Synthesis and experimental study of norfloxacin labeled with technecium-99m as a potential agent for infection imaging

Original Article

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ABSTRACT

Introduction: Differentiation between infection and sterile inflammation is one of the most difficult medical problems and is relevant in many clinical situations. Scintigraphy with radiolabeled antibiotics, especially labeled fluoroquinolones, is a promising tool for diagnosing bacterial inflammation. We have attempted to synthesize ^{99m}Tc-norfloxacin and studied its properties in experiment.

Methods: In present study we labeled norfloxacin with ^{99m}Tc, determined its radiochemical purity by thin-layer chromatography procedure, compared the bactericidal activity of ^{99m}Tc-norfloxacin and unlabelled norfloxacin by disk diffusion, performed in vitro binding assays and evaluated the feasibility of ^{99m}Tc-norfloxacin to image soft-tissue infections in rat modal of thigh muscle infection. Scintigraphy was performed at 30 min, 90 min and 18 h post injection. Abscess –to–normal-site ratios were calculated.

Results: In this study ^{99m}Tc-norfloxacin with high radiochemical purity and low colloid content was synthesized. Disk diffusion method showed that ^{99m}Tc-norfloxacin retains bactericidal properties of unlabelled norfloxacin. In vitro binding studies demonstrated small degree of the tracer binding with S. aureus and ability of ^{99m}Tc-norfloxacin to bind with killed as well as alive bacteria. Uptake of the tracer was clearly visible in infected muscles and not visible in sterile inflammed muscles.

Conclusion: The ^{99m}Tc-norfloxacin retains bactericidal properties of unlabelled norfloxacin. The tracer demonstrated small degree of S. aureus binding both with killed as well as alive bacteria. Studies which were performed in vivo in rats with a model of thigh muscle infection showed good ability of the radiopharmaceutical to image the infection.

Key words: Norfloxacin; Technecium-99m; Infection; Inflammation; Scintigraphy

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INTRODUCTION

Differentiation between infection and sterile inflammation is one of the most difficult medical problems and is relevant in many clinical situations. Modern highly informative radiologic modalities such as computed tomography, magnetic resonance imaging are failed to reveal inflammation on early stages in the absence of anatomical tissue changes. Radionuclide methods were successfully used for inflammation imaging for the past 30 years. Nonetheless the majority of proposed radiopharmaceuticals (labeled autologous leukocytes, labeled anti-granulocyte antibodies, et al) are unable to differentiate between infection and sterile inflammation. This problem can be solved using tracers which bind directly with live bacteria [1]. Labeled antibiotics, particularly labeled fluoroquinolones, are a new generation of radiopharmaceuticals for infection imaging. Firstly ciprofloxacin labeled with ^{99m}Tc, which presumably binds to DNA-gyrase and topoisomerase IV of bacteria, the same as unlabeled ciprofloxacin does, was proposed [2]. This radiopharmaceutical is well studied nowadays, but reported data concerning its specificity for infection imaging are inconsistent [3-7] and methods of labelling are not convenient enough requiring final tracer purification. So other antimicrobal agents such as levofloxacine, sparafloxacine, enrofloxacine and norfloxacine were labelled with ^{99m}Tc in order to get more specific tracer for infection imaging [8-12].

These fluoroquinolones have similar structure as ciprofloxacin (Figure 1) with analogous mechanism of action and broad spectrum of antibacterial activity.

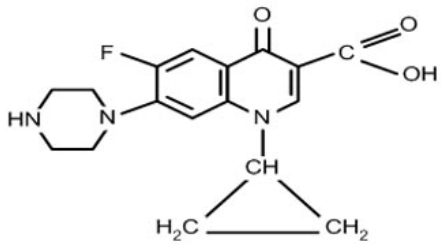

Fig 1. Ciprofloxacin structure.

However, not many publications devoted to the study of these agents are available. In this study we propose a kit formulation for labeling of norfloxacin with ^{99m}Tc.

The bactericidal activity of ^{99m}Tc-norfloxacin and unlabelled norfloxacin were compared by in vitro binding assays to evaluate the ability of ^{99m}Tc-norfloxacin to visualize soft-tissue infections or sterile inflammations in rats.

METHODS

In this study we used norfloxacin hydrochloride (NFH) which was synthesized in the Institute of Organic Synthesis of the Ural Branch of the Russian Academy of Sciences (Ekaterinburg, Russia) as described by Mokrushina G.A. et al [13]. The structure of norfloxacin is presented in Figure 2.

