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REVIEW

Ciprofloxacin: from infection therapy to molecular imaging

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Abstract

Diagnosis of deep-seated bacterial infection remains a serious medical challenge. The situation is becoming more severe with the increasing prevalence of bacteria that are resistant to multiple antibiotic classes. Early efforts to develop imaging agents for infection, such as technetium-99m (^{99m}Tc) labeled leukocytes, were encouraging, but they failed to differentiate between bacterial infection and sterile inflammation. Other diagnostic techniques, such as ultrasonography, magnetic resonance imaging, and computed tomography, also fail to distinguish between bacterial infection and sterile inflammation. In an attempt to bypass these problems, the potent, broad-spectrum antibiotic ciprofloxacin was labeled with ^{99m}Tc to image bacterial infection. Initial results were encouraging, but excitement declined when controversial results were reported. Subsequent radiolabeling of ciprofloxacin with ^{99m}Tc using tricarbonyl and nitrido core, fluorine and rhenium couldn't produce robust infection imaging agent and remained in discussion. The issue of developing a robust probe can be approached by reviewing the broad-spectrum activity of ciprofloxacin, labeling strategies, potential for imaging infection, and structure–activity (specificity) relationships. In this review we discuss ways to accelerate efforts to improve the specificity of ciprofloxacin-based imaging.

Keywords Ciprofloxacin · Therapy · Infection · Antibacterial agent · Broad spectrum · Imaging · Nuclear medicine

Introduction

Antibiotic research was highly praised from 1930 to 1970, as this time period was declared to be the golden age of antibiotic development. Indeed, most of the antibiotic classes in current use were developed then. However, the enormous consumption of antibacterial agents has driven the emergence of bacterial resistance to sulfonamides, β-lactams, and macrolides; resistance now limits the efficacy of antibiotics in general. For some indications, bacterial resistance threatens our ability to control infection. A new round of research is occurring to develop novel antibiotics and improve existing agents.

With the quinolone class, a significant advancement occurred in the late 1970s and early 1980s with the addition of a fluorine moiety to the C6 position, thereby producing norfloxacin. Soon after, the N1 ethyl was changed to cyclopropyl, giving rise to ciprofloxacin, a compound with greater activity and a broader spectrum. In subsequent years, a variety of quinolone derivatives have entered the clinical and veterinary markets [1] and it was identified as second

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largest antibacterial class which share 16.6% global antibacterial drugs market [2].

Ciprofloxacin and other fluoroquinolones share a common mechanism of action that begins with the trapping of gyrase and topoisomerase IV on DNA as a ternary complex. The targets of fluoroquinolones are widely distributed among bacterial species, which allows the fluoroquinolones to have broad-spectrum activity [3]. Ciprofloxacin and other fluoroquinolones are important for controlling infections of lungs, joints, bones, airways, and the urinary tract. Other chronic infections, such as infectious diarrhea, anthrax, and intra-abdominal infections are also being commonly addressed with ciprofloxacin. Broad-spectrum antibacterial potential of ciprofloxacin, later on in 1990s, were considered for bacterial infection imaging by labeling it with ^{99m}Tc.

Initial reports indicated that ^{99m}Tc labeled ciprofloxacin accumulates specifically at sites of bacterial infection rather than at inflammation lesions [2, 4–9], and for a decade (~ 1995–2005) the agent was marketed under the trade name Infecton®. The excitement disappeared when a lack of specificity toward bacterial infection was reported [4, 8, 9].

Recent excitement in the field of infection imaging has emerged from several technical developments. For example, a positron emission tomography/computed tomography (PET/CT) integrated technique is being developed for imaging a broad spectrum of infections, including fever of unknown origin (FUO), spondylitis, discitis or osteitis associated with metallic implants [10, 11]. Other examples include imaging infections caused by *Mycobacterium tuberculosis, Staphylococcus aureus*, and *Escherichia coli* using metabolic tracers, such as the glucose analogue, 2-[^{18}F]-fluoro-2-deoxy-d-glucose (FDG) [12, 13], ^{18}F-2-fluorodeoxy sorbitol (^{18}F-FDS) [14, 15], para-mannitol, 6-^{18}F-fluoromaltose, ^{18}F-manitol and *p*-aminobenzoic acid (PABA) [16], and ^{99m}Tc labeled antimicrobial peptides such as ^{99m}Tc-Ubiquicidin 29–41 [17]. Nevertheless, work is continuing with ^{99m}Tc-fluoroquinolones [18, 19] because the agents are easy to prepare and they have high selectivity and specificity in some situations [20, 21]. Moreover high sensitivity is possible due to the high resolution of integrated SPECT/CT cameras and ^{99m}Tc labeling with a wide variety of available fluoroquinolone derivatives. To address the potential of ciprofloxacin, both as an imaging and a therapeutic agent, we discuss the quinolone mechanism of action, labeling strategies with various radionuclides, and infections imaging results both in animals and patients.

