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RESEARCH ARTICLE

Biodistribution of Technetium-99m Doxycycline Hyclate

Derya Ilem Ozdemir was awarded the Young Scientist prize of FABAD for her article.

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Summary

The aim of the present study is to evaluate doxycycline hyclate biodistribution by gamma scintigraphy in inflamed rats. The Technetium-99m (^{99m}Tc) eluted was used for labeling of doxycycline hyclate. ^{99m}Tc-doxycycline hyclate was prepared with a radiochemical yield greater than 90%, adding ^{99m}Tc to doxycycline hyclate in the presence of stannous tartarate and ascorbic acid. Scintigraphic studies in rats were carried out using experimentally induced inflammation in the left thigh muscle using turpentine oil. Static images were acquired by a gamma camera in different time intervals for 6 hours. For quantitative evaluation of ^{99m}Tc-doxycycline hyclate uptake, the regions of interest were drawn around and uptakes were calculated as counts per pixel. According to in vivo studies, the accumulation of ^{99m}Tc-doxycycline hyclate in the inflamed muscle was found higher than the control muscle.

Key Words: *Technetium-99m, Doxycycline hyclate, Radiolabeling, Inflammation imaging.*

Teknesyum-99m Doksisiklin Hiklat'ın Biyodağılımı

Özet

Çalışmamızın amacı, inflamasyon oluşturulmuş ratlarda doksisiklin hiklatın tutulumunu gama sintigrafi ile incelemektir. Doksisiklin hiklatın Teknesyum-99m eluatı ile işaretlendi. ^{99m}Tc-doksisiklin hiklat, kalay tartarat ve askorbik asit varlığında %90'ın üzerinde bir radyoişaretleme verimi ile işaretlendi. Sol uyluk kaslarında turpentin yağı ile inflamasyon oluşturulmuş ratlarda görüntüleme çalışmaları yapıldı. Gama kamera ile 6 saat boyunca farklı zamanlarda statik görüntüler alındı. ^{99m}Tc-doksisiklin hiklat tutulumunun kantitatif değerlendirmesi için, ilgi alanları çizildi ve piksel başına düşen sayım miktarı hesaplandı. İn vivo çalışmalara göre, ^{99m}Tc-doksisiklin hiklat'ın inflame kasdaki tutulumu normal kasdan daha yüksek bulundu.

Anahtar Kelimeler: *Teknesyum-99m, Doksisiklin Hiklat, Radyoişaretleme, İnflamasyon Görüntüleme.*

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INTRODUCTION

Inflammation is a complex tissue reaction to any kind of injury, in order to bring serum molecules and cells of the immune system to the damaged site. Although different factors lead to injury such as living microbes (bacteria, fungi, viruses), traumatism, physical and chemical agents, the mechanisms of the appearance of inflammations are similar. Infection simply means "contamination with microorganisms". Despite the great strides in management of infectious diseases, infections remain among the most frequently encountered and costly causes of deaths and diseases worldwide, particularly in the developing countries. Timely diagnosis could help in instituting effective treatment and reduce the morbidity and mortality.

In a severely immunosuppressed patient, there can be an infection without any inflammation. Also there can be an inflammation without any infection, since the reaction of the tissues is triggered by products of tissue injury.

Because of the conventional imaging techniques such as radiological techniques including computed tomography (CT), nuclear magnetic resonance (NMR) and ultrasonography (US) are based on important anatomic alterations, they are not able to detect infection and inflammation foci in early phases of development. In contrast, nuclear medicine scintigraphic imaging techniques are based on pathophysilogical and pathobiological changes which occur earlier in the infection process. When anatomical changes are not yet apparent, infection and inflammation foci can be visualized in early phases with scintigraphic imaging techniques. Over the past years, several radiopharmaceuticals have been developed for infectious and non-infectious inflammatory diseases imaging. Today ^{67}Ga scintigraphy ^{99m}Tc-nanocolloid, ^{111}In and ^{99m}Tc in vitro in vivo labeled leukocytes and monoclonal antigranulocyte antibodies are widely available for this purpose. Most of these methods are localizing both bacterial and sterile inflammation.