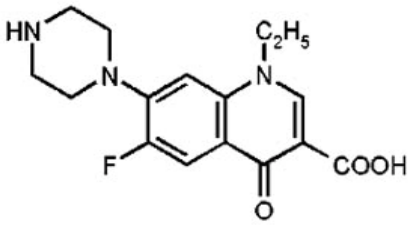

Fig 2. Norfloxacin structure.

We prepared ^{99m}Tc-norfloxacin by the following method [12].

Firstly, in vial A, 0.7 g of stannous (II) chloride dihydrate ($SnCl_2$ $2H_2O$) was added to 0.2 ml of 1M hydrochloric acid (HCl). When dissolution was completed volume was increased with distilled water up to 10 ml. The dissolution was carried out in the presence of the inert gas (argon). Secondly, into the vial B 0.025 ml of $SnCl_2$ solution from vial A and 5 mg of NFH was added. The contents of the vial B then was freeze dried. To obtain the ^{99m}Tc-norfloxacin 500 MBq (in 5 ml 0.9% NaCl) of freshly eluted sodium pertechnetate was added to the vial B and incubated 20 min at room temperature.

Thin-layer chromatography (TLC) procedure

To determine radiochemical purity of ^{99m}Tc-norfloxacin 5 µl of prepared sample was spotted on 3 silica-gel impregnated strip (Sorbfil, Russia), 2×15 cm. To determine pertechnetate content of the radiopharmaceutical sample, first strip was developed using acetone as the mobile phase (time of chromatography 10 min). In this system, pertechnetate migrated with the front of the mobile phase (Rf=1.0). To determine the colloid content of the preparations, the second strip was developed using ethanol:water:ammonium hydroxide (2:5:5) as the mobile phase (time of chromatography 40 min). In this system, the colloid was found at the origin of the strip (Rf = 0). To determine ^{99m}Tc-norfloxacin content third strip was developed using ethylacetate:isopropanol:concentrated ammonia (12:6:4) as the mobile phase (time of chromatography 40 min). In this system, the ^{99m}Tc-norfloxacin migrated with the front of the mobile phase (Rf=0.5). Then all three strips were cut starting from the starting line on the pieces of 10 mm length and their

radioactivity (count rate) was measured on the single-channel amplitude analyzer "Strahlungsmessgerat-20046".

Identifying of unlabeled NFH

The qualitative analysis of unlabeled norfloxacin was performed by the method of Titov I.V. et al [14] and by spectrophotometric analysis. For this purpose 5 ml of the radiopharmaceutical was diluted in 50 ml of H_2O, and then mixed with 0,1M HCl. A necessary part of the solution was placed in a spectrophotometer cuvette and mesured. Maximum of the solution absorption spectrum should be at wavelength of 273 ± 2 nm.

Stability

The stability of ^{99m}Tc–norfloxacin was studied in vitro by mixing of 5 mL of normal serum and 0.5 mL of ^{99m}Tc–norfloxacin following by incubation at 37^0C for 8 h. At different time points (1 h, 4 h and 8 h) 0.2 mL aliquots of complex were removed and evaluated for radiochemical purity using TLC.

Microorganisms

Staphylococcus aureus isolates (B-243, 83A phage III (S. aureus)) were obtained from clinical specimens from the bacterial laboratory of Tomsk Regional Clinical Hospital. Strains were stored as a bacterial inoculation on slant meat-peptone agar (MPA) (GEM, Russia) at room temperature, protected from light. Suspension of S. aureus containing $1{\cdot}10^7$ cfu or $1{\cdot}10^9$ cfu was prepared by washing out the MPA with daily culture using sterile saline and turbidity standard.

Norfloxacin-resistant strain was obtained as follows. S. aureus ($1{\cdot}10^7$ cfu) was spread uniformly onto the plate with MPA. Then the commercial paper disk with 5 µg of norfloxacin was placed onto inoculated agar plate, following by the plate incubation at 37°C for 24 h. Around the disk with norfloxacin, a zone of growth inhibition 24 mm in diameter was formed. Within this area one S. aureus colony resistant to norfloxacin was observed. Resistance to norfloxacin was confirmed by disk diffusion method [15].