Overview of ciprofloxacin synthesis

Since their discovery in the early 1960s [22], the quinolone class of antibiotics has evoked considerable clinical and scientific interest. Nalidixic acid (Fig. 1), the first of the class to be used clinically, was discovered as an impurity during the synthesis of quinine. This agent was found to be bacteriostatic at low concentration and bactericidal at higher concentration [23]. The compound exhibited a narrow spectrum of clinical activity that focused on Gram-negative bacteria.

Structurally, the two rings of nalidixic acid each contain a nitrogen atom, which makes the compound a naphthyridone rather than a quinolone. From this progenitor compound two drug classes emerged, the quinolones and the naphthyridones. These classes were developed by stepwise modification in the basic structure, in many cases using the well-known Gould–Jacobs method [24], to improve efficiency and reduce cost [25, 26].

Ciprofloxacin, a member of the third generation quinolones (norfloxacin is a common representative of the second generation), was recognized as having particularly good activity against Gram-negative bacteria [27]. Although few of the fluoroquinolone substituents can be assigned unique functions, several dominant features have been identified in Fig. 2.

Clinical properties

Ciprofloxacin, launched into clinical practice in 1987, is listed as an essential medicine by the World Health Organization (WHO). Since its introduction to clinical practice, more than 250 million patients have been treated along with enormous research investigation—as reflected in more than 32,000 publications [28]. Ciprofloxacin is administrated both through intravenous and oral routes.

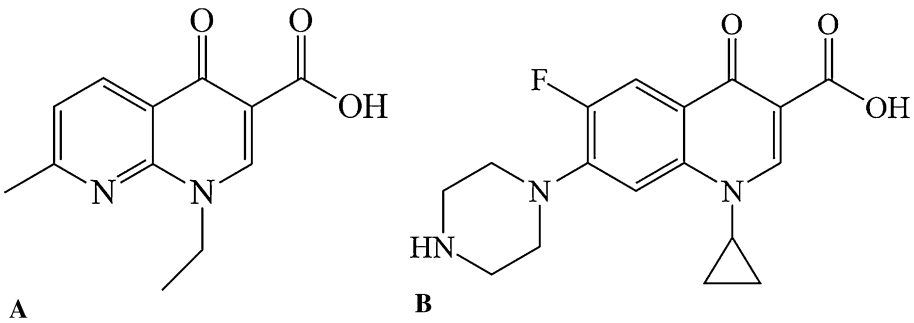

Fig. 1 Structure of nalidixic acid (**a**) and ciprofloxacin (**b**)

