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THE INVESTIGATION OF *IN VITRO* AND *IN VIVO* RELEASE OF ^{131}I-ORNIDAZOLE FROM POLYETHYLENE GLYCOL-6000 SUPPOSITORIES

POLİETİLEN GLİKOL SUPOZİTUVARLARDAN *İN VİTRO* VE *İN VİVO* ^{131}I-ORNİDAZOL SALIMININ İNCELENMESİ

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In this study, ornidazole and polyethylene glycol-6000 (PEG), a suppository base, were labeled with ^{131}I and ^{99m}Tc, respectively. The rectal suppositories containing either labeled or unlabeled ornidazole were prepared by fusion method using PEG 6000. The in vitro release of ^{131}I-ornidazole from ^{99m}Tc-labeled PEG 6000 was investigated in intestinal fluid at 37C. The rotating rate of stirrer was 100 rpm. Gamma peaks of ^{99m}Tc and ^{131}I were counted by NaI(Tl) scintillation detector and the in vitro release profiles were plotted. In addition, the release profile of unlabeled ornidazole was obtained spectrophotometrically. All the release profiles obtained were compared and evaluated. On the other hand, to investigate the in vivo release, ^{131}I-labeled ornidazole suppositories were administered by rectal route to rabbits. Scintigraphic images were taken at predetermined time intervals. In vivo release results were compared with in vitro results and a good correlation was obtained.

Bu çalışmada, ornidazol ^{131}I, *polietilen glikol ise ^{99m}Tc ile işaretlendiler. İşaretli olan ve işaretli olmayan ornidazol içeren suppozituvarlar PEG kullanılarak hazırlandılar. ^{99m}Tc ile işaretlenen PEG sıvağından ^{131}I ile işaretli ornidazolün in vitro salımı bağırsak sıvısında 37C ve 100 devirdakika dönüş hizinda incelendi. ^{99m}Tc ve ^{131}Iun gama pikleri NaI (Tl) sintilasyon dedektöründe sayıldı ve ornidazolün in vitro salım profilleri oluşturuldu. Ayrıca, işaretli olmayan ornidazolün in vitro salımı aynı dissolüsyon ortamı kullanılarak spektrofoto-metrik olarak da incelendi. Elde edilen tüm salım sonuçları birbiriyle karşılaştırıldı ve değerlendirildi. ^{131}I işaretli ornidazol supozituvarlar tavşanların rektumlarina uygulandı. Belirli zamanlarda sintigrafik görüntüler alındı. İn vivo salım sonuçları ile in vitro salım sonuçları karşılaştırıldı ve iyi bir korelasyon bulunduğu gözlendi.*

Keywords: *Radiolabeling; Ornidazol; Iodine-131; Technetium-99m; Release studies; In vitro-in vivo correlation*

Anahtar kelimeler: *Radyoişaretleme; Ornidazol; İyot-131; Teknesyum-99m; Salım çalışmaları; in vitro-in vivo korelasyon*

Introduction

Ornidazole (ORN) is a 5-nitroimida-zole derivative, which has antimicrobial activity and is used in the treatment of susceptible protozoal infections and in the treatment and prophylaxis of anaerobic bacterial infections. It is administered orally as tablets, vaginally as a pessary or intravenously(1). ORN suppositories are a cheap method of preventing postoperative infection in cases of unperforated appendix (2). Administration by oral and rectal routes is preferred rather than by the intravenous route on the basis of efficacy, safety and cost; this recommendation is applicable to both loading and maintenance dosing. Intravenous administration should be restricted to emergency preoperative loading (single 500mg dose);

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to patients with proven anaerobic infections and serious sepsis associated with an unidentified organism; and also to patients who are unable to take medication by mouth and those without a functional rectum or with diarrhea and patients with leukemia who are vomiting. These drugs are remarkably safe under conditions of acute use if the intravenous route is avoided. It can be concluded that the nitroimidazoles are effective, cheap and safe drugs for the shortterm treatment of protozoal and bacterial (anaerobic) infections(3).

Polyethylene glycols are water-soluble polymers of ethylene glycol. They are widely used in pharmaceuticals as solvents, as tablet binders and coatings, as cosmetic bases and as ointment and suppository vehicles. When used as suppository vehicles, they release incorporated medicament by dissolving in the rectal fluids (4).