Comparison of ^{99m}Tc- norfloxacin and unlabeled norfloxacin bactericidal activity

Disc diffusion method [15] was used for comparing ^{99m}Tc-norfloxacin and unlabeled norfloxacin antimicrobial activity. The suspension of S. aureus ($1{\times}10^7$ cfu) was spread uniformly onto peptone agar medium plates. Three blank paper disks 10-mm in diameter were impregnated with 30 µl of ^{99m}Tc-norfloxacin (1MBq), 30 µl of unlabeled norfloxacin (concentration of norfloxacin 1 mg/ml in both solutions), 30 µl of $Na^{99m}TcO_4$ (1MBq) were placed onto the inoculated agar plates. The fourth commercial disk with 5 µg of norfloxacin (NICF, Russia) was placed onto the same plate. The plates were then incubated at 37°C, and the diameters of the growth zones inhibition were measured at 24 h.

In vitro cell binding studies

A prefiltered through Minisart ® syringe filter with a pore diameter of 100 nm (Sartorius Stedim Biotech) 40 MBq 1 ml of ^{99m}Tc-norfloxacin was added into 6 sterile vials containing $1{\cdot}10^7$ cfu S. aureus susceptible to norfloxacin, into 6 sterile vials $1{\times}10^7$ cfu S. aureus susceptible to norfloxacin heat-killed S. aureus, into 6 sterile vials $1{\times}10^7$ cfu norfloxacin resistant S. aureus. Then 3 groups of 3 vials containing each of the S. aureus isolates were incubated at 37°C for 15 min, 30 min and 60 min. As controls 9 vials containing each of the S. aureus isolates were incubated with 20 MBq 1 ml prefiltered $Na^{99m}TcO_4$ at 37°C for the same terms.

At the end of the incubation period the content of each vial was placed in a sterile syringe and passed through a syringe filters with a pore diameter 200 nm, followed by filters washing with 10 ml of 0.9% NaCl solution. The radioactivity of filters and vials with washing solution was measured on the dose calibrator «Isomed 1000» (Nuklear Medizintechnik, GMBH). As a control we used a filter and performed the following procedure: 1 ml (40 MBq) prefiltered through Minisart ® syringe filter with a pore diameter of 100 nm ^{99m}Tc-norfloxacin was passed through the control filter, following by washing with 10 ml of 0.9% NaCl solution. The radioactivity of the control filter was subtracted from the radioactivity of filters with radio-labeled bacteria.

Thigh muscle infection model

In 5 Wistar rats (weight, 200–250 g) inflammation was induced in the left thigh muscle. Firstly, rats underwent immunosuppression by injection of cyclophosphamide (20 mg in 1 ml 0.9% NaCl). The immunosuppression was done, because we were unable to induce an adequate model of abscess (with abundant S aureus content in tissue) without this procedure, probably due to the high regenerative capacity of rats. For this reason immunosuppression of rats is a limitation of our study. Five days later the tissue of left thigh muscle was damaged by a sterile needle, then S. aureus ($1{\times}10^9$ cfu) was injected into the wound. As a control 5 rats were injected with sterile turpentine into the left thigh muscle for the modeling of a sterile inflammation. On the 4th day when the swelling of the inoculated muscle has appeared, 40 MBq of ^{99m}Tc-norfloxacin was injected into the tail vein. All animal studies were performed

according to requirements of the Helsinki Declaration of the World Medical Association (2000). All rat procedures were approved by the local Animal Care and Use Committee and performed in accordance with institutional politics and the rules accepted by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

Scintigraphy and biodistribution

Biodistribution of ^{99m}Tc-norfloxacin was studied on 45 healthy male Wistar rats (weight, 200–250 g) by organ-activity measurement, after injection of 40 MBq (0.5 ml) of the radiopharmaceutical into the tail vein of rats. At 1, 3, 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180 min and 18 h after injection groups of three animals per each term were decapitated. Tissue samples (blood, muscle, lung, spleen, kidney, liver, and intestine) were dissected and packaged in vials for weighing and radioactivity measurement on the dose calibrator «Isomed 1000». Aliquots of the injected dose were counted simultaneously. The radiopharmaceutical content was calculated in percentage of the injected dose (%ID) per 1 g of extracted tissue.