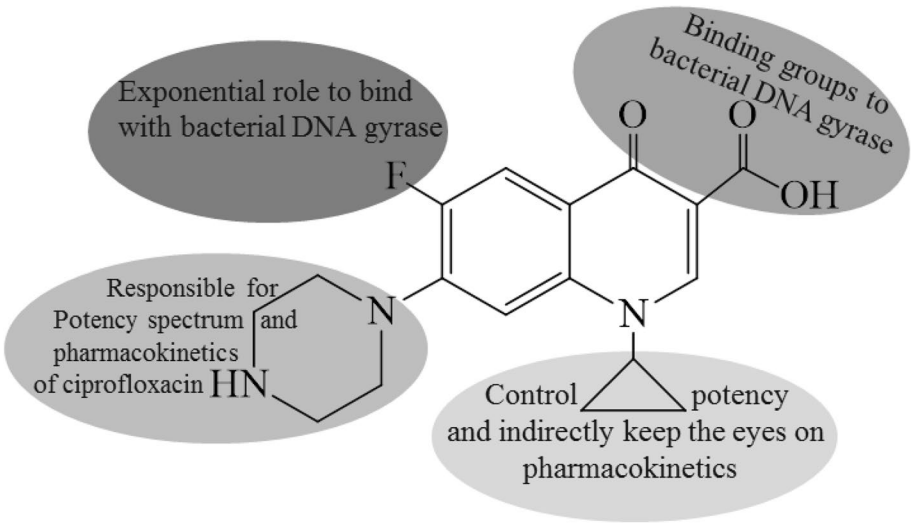

Fig. 2 Structure activity relation of different groups of ciprofloxacin

Pharmacokinetic studies indicate that drug absorption from the gastrointestinal tract provides 70–85% bioavailability, reaches a peak plasma concentration within an hour [29–35], and shows low plasma protein binding (25–30%) [31]. The pharmacokinetic characteristics are rapid and effective distribution within patients, and significant concentration in infected tissues, which can exceed serum levels by several fold [31, 36]. Metabolic studies reveal that about 10% of the absorbed dose metabolizes via conjugation in the liver, while 30–50% is excreted unaltered via urine (when administered intravenously, the excreted value increases to > 57% and peak plasma concentration is reached within one minute [29]). Neutrophils may play a role in the distribution of ciprofloxacin by up-taking and releasing it at infection sites, thereby contributing to the therapeutic value of ciprofloxacin (some other antimicrobial agents, such as β-lactams, do not penetrate into neutrophils [36]).

Ciprofloxacin reaches infectious tissues directly through body fluids (Fig. 3a) or encapsulation by neutrophils (Fig. 3b). The later moieties phagocytose foreign particles and microorganisms (Fig. 3c) to deactivate or eradicate by killing [37]. However, some microorganisms resist to phagocytosis-mediated killing and damage neutrophils (Fig. 3e). That releases the fluoroquinolone into the intracellular matrix where the bactericidal agent (the second-line of defense) encounters the pathogen. The damaged neutrophils are then released into blood (Fig. 3f), recognized by the liver, and expelled as non-functional moieties. This release of damaged neutrophils is the basis of a biochemical test for infection. Ciprofloxacin-loaded neutrophils work efficiently to kill bacteria, and the dead-cell debris is expelled through an exocytosis process (Fig. 3d) [38].

Mechanism of action

As with other fluoroquinolones, ciprofloxacin acts by trapping the type II topoisomerases, DNA gyrase and topoisomerase IV, on DNA. These enzymes solve topological problems associated with DNA biology, including chromosome replication [40]. The ternary drug–enzyme–DNA complexes (cleaved complexes) rapidly inhibit DNA synthesis but reversibly complexes (Fig. 3g) [39]. Cell death derives from chromosome breakage, in part from the accumulation of toxic reactive oxygen species [41–44].

Rationale for the use of technetium-99m-labeled ciprofloxacin

Radiolabeled (Indium-111 (^{111}In) or ^{99m}Tc) white blood cell (WBC) imaging is successful for imaging a variety of infections, [45, 46] but, as pointed out above, it cannot be used to distinguish bacterial infection and inflammation [5, 45, 47–49]. Radiolabeled monoclonal and polyclonal antibodies were also tested to differentiate between infection and inflammation, but these also tend to localize at sites of inflammation. Moreover, ^{99m}Tc-antibodies were not better than tagged leukocytes in terms of specificity for bacterial infections [47]. In contrast, fine-needle aspiration appears to give a definitive diagnosis of infection; however, it is invasive and difficult to apply with critically ill patients [50].

^{99m}Tc-labeled ciprofloxacin has been observed to discriminate non-invasively between infection (a microbiological process) and sterile inflammation (a histological