Radioisotopes have been extensively used in the testing of various pharmaceutical dosage forms. The literature contains several reports of evaluative methodology regarding *in vivo* and *in vitro* uses of radioisotopes in various dosage forms(5). Various investigators evaluated the dissolution rate of active substances using radioisotopes(6-8). Relationships were found between *in vitro* and *in vivo* dissolution data (6, 9-11). Data acquired from the scintigraphic evaluation of pharmaceutical dosage forms are now being used increasingly at all stages of product development, from the assessment of prototype delivery systems to supporting the product licence application. Prototype drug delivery systems can be tested in vitro using various techniques designed to study drug release, either in a passive or active manner(12).

The purpose of this study was to compare the *in vivo* and *in vitro* release rates of labeled and unlabeled ORN from PEG 6000 suppository base by spectrophotometric method and NaI(TI) Scintillation detector and also to establish a correlation between *in vitro* and *in vivo* release results.

Materials and Methods

ORN was a gift from Roche (Germany). Na ^{131}I was obtained from the Department of Nuclear Medicine (Ege University, Turkey). Iodogen was purchased from Sigma (USA). PEG 6000 was obtained from Hoechst (AG Werk Gendorf, Burgkirchen). Tc-99m activity was taken from Amersham (UK) MoTc generator. All chemicals used were of analytical grade. ORN and poly-ethylene glycol were in pharmaceutical grade and were used as received from the supplier.

Preparation of active and inactive suppositories

1.50 g PEG-6000 was melted at 60C in a water bath by stirring continuosly. 50 l freshly prepared $SnCl_2$ solution and 2 mCi Na $^{99m}TcO_4$ were added and thus PEG was labeled. ORN was also labeled with ^{131}I(13). The mixture of ^{99m}Tc-labeled PEG 6000 and ^{131}I-labeled ORN (2mCi) was poured into a mould and then frozen in a refrigerator. The prepared cylindrical suppositories containing 0.003 g drug were 0.7 cm in length having a diameter of 0.5 cm. Unlabeled suppositories were prepared using the same procedure mentioned above with 0.9% NaCl solution instead of Na $^{99m}TcO_4$ and Na ^{131}I.

In vitro release studies

A suppository containing unlabeled ORN was placed into a 50 mL beaker in a water bath at 370.1C. The rotating rate of stirrer was 100 rpm. The dissolution medium was 10 mL intestinal fluid (pH=7.50.1) (14). 0.5 mL samples were withdrawn at predetermined time intervals (1,2,3,4,5,10,15 and 20 minutes) and the sample volumes were adjusted to 10 mL with intestinal fluid at each intervals. 0.5 mL intestinal fluid was added to the dissolution medium to compensate for sampling. The

absorbances of these solutions were measured at 318 nm spec-trophotometrically (Shimadzu Double-Beam 150-02) against the blank sample. The labeled suppositories were also tested in the same dissolution conditions. 50 L samples were withdrawn and gamma peaks of ^{99m}Tc and ^{131}I were counted by NaI(TI) scintillation detector. After the dissolution tests, the release profiles were plotted as a function of time. The obtained results are the mean of at least three experiments.

In vivo studies

Male rabbits (n=3) weighing approximately 2.5 kg were fasted for 8 hrs prior to the experiments, but were allowed free access to water. The suppository containing ^{131}I-ORN was administered into the rectum. After rectal administration of suppositories whole body images were obtained for 14 hrs using gamma camera (Sophy Gamma Camera DSX). The radioactivity remaining in the suppository was counted and *in vivo* release of ^{131}I-ORN was calculated.

Results and Discussion

In vitro studies

A dual labeling procedure is often used to follow the release rates of drugs and vehicles (7). For this purpose, ORN was labeled with ^{131}I. Also PEG 6000 was labeled with ^{99m}Tc and the *in vitro* release rates were examined by the NaI(TI) scintillation detector in this study. Furthermore, the *in vitro* release of unlabeled ORN was investigated Spectrophotometrically and the

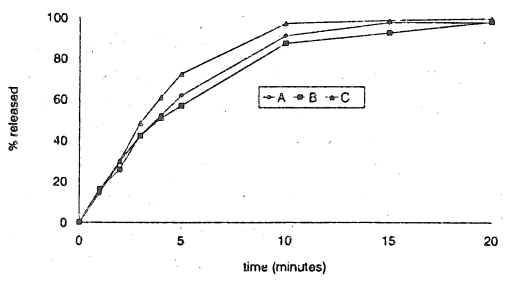


Fig.1. The release profiles of ^{99m}Tc-PEG(A), ^{131}I-ORN (B) and unlabeled ORN (C) as a function of time

in vitro release pattern results so obtained were compared with those of nuclear *in vitro* method. The results are shown in Fig.1.