The scintigraphy of rats with thigh muscle infection model and sterile inflamed thigh muscle was performed by γ-camera at 30 min, 90 min, and 18 h after injection of 40 MBq (0.5 ml) ^{99m}Tc-norfloxacin . Rats were placed prone on a dual-head γ-camera Forte (Philips Medical Systems, Netherlands) equipped with a parallel-hole, low-energy, all-purpose collimator. Images were obtained with a 15% symmetric window over the 140-keV energy peak of ^{99m}Tc. After acquisition of 100,000 or 300,000 counts, the images were stored in a 256 x 256 matrix and processed with the software package JetStream® Workspace Release 3.0 (Philips Medical Systems, Netherlands). Regions of interest (ROIs) were drawn on each anterior scintigram: one region over the pathologic site and one over the normal contralateral site. Mean activity per pixel was determined in each ROI. Then, the abscess–to–normal-site activity ratios were scored. During scintigraphy animals were anesthetized with diethyl ether.

Postmortem microorganism cultivation

The control of microorganism's presence in muscle tissue was performed both in rats with infection model and rats with sterile inflammation model. The infected or sterile inflamed thigh muscle from rats was aseptically removed, 2 mL of saline were added, and the tissue was homogenized using a tissue homogenizer. Then 0.1 ml of tissue suspension was inoculated on plates with the MPA and incubated at 37^0C for 24 h following by the cfu counting. As a control, tissues of uninfected thigh muscle were examined simultaneously. Only rats with abundant growth of S. aureus after abscess modeling and rats with no growth of any bacterium after sterile inflammation modeling were included in the study.

Statistical analysis

All mean values are expressed as %ID/g ± SD. Data were analyzed statistically using methods of general statisticst with a commercially available software package «Statistics for Windows» (StatSoft Inc., Version 6.0).

RESULTS

Radiochemical purity

Quality control of ^{99m}Tc-norfloxacin with TLC procedure indicated that the preparation contained < 3% of pertechnetate (TcO_4) and ≈ 5% of colloids (TcO_2). The radiochemical purity of radiotracer was >92%. The radiochromatogramm of ^{99m}Tc-norfloxacin is shown in Figure 3.

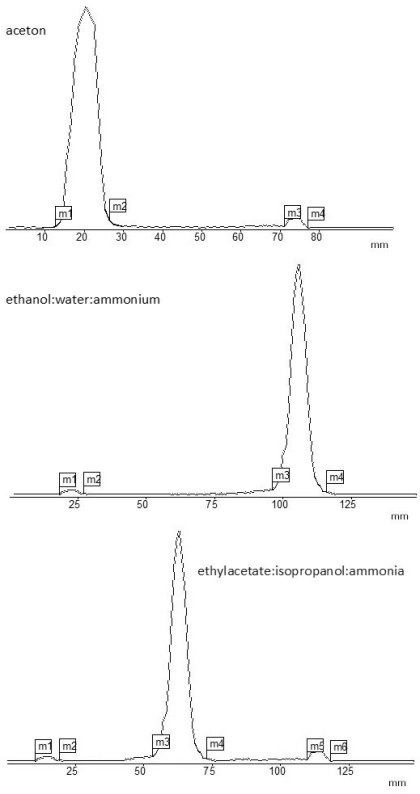

Fig 3. Radiochromatogramm of ^{99m}Tc-norfloxacin in three solvent systems.

Table 1. In vitro stability of ^{99m}Tc-norfloxacin in human normal serum.

(%)	1 hour	4 hours	8 hours
Radiochemical purity	94.3 ± 1.8	92.8 ± 2.1	91.6 ± 2.3
Released radioactivity	5.2 ± 0.7	6.7 ±1.0	7.9 ± 1.1

Identifying unlabeled NFH

Maximum of absorbtion spectrum of the ^{99m}Tc-norfloxacin solution was at a wavelength of 273 nm, which correspond to unlabeled norfloxacin.

Stability

Stability test showed (Table 1) that complex ^{99m}Tc–norfloxacin was stable in normal serum at least for 8 h. Radiochemical purity of the tracer at the end of the experiment was 91.6 ± 2.3% and the release of radioactivity 7.9 ± 1.1%.

Comparison of ^{99m}Tc- norfloxacin and unlabeled norfloxacin bactericidal activity

Results of bactericidal activity of ^{99m}Tc-norfloxacin and unlabeled norfloxacin by disk diffusion method are shown in Table 2. The diameters of sterile zones were both 27 mm around disks for ^{99m}Tc-norfloxacin and unlabeled norfloxacin. The diameter of the sterile zone around the commercial disk with norfloxacin was 25 mm, confirming the quality of used norfloxacin. Solution of $Na^{99m}TcO_4$ did not show any bactericidal activity - diameter of sterile zone around disk was 0 mm.