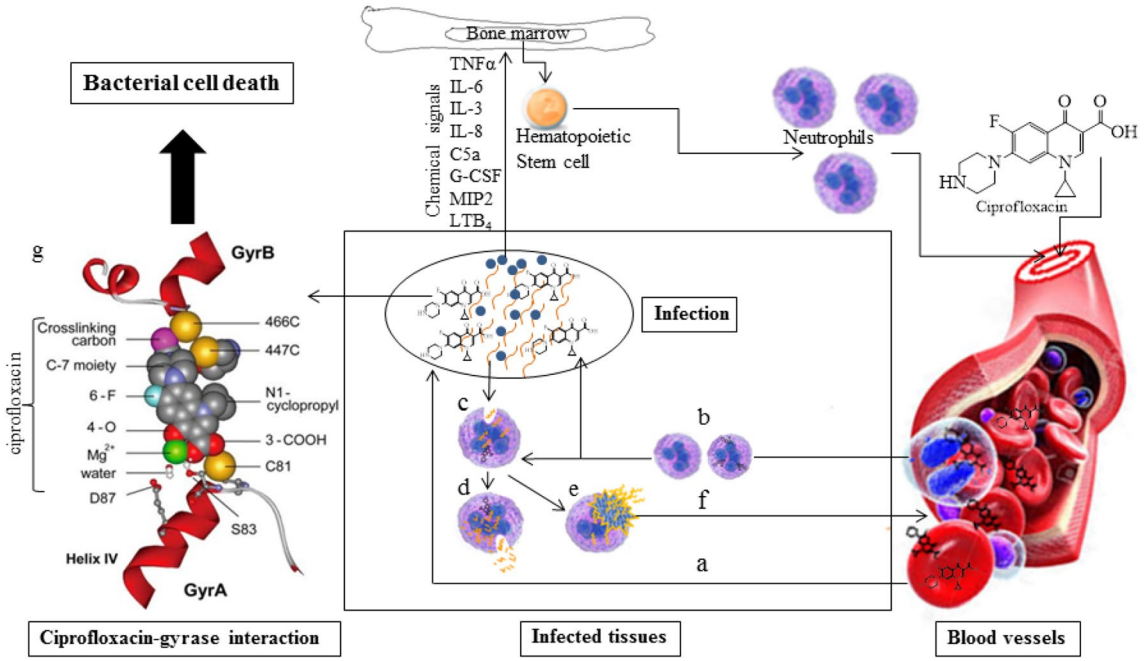

Fig. 3 Mechanism of action of ciprofloxacin (*a*) release of ciprofloxacin at infected tissues; (*b*) release of ciprofloxacin at infected tissues through neutrophil encapsulation; (*c*) engulfing of bacteria by ciprofloxacin loaded neutrophils; (*d*) release of dead bacteria by the action of neutrophil and ciprofloxacin loaded neutrophils; (*e*) release of live bacteria after killing neutrophils; (*f*) entrance of dead neutrophils into blood that make the bases for biochemically detection of infections through blood test; and (*g*) showing the binding of ciprofloxacin with bacterial DNA gyrase [39]

event) [51]. Sterile inflammation is a common histological event, which is triggered by physical, chemical or metabolic noxiae (in radiopharmaceutical development studies it is commonly induced using chemicals or heat-killed bacteria) [52]. There are several reasons to distinguish infection from inflammation, the most important of which is to make sure the presence of infection before starting antibiotic therapy which eliminate the chance of unnecessary use of antibacterial agents and consequently the appearance of microbial resistance [53, 54]. In case of deep-seated bacterial infections e.g. intra-abdominal abscesses, osteomyelitis, and endocarditis,most of the imaging methods show conflicting informations, whereas ^{99m}Tc-ciprofloxacin diagnose precisely [8]. In vitro experiments and infection induced animal model studies, ^{99m}Tc-ciprofloxacin showed promising accumulation at bacterial infection foci while at sterile inflammation only blood pool activity has been reported [2, 55]. The infection imaging specificity of ^{99m}Tc-ciprofloxacin in animal models is shown in Table 1 [56–58]. Below we review labeling strategies and investigations of imaging in patients that will helpful to conclude imaging specificity and validation of ^{99m}Tc-ciprofloxacin.

Table 1 Infection imaging potential of ^{99m}Tc-ciprofloxacin using infection induced animal models

Radio-pharmaceutical yield (%)	Stability in serum (%)	Bacterium tested	Animal model	T/NT ratio	Reference
78		*S. aureus*	Rat	NA	[59]
NA	> 95	*S. aureus* *M. tuberculosis*	Rabbit	NA	[60]
> 90%	84.2%	*S. aureus*	Mouse	3.23 ± 0.05	[61]
99.6	NA	*P. aeruginosa* *S. typhi* *E. coli*	Rabbit	5.425 ± 0.17 5.397 ± 0.15 4.890 ± 0.13	[62]
≥ 90	≥ 90	*S. aureus*	Rats	NA	[63]
> 95%		*S. aureus*	Rabbits	4.28	[64, 65]

Methods for radiolabeling ciprofloxacin

With the encouraging development of ^{99m}Tc-ciprofloxacin as an imaging agent and the gradually increasing popularity of non-invasive methods of infection diagnosis [66], studies were carried out in variety of animal infection models using a variety of radiolabeling methods. These strategies have been included, labeling with ^{99m}Tc (using oxo, carbonyl, or nitrido cores), and fluorine-18/fluorine-19 (^{18}F/^{19}F). In addition, the ciprofloxacin was labeled with rhenium-188 (^{188}Re) to elucidate the ^{99m}Tc-ciprofloxacin structural elucidation issue.