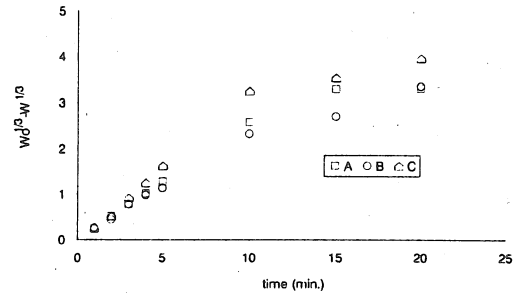


Fig.2. The kinetic evaluations of the release results.(A: ^{99m}Tc-PEG, B: ^{131}I-ORN, C: unlabeled ORN).

It was observed that the formulations released approximetely 80-90% of drug within 10 minutes. When the dissolution results were evaluated kinetically, Hixson-Crowell kinetic model was found to fit as the best model. The relationships seemed to be linear, especially for the first 10 minutes (Fig. 2).

The release results obtained were also investigated statistically. No significant difference was observed between spectrophotometric and nuclear results. The relationship between these results were examined (Fig.3) and very significant correlations were found ($p<0.001$).

Few drug molecules contain isotopes of radionuclides suitable for imaging with a gamma camera. It may be possible to radiolabel the compound, for example, by iodination, but it must be noted that the radiolabeled molecule may behave differently from the nonlabeled compound and the tracer may become detached. Similar considerations apply when radiolabeling other components of drug formulations. Most

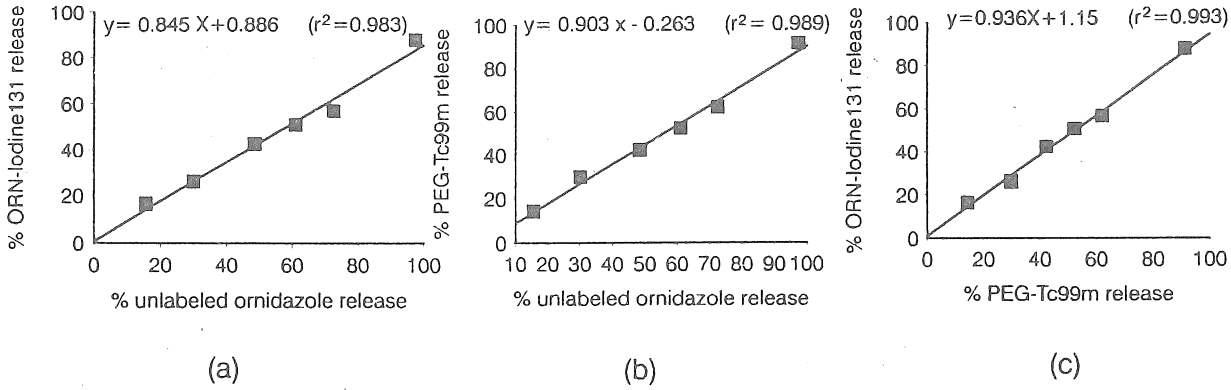
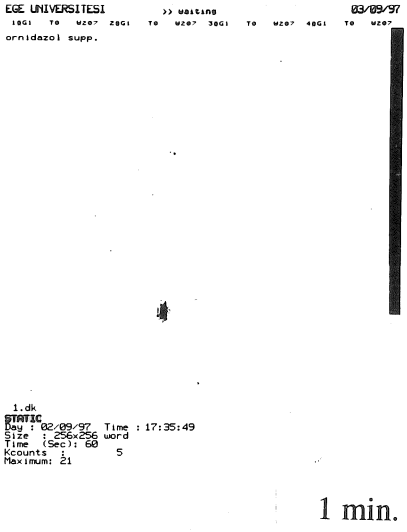


Fig.3. The correlations of the release results obtained with different assay methods

dosage forms, however, do not contain an ingredient that can be readily labeled with a suitable tracer. Radiolabeling is, therefore, usually achieved by the addition of radiolabeled material that reflects the properties of the system under investigation. For example, with a sustained-release preparation it may be possible to match the release rate of a technetium compound to that of drug (15,16). In our study, it was also found that PEG suppository base labeled with ^{99m}Tc showed the same *in vitro* release patterns as those of ^{131}I labeled drug. A very good correlation was obtained between the results as shown in Fig. 3-c. On the other hand, the results obtained from the unlabeled ORN were not very different from the labeled ORN results. These results shown in Fig.3a-b can give some information about the release pattern of ORN when it is not possible to investigate the release of drug by nuclear method, and they can also be correlated with the nuclear results. The nuclear method was found to be more precise and also it needed less sample volume to determine the amount of release. The use of radiation detection method for determination of a drug will provide analytical sensitivity for accurate measurement in even very low concentrations.