Table 2. Results of disk diffusion showing ^{99m}Tc-norfloxacin and unlabeled norfloxacin bactericidal activity.

Type of disk impregnation	Diameter of sterile zone (mm)
^{99m}Tc- norfloxacin	27
Unlabeled norfloxacin	27
Commercial disk with norfloxacin (5 µg)	25
$Na^{99m}TcO_4$	0

In vitro cell binding studies

Generally the bacterial tracer binding rate was ≈ 10%. Incubation results of susceptible to norfloxacin, susceptible to norfloxacin heat-killed and norfloxacin resistant S. aureus with ^{99m}Tc-norfloxacin or $Na^{99m}TcO_4$ are shown in Figure 4. At the same time 15 min after incubation with bacteria ^{99m}Tc-labeled norfloxacin accumulated more in inactivated isolates of S. aureus, rather than in alive antibiotic sensitive or resistant bacteria. However, over the time radioactivity of heat-killed bacteria decreased and radioactivity of alive isolates increased. This could be due to the reversible interaction of ^{99m}Tc-labeled norfloxacin with killed bacteria and its irreversible binding with live cultures. These results indicate the non-specific potential ^{99m}Tc-labeled norfloxacin accumulation in sites of infection. By 60 min of incubation, the amount of ^{99m}Tc-norfloxacin in all types of isolates became identical. Incubation of $Na^{99m}TcO_4$ with above mentioned isolates showed low uptake of radiopharmaceutical by S. aureus. In control filters radioactivity was also low.

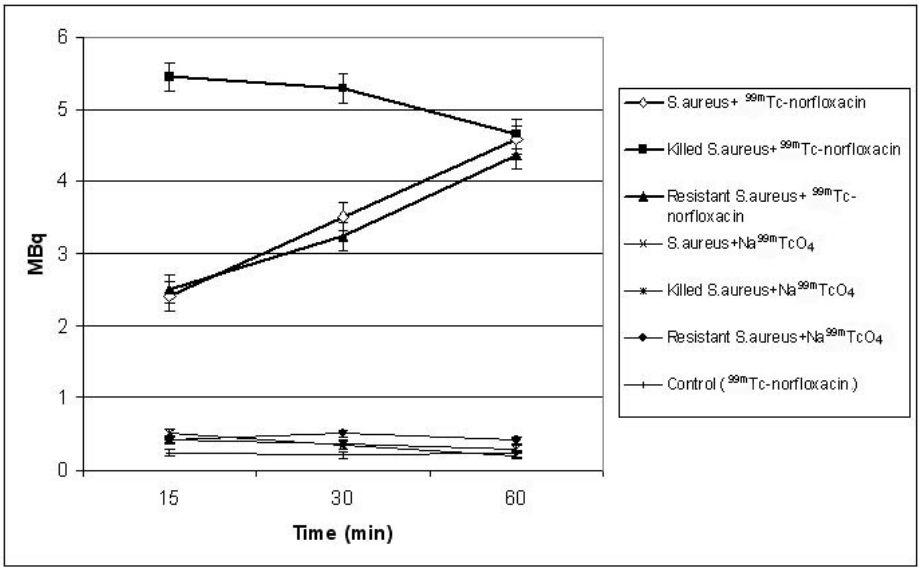

Fig 4. The dynamics of ^{99m}Tc-norfloxacin and $Na^{99m}TsO_4$ uptake (MBq) in different S. aureus isolates.