Doxycycline hyclate is a well-known broad-spectrum tetracycline antibiotic obtained through modification of the oxytetracycline molecule. It has bacteriostatic activity against a wide variety of microorganisms, including aerobic and anaerobic Gram-positive and Gram-negative bacteria, chlamydiae, rickettsiae and mycoplasmas. It exerts bacteriostatic effect by inhibiting the bacterial protein synthesis due to the disruption of transfer RNA and messenger RNA at the ribosomal sites.

The aim of the present study is to evaluate radiolabeled doxycycline hyclate biodistribution by gamma scintigraphy in inflamed rats.

MATERIALS and METHODS

Doxycycline hyclate was obtained from AppliChem (Germany). Stannous tartarate purchased from Sigma-Aldrich (USA) and ascorbic acid was purchased from Sigma-Aldrich (United Kingdom). ^{99m}Tc-pertechnetate was obtained from the daily milking of a ^{99}Mo/^{99m}Tc-generator (Nuclear Medicine Department of Ege University). Radioactive samples were counted in a counting unit (Atomlab 100 Dose Calibrator Biodex Medical Systems).

Radiolabeling of Doxycycline Hyclate

Doxycycline hyclate was chosen due to its broad spectrum activity against both gram positive and gram negative bacteria. Optimum radiolabeling conditions were investigated with different concentrations of reducing and antioxidant agents. Ready to use kit was prepared by the specified amounts of doxycycline hyclate, stannous tartarate and ascorbic acid. The kit was reconstituted with 0.2 mL ^{99m}Tc solution having an activity of 370 MBq.

Radiochemical Purity

Radiochemical purity of ^{99m}Tc-doxycycline hyclate was checked by thin layer chromatography (TLC) using Instant thin layer chromatography silica gel (ITLC-SG) plates as the stationary phases and acetone and acetonitrile/water/trifluoroacetic acid (ASN/W/TFA) (50/25/1.5) as the mobile phases. The radioactivity on plates was measured using a TLC scanner (Bioscan AR 2000) and% radiochemical purity (RP) of ^{99m}Tc- doxycycline hyclate was calculated from the following equation by subtracting from 100 the sum of measured impurities percentages.

% RP= 100 – (% Free + % Hydrolyzed/reduced ^{99m}Tc-pertechnetate)

Stability of ^{99m}Tc- doxycycline hyclate

After labeling doxycycline hyclate with ^{99m}Tc, the preparation was left at room temperature for six hours. The labeling stability of ^{99m}Tc-doxycycline hyclate was evaluated by TLC studies every hour.

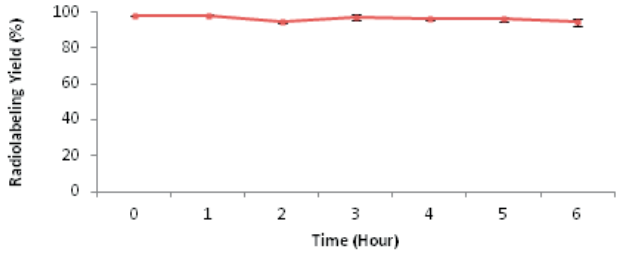

Figure 1. Stability of ^{99m}Tc-doxycycline hyclate.

Animal Gamma Scintigraphy Studies

Experiments were performed on rats according to a protocol approved by Ethical Committee for Animal Research, University of Ege (Registration number: 2010-37, 2010). Wistar rats (200-250 g) were anesthetized by a mixture of Ketamine/Xylazin. Inflammation process was induced by direct injection of 0.2mL turpentine oil into the left thigh muscles of 6 rats. The right thigh muscles were used as controls. After 24h, 3.7 MBq ^{99m}Tc-doxycycline hyclate injected in to the tail veins of the rats and accumulation of the tracer in inflamed areas were assessed by gamma scintigraphy studies at different time intervals after the injections (0, 1h, 2h, 3h, 4h, and 5h).

Statistical analysis

The means and standard deviations were calculated on Microsoft Excel. *t*-test was used to determine statistical significance. Differences at the 95% confidence level ($p<0.05$) were considered significant.