Ciprofloxacin labeling with technetium-99m

Labeling using technetium oxo core

Sodium pertechnetate ($NaTcO_4$), eluted from a ^{99}Mo/^{99m}Tc generator, is commonly used as a source of ^{99m}Tc for labeling ciprofloxacin. As pertechnetate, ^{99m}Tc exists in a high oxidation state (i.e. +7), the principle factor controlling the chemistry of technetium. At this oxidation state, technetium is unable to form coordination complexes with ligands. However, at lower oxidation states, it complexes with variety of ligands. The reduction in oxidation state of $^{99m}TcO_4^{-1}$ is performed by using an appropriate reducing agent. However, from its discovery in 1943, many reducing agents have been tested for obtaining appropriate oxidation state to complex with ligand molecule. The selection of a suitable reducing agent is based on lack of toxicity, water solubility, and suppression of ^{99m}Tc-colloid formation [67]. In the early period of ^{99m}Tc radiopharmaceutical development, ferric chloride and ascorbic acid were used to prepare ^{99m}Tc-labeled albumin. Subsequently, sodium borohydride, cyanoborohydride, sodium nitrite, sodium amalgam, dithiothreitol, and propionaldehyde were also tested. Most of these agents failed to reduce $^{99m}TcO_4^{-1}$ to the required oxidation state; consequently, little ^{99m}Tc bound (Table 2) [68].

Table 2 Results of reactions between TcO_4^{-1} and various reducing agents in the presence of phenyl phosphonate or nitriolotris(methylene)-triphospha ligands

Sr. #	Reducing agent	Temperature (°C)	Bound %	TcO_2 %	TcO_4^{-1} %
1	Sodium brohydride	25	50	35	15
2	Cyanoborohydride	95	0	0	100
3	Sodium nitrite	25	0.3	0	99.7
4	Sodium amalgam	25	7.0	2.0	91.0
5	Dithiothreitol	58	84.0	13.5	2.5
6	Propionaldehyde	25	41.0	1.5	57.5

Later, formamidine sulfinic acid (FSA) and stannous(II) chloride di-hydrate ($SnCl_2{\cdot}2H_2O$) showed promising reducing action during labeling of nicotinic acid. The development of ^{99m}Tc-ciprofloxacin, initially in 1993, was carried out using FSA as a reducing agent and it was favored due to its high reducing power, high radiochemical yield, and low free and hydrolyzed ^{99m}Tc. However, $SnCl_2{\cdot}2H_2O$ remained in use for the development of new tracer agents. In the same period, stannous(II) tartrate was reported to be a potentially useful reducing agent. In subsequent studies, a variety of other tin salts, such as stannous fluoride, stannous oxalate, and stannous phosphate, were tested [2] – out of these, however, $SnCl_2{\cdot}2H_2O$ and stannous tartrate were used frequently for the development of ^{99m}Tc-labeled radiopharmaceuticals. Both reducing agents showed excellent properties in terms of ease of preparation, high radiochemical purity, and biological stability of ^{99m}Tc-labeled compounds. Below we describe examples of infection imaging using FSA, $SnCl_2{\cdot}2H_2O$, and stannous tartrate as reducing agents for preparing ^{99m}Tc-ciprofloxacin.

Labeling using FSA In 1993, FSA became a prominent reducing agent for ^{99m}Tc-labeling of ciprofloxacin. Using FSA reducing agent, the labeling process required heating at 100 °C for 10 min. However, the agent showed incomplete reduction of $^{99m}TcO_4^{-1}$ which leave enough free $^{99m}TcO_4^{-1}$ that need an extra purification step (Sephadex chromatography) to obtain maximal radiochemical yield [2, 69]. The resulted radiochemical showed a specificity 71% in abdominal infection, 91% in skeletal infection, and 100% with infective endocarditis imaging [2]. In other studies, slightly different procedures were opted e.g. heating procedure, pH changes, and reducing agent concentration, to obtain a satisfactory radiochemical yield (~90%) without extra purification step and imaging specificity [63].

Labeling using $SnCl_2{\cdot}2H_2O$ This reducing agent is a widely accepted agent for routine radio-synthesis of ^{99m}Tc-labeled compounds. It was preferred over FSA for a single-vial ^{99m}Tc-ciprofloxacin cold kit under mild reaction conditions, *i.e.* incubation of labeling mixture for 10 min at room temperature, thereby producing a promising radiosynthesis yield and shelf-life. ^{99m}Tc-ciprofloxacin prepared using $SnCl_2{\cdot}2H_2O$ showed encouraging infection imaging results. For example, in a murine infection model, ^{99m}Tc-ciprofloxacin clearly accumulated in infected tissues [70]; in a 16-patient scintigraphy study, one patient showed a false negative result while 15 showed true positive results. The work showed 88% sensitivity and 85% specificity for hip infection, while for knee infection the two parameters were 100 and 50%, respectively [2, 8, 58, 71, 72]. While labeling antibiotics of other classes, $SnCl_2{\cdot}2H_2O$ was also remained a first order choice [73–79].