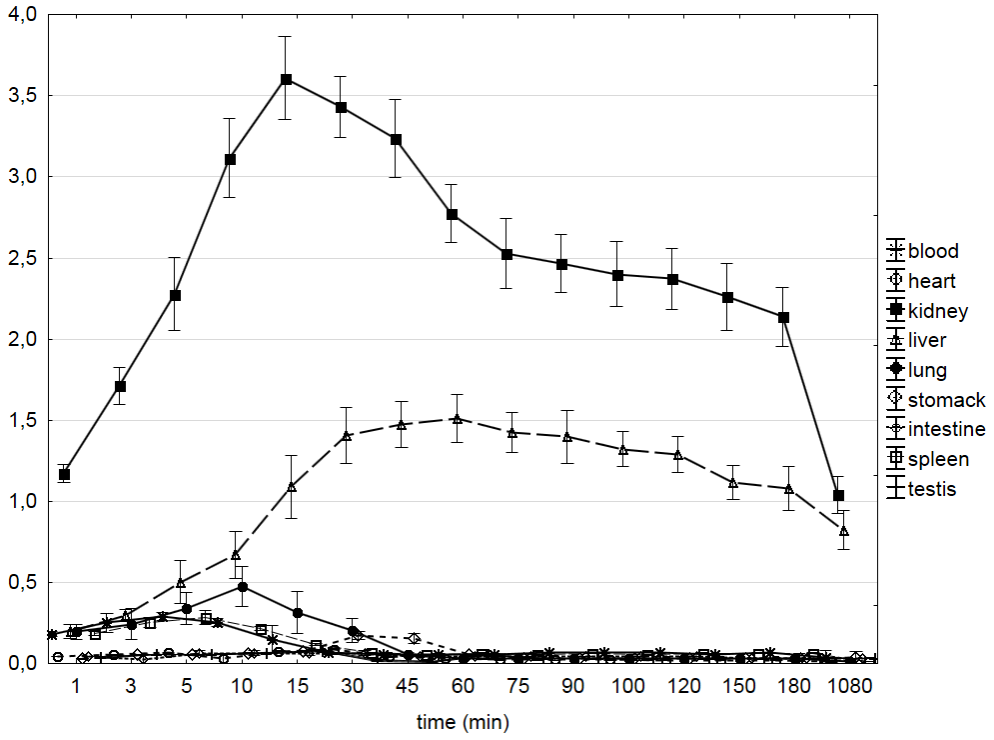

Fig 5. Biodistribution of ^{99m}Tc-norfloxacin (data obtained from abduction tissue countings).

Biodistribution studies

Results of biodistribution studies are shown in Figure 5.

The highest uptake of the tracer was observed in kidneys and in a liver, which corresponds to pharmacokinetics of unlabeled norfloxacin [16]. Accumulation of ^{99m}Tc-norfloxacin by kidneys has begun immediately after injection with maximum uptake at 15 min (3.607%ID/g). By the end of the experiment (18 hours post injection) 1,041±0,114 % ID/g of the tracer was detected in kidneys. Maximum of ^{99m}Tc-norfloxacin uptake (1.504% ID / g) was observed in liver at 60 min of the study and remained high up to the end of the experiment.

The blood clearance of the radio-labeled antibiotic was rapid – by 15 min of the study about 0.12% ID/g of the tracer was detected in blood. In other internal organs at all stages of the experiment accumulation of the radiopharmaceutical was low.

Scintigraphy of rats with thigh muscle infection model

The scintigraphy of rats with thigh muscle infection and sterile inflamed thigh muscle was performed on the 4th day after inflammation modeling. All rats had hyperemic papule in right thigh muscle, three infected rats had visible suppuration.

Rats were scanned by γ-camera at 30 min, 90 min, and 18 h post injection. At all stages, intense renal and liver uptake was observed. In addition, at 90 min and 18 h post injection accumulation of the radiopharmaceutical in intestine was detected. Uptake of the tracer in the infected muscle was seen (Figure 6) in all 5 rats with visible suppuration.

Accumulation of ^{99m}Tc-norfloxacin visualized in all stages of the study. Infected/background and inflamed/background ratios are presented in Table 3.

Table 3. Infected/background and Inflamed/background ratios from ROI analysis of ^{99m}Tc-norfloxacin at various times post injection.

Time	Infected/background	Inflamed/background
30 min	2,30±0,65	1,20±0,32
60 min	2,46±0,73	1,22±0,29
90 min	2,51±0,76	1,10±0,15
18 h	2,87±0,80	1,0±0,14

Data are expressed as mean±SD

By 18 h of the experiment infected/background ratio in rats with thigh muscle infection increased up to 2.87±0.80.

In rats with sterile inflamed muscle the tracer uptake was not visible during the study (Figure 7) and inflamed/background ratio was at 30 min 1.2±0.3, at 18 h – 1.0±0.2 (Table 2).

Microorganism culturing

Culturing of infected tissue suspension showed the presence of viable S. aureus in all cases (>0.5*10^7 cfu). Culturing of suspension of uninfected and sterile inflamed thigh muscle tissue indicated an absence of microorganism growth.