In vivo studies

Scintigraphic images were taken at predetermined time intervals (Fig.4). The initial count rate in rectum was considered as 100% of the administered dose. The other count rates were compared with the initial values. The percentage of remaining ^{131}I-labeled ORN in rectum at each time was calculated and the release results were obtained. The so obtained values were plotted as a function of time to estimate the *in vivo* release. But after five minustes, the remaining radioactivity in the suppository in rectum was not detected accurately as it completely dissolved. These results could be used only for the determination of drug dispersion. According to these findings, it was seen that there was a significant correlation between the *in vitro* and *in vivo* release rates in the first five minutes. The suppository dosage forms released nearly 60% of the drug *in vitro* and *in vivo* in 5 minutes. *In vitro* release rates were found to be 0.375, 0.345 and 0.444 mg/min for ^{99m}Tc-PEG, ^{131}I-ORN and unlabeled ORN, respectively. *In vivo* release rate of ^{131}I-ORN was found to be 0.336 mg/min. The *in vitro* release value of 0.345 mg/min obtained for ^{131}I-ORN was found to be of significant value for determining the *in vivo* release of drug. The *in vivo* release of ^{131}I-ORN and it's correlation with the *in vitro* release results are shown in Figs. 5 and 6.



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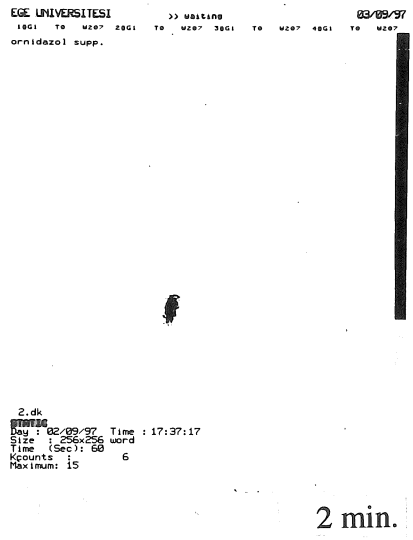

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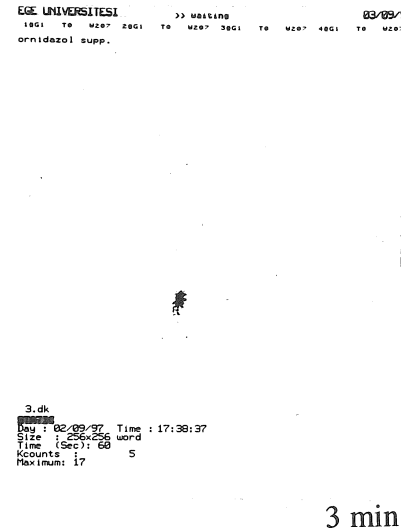

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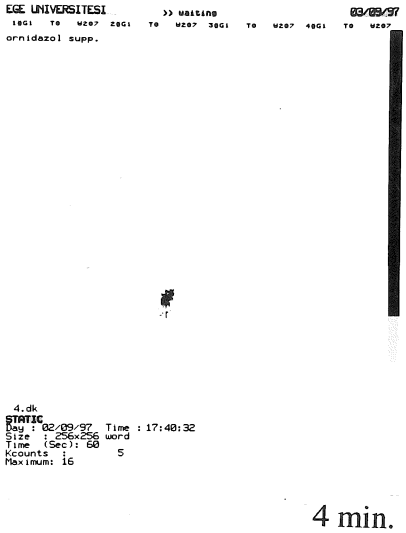

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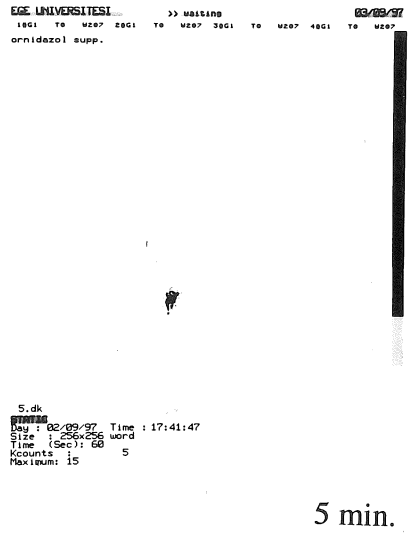

