0.4 µm filter. Samples were diluted and assayed spectrophotometrically (Shimadzu UV-1208) at 320 nm. Calibration curves were used to determine the amount of solubility. The results are the average of six separate experiments.

3.3. Partition coefficient determination

The partition coefficient (P_o) determination experiments (Gao et al. 2005) were carried out in aqueous phosphate (pH 7) and lactate (pH 4.5) buffer solutions for both MTZ and ONZ, separately. The buffer solutions were presaturated with *n*-octanol. The drug substances were dissolved in these buffer solutions at concentrations of about 10^{-2} M. The buffer phase:*n*-octanol ratio was adjusted to 1 : 1. Six vials for each of the drug substances were sealed and agitated in a magnetic stirrer for about 6 h, at 600 rpm. After stirring the vials the phases were separated by centrifugation at 3000 rpm for 20 min (Kim et al. 2001; Mrestani et al. 2004). UV–Vis absorption spectra of the compound at the aqueous phase before and after partition were collected. The P_o value was calculated by the following Eq. (1).

$$P_o = \left\{ \left(\frac{A_1 - A_2}{A_2} \right) \left(\frac{V_w}{V_o} \right) \right\} \quad (1)$$

where A_1 and A_2 represent the UV–Vis absorption value of the compound at the aqueous phase before and after partition, respectively, while V_w/V_o represents the aqueous phase : *n*-octanol phase ratio. The aqueous phase : *n*-octanol ratio employed was kept the same. Samples, before and after partition, were quantified by using UV–Vis spectrophotometry, at 320 nm. The partition coefficient in water was also determined according to the method mentioned above.

3.4. Labeling of metronidazole and ornidazole with ^{99m}Tc

Labeling of MTZ and ONZ with ^{99m}Tc was accomplished by the Ligand exchange method (Deckart and Cox 1987). Therefore, 50 mg pyrophosphate was dissolved in 2 ml bidistilled water, and 0.4 ml $SnCl_2 \cdot 2\,H_2O$ (10 mg/10 ml) solution was added to pyrophosphate media, then pH was adjusted to 7.0. After preparing pyrophosphate (PYP) and stannous mixture solution, freshly eluded ($^{99m}TcO_4$) – pertechnetate was added to this mixture and was left for incubation for 10 min. When ^{99m}Tc-PYP labeled compound occurred (Checked by W31ET/SF PC chromatography), conditions were ready for exchange reaction. Then 20 mg of each drug were dissolved in 2 ml bidistilled water and the solution was added to the previous reaction mixture, separately. New reaction composition was put in the boiling water bath in special lead shield for 10 min. After heating, the vial was cooled to room temperature. The radiochemical purity was confirmed with instant thin-layer chromatography. A small amount of mixture was taken from vial to syringe and dropped to origin of the (1 × 10 cm) W31ET/SF chromatography strips to check the profile of bound MTZ and ONZ with ^{99m}Tc; in the same way an ITLC-SG/acetone system was used to check the amount of unbound ^{99m}Tc part (Robbins 1984). Static images were obtained from strips for 15 min, with a gamma camera and the ROI (Region of Interest) process was performed on images of chromatography strips. By the ROI process, special data were obtained from strip images and chromatographic profile and bonding efficiency was easily calculated (Steves 1996; Taner et al. 2000).

3.5. Preparation of the vaginal suppositories

All unlabeled vaginal suppositories containing 500 mg of the drug were prepared by the fusion method with Witepsol H15. The fusion method was also accomplished in composition of the labeled dosage forms. 100 µg

MTZ and ONZ labeled with ^{99m}Tc, ~1 mCi for each, were used to prepare vaginal suppositories. The drugs were mixed thoroughly in the melted Witepsol H15 separately, and the resultant mixture was poured into moulds and solidified at +4 °C in the refrigerator. The average weights, heights, thickness and hardness (by Erweka supporitory hardness tester) of the formulations were measured.

3.6. Dissolution tests

The unlabeled suppositories were examined using the basket (Apparatus I, USP 24) method at 37 ± 0.5 °C in terms of dissolution rates. The rotation speed was 100 rpm and the dissolution medium was 900 ml, pH 4.5 lactate and pH 7 phosphate buffer solutions at 37 ± 0.5 °C (Ondracek et al. 1988; Gjellan and Graffner 1994).
For all dissolution tests, filtered samples of dissolution medium were taken at predetermined time intervals and the drug content was determined spectrophotometrically against blank, at 320 nm for MTZ and ONZ. The averages of the released amounts of six samples were calculated.

3.7. Application of vaginal suppositories in rabbits and imaging studies

Animal investigations were carried out with the approval of the Ethical Committee for Animal Research, University of Ege (Document No: 2001-04). The single dose suppositories of labeled MTZ and ONZ prepared with Witepsol H15 were administered intravaginally to six, white, New Zealand female rabbits weighing 2.5–3 kg, and radiation activity was evaluated with a gamma camera. Six animals were placed in a supine position under the detector of the camera. Scintigraphic images were collected from the initial value of the radioactivity at 15, 20, 30, 60, 90 and 120 min. At the same geometry the images were monitored using SPECT gamma fitted with a low energy, high-resolution parallel hole collimator.

3.8. Biodistribution studies

Six, white, New Zealand female rabbits, weighing 2.5–3 kg were used for biodistribution studies. After the imaging studies were completed, the rabbits were sacrificed with an overdose of anesthetic agent. The organs (muscle, intestine, colon, lungs, liver, heart, kidneys, ovary, uterus, and bladder) were dissected, weighed and their radioactivity was measured using a SOPHY DSX gamma camera. The results were expressed as percent of administered dose per gram tissue (AD/g%).

3.9. Statistical analysis

The results of dissolution data obtained in each formulations were subjected to statistical analysis using independent-t Test. The partition coefficient data analysis was conducted with one way ANOVA. The chosen level of significance was $P < 0.05$ for both statistical analysis.

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