DISCUSSION

In this study, norfloxacin was labeled with ^{99m}Tc with high radiochemical purity > 92%. The amount of ^{99m}Tc-colloids, which were formed during labeling, was small ≈ 5%. That allowed using the tracer without preliminary purification and opening up as opportunities to make a kit for commercial use.

Presumably ^{99m}Tc-norfloxacin must bind to DNA-gyrase and topoisomerase IV of bacteria as unlabelled norfloxacin does. However, it should be noted that the molecule of the antibiotic undergoes modification, owing to addition the molecule of ^{99m}Tc. For radiolabeling of norfloxacin we used $SnCl_2$ $2H_2O$ as a reducer. Reduced ^{99m}Tc molecule reacts with carboxyl and carbonyl groups of norfloxacin, which may affect the bactericidal properties of the antibiotic and, accordingly, the mechanism of the tracer interaction with microorganisms. Therefore, we compared the antimicrobial activity of ^{99m}Tc-norfloxacin and unlabelled norfloxacin against S. aureus by disk diffusion. In our study the diameter of sterile zones did not differ between disks with the tracer, unlabeled antibiotic and commercial norfloxacin disk. Solution of $Na^{99m}TcO_4$ did not show any bactericidal activity. These facts indicate that radiolabeling with ^{99m}Tc does not affect chemical structure and antibacterial properties of norfloxacin. In vitro cell binding studies showed that on early terms of ^{99m}Tc-norfloxacin incubation (15 - 60 min) with bacteria the tracer accumulated more in heat-killed isolates of S. aureus, than in live bacteria.

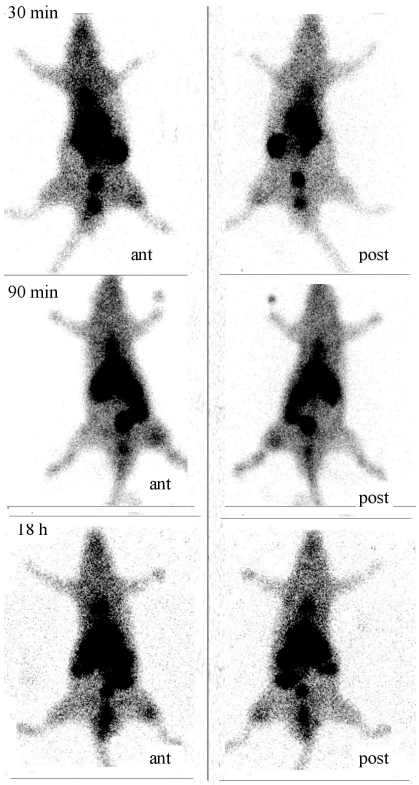

Fig 6. Images of a rat with left thigh muscle infection model at different stages after injection of 40 MBq (0.5 ml) of ^{99m}Tc-norfloxacin. The 300,000 counts acquisition. Rat was anesthetized with diethyl ether.

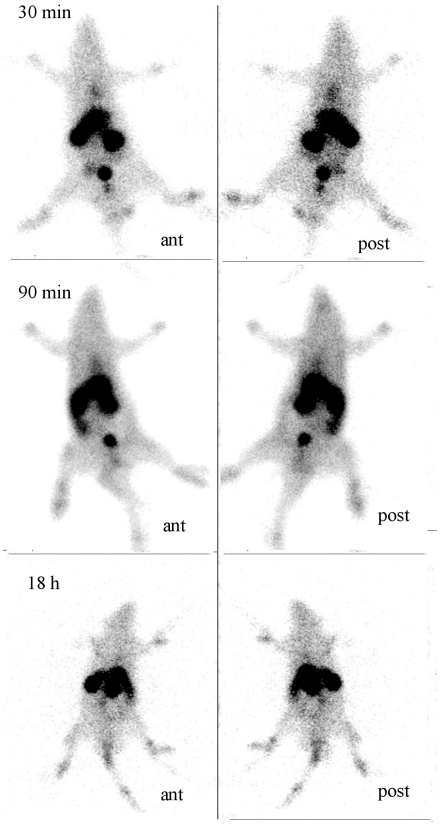

Fig 7. Images of a rat with left thigh muscle sterile infection model at different stages after injection of 40 MBq (0.5 ml) of ^{99m}Tc-norfloxacin. The 300,000 counts acquisition. Rat was anesthetized with diethyl ether.