Table 3 Merits and demerits of FSA and Sn-salts as reducing agents in ^{99m}Tc labeled radiopharmaceuticals

Reducing agent	Merits	Demerits
FSA	Less toxic High and stable reducing power Maximum labeling activation Stable to hydrolyze	Reducing agent at high temperature Decomposes on heating Compete with added ligand to approach ^{99m}Tc; results unexpected ^{99m}Tc-complex biodistribution
Sn-salts ($SnCl_2{\cdot}2H_2O$ and Sn-tartrate)	Required no heating to reduce ^{99m}Tc Provide simple labeling methods Effective at mild pH conditions Formation of a single-component complex with distinct oxidation state Show no interference in complexation process Long shelf-life (during storage of kit) No unexpected and side ^{99m}Tc complex produce	Toxic due to presence of tin as a foreign atom Undesirable side product may form due to the reaction between tin and Tc Colloidal tin oxide interfere during biodistribution when weak ligands are used for ^{99m}Tc labelling Hydrolyzed to form polydispersed colloidal particles formation

Labeling using stannous tartrate Stannous tartrate was frequently reported in clinical studies of ^{99m}Tc-ciprofloxacin soon after the development of ^{99m}Tc-ciprofloxacin. FSA was replaced with stannous salts due to multiple serious disadvantages (Table 3) [80]. In other studies, more than 1500 patients were examined using ^{99m}Tc-ciprofloxacin in which TcO_4^{-1} was reduced using stannous tartrate; the overall sensitivity was 85.4% and specificity was 81.7% for detecting infective foci [58]. The chemistry of ^{99m}Tc reduction by the two stannous salts is similar, with the main difference being the production and elimination of counter ions. Tartrate and chloride ions are removed in different ways; tartrate is an organic moiety and offers no serious interference. However, with both reducing agents Sn(II) gets oxidized from [Sn(II) to Sn(IV)] during exposure to oxygen. That oxidation results in a decrease in Sn(II) ion concentration and therefore poor reduction of TcO_4^{-1}, loss of radiochemical yield, and low target specificity. A post-reduction step was introduced to fix this problem and obtain maximal reduction benefits [9, 81–85].

Labeling using technetium carbonyl core

To improve the infection imaging specificity, other ^{99m}Tc-labeling strategies were also introduced. One, was the use of ^{99m}Tc-tricarbonyl core [86], which does not require prior conventional $^{99m}TcO_4$-reduction, labeling with fluorine-18, and labeling with galium-67 [18, 87, 88]. In recent years, many studies have been carried out to evaluate ^{99m}Tc-tricarbonyl-ciprofloxacin radiopharmaceuticals in vitro and in vivo [64]. For example, Halder et al. conducted a competitive study between $^{99m}Tc(CO)_3$-ciprofloxacin, $^{99m}Tc(CO)_3$-nitrofuryl thiosemicarbazide (NFT) and ^{99m}Tc(V)-ciprofloxacin. The mean ratios of uptake in infected/non-infected thighs were 3.87, 3.41 and 3.17, respectively. The advantages of the fac-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ in labeling are ease of synthesis, the availability of three labile aqua groups, which ensure complex formation according to the number of electron donor moieties, small size, and inertness [64, 89, 90].

Labeling using technetium nitrido core

The $[Tc{\equiv}N]^{2+}$ core, typically isoelectronic with $[Tc{=}O]^{3+}$, exhibits a very high chemical stability toward basic ^{99m}Tc labeling reaction conditions such as oxidation–reduction reactions and pH variations. The labeling with technetium nitride core plays critical role in alteration of the biological behavior of a radiopharmaceutical as has been extensively investigated both in sterile and pyrogen free conditions [91]. Ciprofloxacin was labeled with 99mTc using $[Tc{\equiv}N]^{2+}$ core with high yield and good accumulation potential at infection foci with 1.78 infected muscle-to-normal muscle ratio [65]. Thus technetium nitrido core also opens a new avenue for the exploration of infection imaging behavior of ciprofloxacin.