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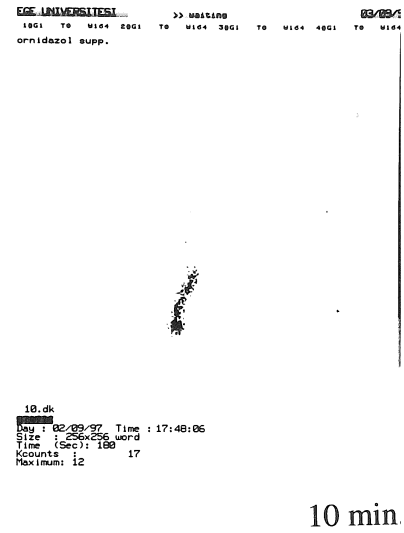

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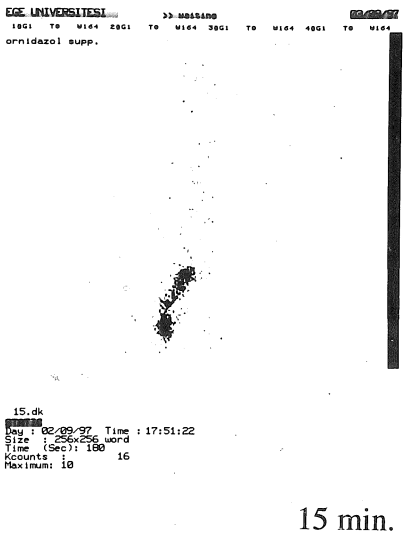

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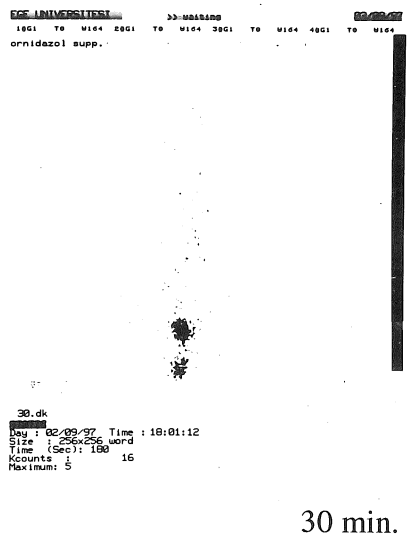

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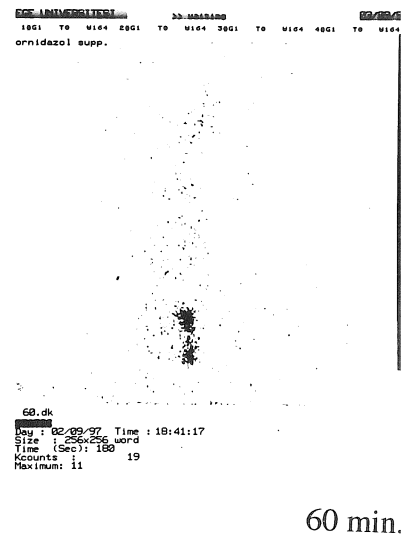

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Fig.4. The scintigraphic images

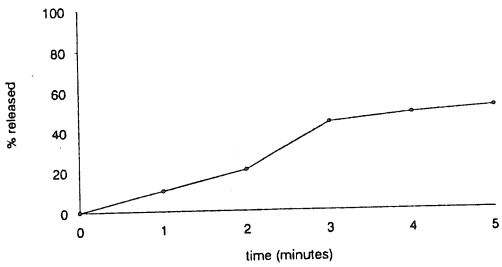

Fig.5. The in vivo release of ^{131}I-ORN

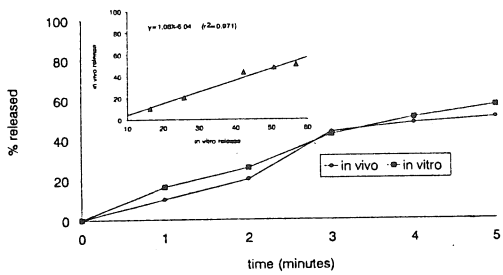

Fig.6. The comparison of the *in vitro* and *in vivo* release of ^{131}I-ORN (insert: The correlation of in vitro and in vivo release results)

In conclusion, the results of the present study clearly showed that *in vitro* release profiles for ^{99m}Tc-PEG, ^{131}I-ORN and unlabeled ORN were similar. In addition, the radiation detection method was found to be more sensitive than the spectrophotometric method for accurate measurements.However, the spectrophotometric method applied for unlabeled ORN can also be used for the determination of release pattern of labeled ORN when correlated with nuclear methods. A good correlation was obtained between *in vitro* and *in vivo* release results for the first five minutes in which approximately 60% of the drug was released. Furthermore, the obtained results demonstrated that a rectally administered ORN was absorbed well and gamma scintigraphy can be used to evaluate the *in vivo* performance of ORN administered rectally.

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