This can be explained by an increased cell membrane permeability of the heat-damaged microorganisms, which facilitates the penetration of the tracer into the bacterial cytoplasm. Meanwhile, it was demonstrated that over time the tracer had a tendency to wash out of heat-killed and to increase its accumulation in living isolates of S. aureus. It should be noted that at 60 min of incubation the accumulation of ^{99m}Tc-norfloxacin in living and heat-killed isolates of S. aureus didn't differ significantly. These findings assume a reversible interaction of the radiopharmaceutical with killed bacteria and its irreversible binding with live bacteria. Our results are consistent with results of previous works [17, 18] and partially in agreement with results of Ibrahim I.T. et al [8], who evaluated binding of ^{99m}Tc-norfloxacin in both living and heat-killed S. aureus at later stages of incubation, from 1 to 24 hours. In the above mentioned work ^{99m}Tc-norfloxacin did not show significant differences in binding to both isolates.

It is known that the resistance of bacteria to fluoroquinolones may be associated with changes in the structure of the target enzyme (topoisomerase), increased permeability of porin channels, or active excretion of antibiotic from cells [16]. Since topoisomerases have different functions, for suppression of the microbial cell vital activity, it is enough to inhibit only one of enzymes, while another topoisomerase can remain active. Therefore, it is accepted that all quinolones have primary and secondary action targets. Primary target is an enzyme to which quinolone exhibits the greatest affinity. In our study ^{99m}Tc-norfloxacin equally accumulated in a resistant and sensitive culture of S. aureus. This can be explained by interaction of radiolabelled antibiotic with secondary target enzyme. In addition, the mechanism of norfloxacin resistance to S. aureus grown in our study probably was not associated with the penetration abnormality or active efflux of antibiotic from bacteria. Therefore, the resistance of S. aureus to norfloxacin should not affect the intensity of the tracer uptake in the infected foci or the quality of scintigraphic images.

The possibility of accumulation of $Na^{99m}TcO_4$ in S. aureus was excluded by results of our experiment.

Biodistribution study showed that ^{99m}Tc-norfloxacin eliminates mainly through urinary system and gastrointestinal tract as unlabelled antibiotic does [16]. Increased accumulation of the tracer by the liver may be also due to the content of colloid in the radiopharmaceutical, which was about 5%. In our opinion, intense uptake of the tracer by kidneys and liver may potentially complicate imaging of an abdomen infection. High uptake in these organs suggests that the tracer is nonspecifically taken up by these tissues. Results of our biodistribution study are in agreement with previous works [8].

Results of the study which were performed in rats with intramuscular infection or sterile inflammation showed the ability of ^{99m}Tc-norfloxacin to accumulate specifically in sites of infection. Thus, infected/background ratios of rats with visible tracer uptake in infected muscle were maximum on delayed (18 h post injection) images. At the same time, in rats with sterile inflamed muscle, the tracer uptake was not visible in all stages of the experiment and inflamed/background ratios didn't change significantly. Converse results for ^{99m}Tc-norfloxacin were reported by Ibrahim I.T et al [8]. The inability of the tracer to differentiate between infection and sterile inflammation was demonstrated in radiometric biodistribution study. Differences with our results may be due to different methods used for ^{99m}Tc-norfloxacin radioactivity measurement (radiometric versus scintigraphic) in a site of infection, differences in methods for infection modeling and bacterial count in abscess.

The results of our study have shown the principal possibility of using ^{99m}Tc-norfloxacin for inflammation/infection imaging. Our findings are in line with several previous in vitro and in vivo studies describing possibilities of radio-labeled fluoroquinolones, and in particular norfloxacin, for diagnosis of infection [2, 4, 7, 9].

CONCLUSION

In this study ^{99m}Tc-norfloxacin with high radiochemical purity and low colloid content was synthesized. Disk diffusion method demonstrated that ^{99m}Tc-norfloxacin retains bactericidal properties of unlabelled norfloxacin. In vitro binding studies demonstrated small degree of the tracer binding with S. aureus and ability of ^{99m}Tc-norfloxacin to bind with dead as with live bacteria. However, studies which were performed in vivo in rats with a model of thigh muscle infection showed good ability of the radiopharmaceutical to detect sites of infection. ^{99m}Tc-norfloxacin is a promising radiopharmaceutical for infection imaging.

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