^{18}F-labeled ciprofloxacin

Due to its promising sensitivity and specificity for bacterial infection, ciprofloxacin was labeled with the positron emitting radionuclide fluorine-18 as a positron-emission-tomographic (PET) agent for studies of drug pharmacokinetics and biodistribution in human tissues [28, 66]. Consequently, addition of ^{18}F expanded the imaging and diagnostic potential of radiolabeled ciprofloxacin. Furthermore, ^{18}F-ciprofloxacin can easily be prepared in two steps by a nucleophile substitution reaction, because ciprofloxacin contains a single

exchangeable fluorine atom. This labeling is more difficult with trovafloxacin, fleroxacin, and lomefloxacin, which have also been labeled with F-18, because they contain two exchangeable fluorine atoms [92–94].

Ciprofloxacin labeling using rhenium

The structure of ^{99m}Tc-ciprofloxacin is still unknown; consequently, the ^{99m}Tc-ciprofloxacin structure was examined by labeling ciprofloxacin with rhenium (Re) to correlate the lack of infection imaging specificity of ^{99m}Tc-ciprofloxacin with structural information. Re is chemically similar to ^{99m}Tc, and complexing with ciprofloxacin was carried out to obtain ^{99m}Tc-ciprofloxacin structural information [95]. The study showed that ciprofloxacin forms a bi-dentate coordination with ^{99m}Tc, one interaction through the carboxyl oxygen atom and the other through the exocyclic carbonyl oxygen atom, as shown in Fig. 4.

^{99m}Tc-ciprofloxacin infection imaging

One clinical challenge has been to identify the cause of FUO. It has been thought that 20–30% might be due to infection. Although ^{67}Ga-citrate, ^{99m}Tc-methylene diphosphonate (^{99m}Tc-MDP), ^{111}In-oxine- and ^{99m}Tc-hexamethylpropyleneamine oxime (HMPAO)-labeled autologous leukocytes and ^{99m}Tc-labeled leukocyte are useful infection imaging agents, considerable effort has been carried out to determine whether ^{99m}Tc-ciprofloxacin can replace leukocyte-based imaging. In an early investigation, 56 patients were examined for possible infection of skin and soft tissue, respiratory tract, abdomen, and skeleton. Out of 56 cases, 39 were found to be in concordance with results found with radiolabeled leukocytes, while 18 cases were not. Out of these 18 patients 9 were clinically documented to have infections; 8 showed a positive ^{99m}Tc-ciprofloxacin image, while 9 showed a negative ^{99m}Tc-leukocyte image. The other 9 also showed a positive ^{99m}Tc-ciprofloxacin image. This early, direct comparative study between ^{99m}Tc-ciprofloxacin and ^{99m}Tc-leukocyte revealed sensitivity, specificity, and accuracy of ciprofloxacin as 84, 96 and 90%, respectively, while leukocytes showed 81, 77 and 79%, respectively [5]. In a subsequent study involving 99 patients, 56 were tested with ^{99m}Tc-leukocytes, and some were given with antibiotic therapy for 5–14 days. The latter were expected to give true-negative results. For imaging with ^{99m}Tc-ciprofloxacin, the results were 83% sensitivity and 91% specificity [2]. In another study, aimed to determine the sensitivity and specificity of ^{99m}Tc-ciprofloxacin, 90 patients were administrated with 300–400 MBq ^{99m}Tc-ciprofloxacin. Whole-body images were taken at 1 and 4 h post injection. The results showed the infection-imaging sensitivity of 70.3% and specificity of 93.1% [56]. The claim regarding the specificity for viable bacterial infection was further tested in three patients with axial skeleton infection; ^{99m}Tc-labeled leukocytes failed to show a hot spot at the infection site, while in all three patients ^{99m}Tc-ciprofloxacin revealed a hot spot [57]. The usefulness of ^{99m}Tc-ciprofloxacin was further tested in 51 patients with suspected bone or joint infection. ^{99m}Tc-ciprofloxacin identified 30 as having infections. Four false positive, 20 true negative, and 2 false negative results were obtained, which indicated sensitivity, specificity, and accuracy of 94, 83 and 89%. In contrast, labeled leukocytes showed values of 63, 96 and 77%, respectively. In same study 5 out of 6 hot spots of vertebral osteomyelitis infection were detected with ^{99m}Tc-ciprofloxacin, while with tagged-leukocytes 4 hot spots were seen with lower radioactive signal [47]. In the same year (2001), Yapra et al. compared ^{99m}Tc-ciprofloxacin with Ga-67 scintigraphy in 22 orthopedic infection patients—sensitivity, specificity, and accuracy were found 85, 92 and 88% for ^{99m}Tc-ciprofloxacin while for gallium-67 the values were 78, 100 and 90%, respectively. Although in this study specificity for ^{99m}Tc-ciprofloxacin was 8% lower than for Ga-67, the former was seen as an