RESULTS

Using the described protocol, doxycycline hyclate were labeled with labeling efficiency of 96% ± 2%. In ITLC system, using acetone as the mobile phase, free ^{99m}Tc-pertechnetate moved with the solvent front (Rf=1), while ^{99m}Tc-doxycycline hyclate and hydrolyzed/reduced ^{99m}Tc-pertechnetate remained at the spotting point. Reduced hydrolyzed technetium was determined by using ASN/W/TFA (50/25/1.5) as the mobile phase where the reduced hydrolyzed technetium remained at the point of spotting (Rf=0) while free $^{99m}TcO_4^-$ and ^{99m}Tc- doxycycline hyclate moved with the solvent front (Rf=1).

During incubation at room temperature, the data did not show significant differences between the samples investigated for the stability studies up to 6h. The results for the radiochemical purity and stability of the ^{99m}Tc- doxycycline hyclate are shown in Figure 1.

According to scintigraphy studies, ^{99m}Tc-doxycycline hyclate remained at the inflamed thigh muscle during the whole experiment. Table 1 shows target to background ratios obtained from region of interest (ROI) analysis of ^{99m}Tc-doxycycline hyclate at different time intervals after injection.

Ratios of 2.57±1.54, 3.18±1.26, 4.55±2.61, 3.69±2.24, 3.64±2.60 of the target/no-target show that there was a significant uptake of ^{99m}Tc-doxycycline hyclate by

Table 1. Target, No- Target and Target/ No Target Ratios from ROI Analysis of ^{99m}Tc-doxycycline hyclate at different time intervals (Values expressed as the mean ± sd (n=6 rats), $p<0.05$)

Time After Injection	Inflamed Thigh Muscle (Target)	Control Thigh Muscle (No-Target)	Ratio Target/No-Target
0	0,25±0,11	0,17±0,06	1,55±0,85
1	1,06±0,37	0,41±0,19	2,57±1,54
2	0,75±0,25	0,28±0,15	4,55±2,61
3	0,89±0,32	0,28±0,15	4,55±2,61
4	0,79±0,79	0,27±0,14	3,69±2,24
5	0,69±0,69	0,22±0,06	3,64±2,60

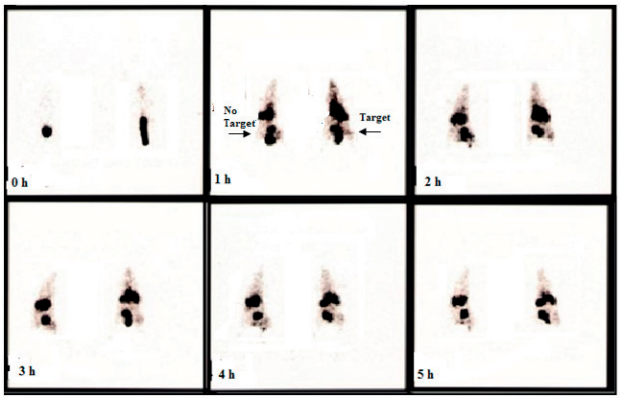

Figure 2. Sintigrams of left thigh muscle's inflamed rats up to five hours after injection.

the inflammation site. As can be seen in Figure 2, the accumulations of ^{99m}Tc-doxycycline hyclate in the inflamed muscles were found higher than the control muscles. The uptake of ^{99m}Tc-doxycycline hyclate until five hours after injection is shown in Figure 2.

DISCUSSION

In this study, doxycycline hyclate was labeled with ^{99m}Tc with high radiolabeling yield over 90%. The results obtained in this work demonstrated that the labeled product was stable at room temperature up to six hours.

Our studies in rats indicated, rapid distribution of ^{99m}Tc-doxycycline hyclate throughout the body and greater uptake by the inflamed muscle than of the control. As shown in Table 1, during the experiments, target/no-target ratios indicated that the labeled product remained at the inflammation site. This observation can probably be explained by the hypothesis that, the increased blood flow and vascular permeability at the inflammation site would increase the drug uptake. ^{99m}Tc-doxycycline hyclate may be applied for the inflammation imaging.

CONCLUSION

In this study, labeling of doxycycline hyclate was performed, using stannous tartarate as a reducing agent. The resulting complex is stable and labeling yield is maintained over 90% up to six hours. This radiopharmaceutical showed grater uptake in the inflamed muscle which is high enough to be distinguished from the background tissue. These promising characteristics make our new radiopharmaceutical a suitable agent for diagnosis of the inflamed foci. Our studies with ^{99m}Tc-doxycycline hyclate to distinguish between infection and inflammation foci will be published in the near future.

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