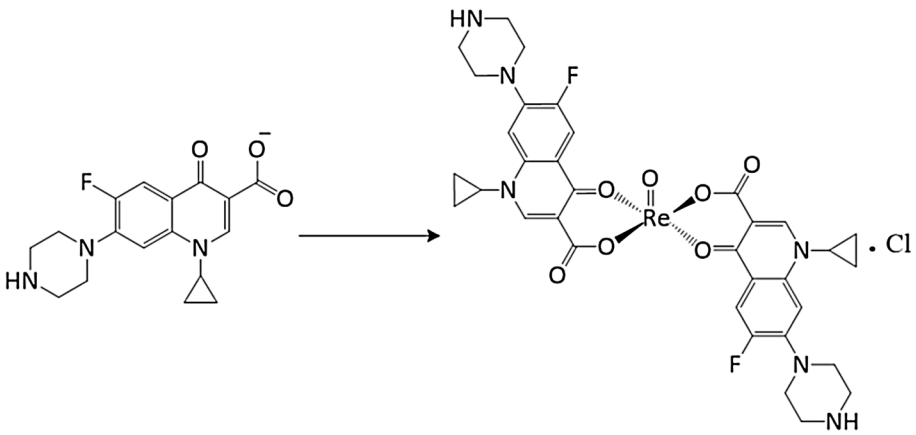

Fig. 4 Chemical structure of Re-ciprofloxacin

Table 4 Sensitivity and specificity of ^{99m}Tc-ciprofloxacin in different cases of infections during patient study reported by Britten and co-workers in 2002

Suspected infection	Number of patients studied	Sensitivity (%)	Specificity (%)
Osteomyelitis	228	90.5	72.8
Orthopaedic prosthesis	194	96	91.6
Tuberculosis	131	79.5	76.9
Soft tissue	45	82.4	72.7
Abdominal	44	87	77
Surgical wound	27	81	100
Septic arthritis	27	94.7	75
Endocarditis	26	62.5	100

attractive agent due to ease preparation, short investigation time and body compatible biodistribution [7].

Enthusiasm for developing Infecton® as an infection-imaging agent was halted when Sarda et al., reported non-specific behavior in detecting prosthetic joint infection in a rabbit model. In this work, involving 6 prosthetic joint-infected rabbits, 3 of 5 rabbits showed uptake on day 5, and all 5 on days 12 to 19; however, one did not show a response to ^{99m}Tc-ciprofloxacin. The mean infected knee to non-infected knee uptake ratio was 1.8 ± 0.4 [96].

The validity in bacterial infection imaging of ^{99m}Tc-ciprofloxacin was further tested using 879 patients from a variety of countries (Argentina, Chile, Egypt, Greece, India, Indonesia, Singapore, and UK) having suspected bacterial infections, such as osteomyelitis, orthopaedic prosthesis, tuberculosis, soft tissue, abdominal, surgical wound, septic arthritis, endocarditis, primary bloodstream, ear, nose, and throat, genitourinary, lower respiratory, enteric, and intracranial (for data see Table 4).

It is obvious from the table that Infecton® showed specificity 91.6, 100 and 100%, for orthropaedic prosthesis, surgical wound and endocarditis infections, respectively and specificity for other infections remained between 72 and 77%. However, the overall sensitivity and specificity were 85.4 and 81.7, respectively. But in subsequent reports, the good sensitivity and specificity was sometimes supported and sometimes challenged. For example, when Larikka et al. studied 16 patients using ^{99m}Tc-ciprofloxacin, sensitivity was 85% and specificity was 78%; Malamitsi et al. studied 45 patients with suspected bone infection, reporting a sensitivity of 97.2% and specificity of 80% [97]; Sarda et al. reported the results of 37 patients studied in two groups, one having septic artiritis/osteomyelitis ($n = 16$) and the second ($n = 11$) having aseptic signs. This work indicated 100% sensitivity, 37.5% specificity and 63% accuracy [8]. Appelboom et al., while studying > 100 patients with arthritis, crystal arthropathy, osteoarthritis, or renal infection, reported that ^{99m}Tc-ciprofloxacin is not a specific infection-imaging agent, but it could be used to indicate inflammation within the joints [98].Gemmel et al. reported data for 22 spinal infection patients, including 9 with deep-seated infections—the planar and SPECT scan at 3 h post injection indicated sensitivity, specificity, and accuracy of 78, 69, 73% and 100, 54 and 73%, respectively [99]. De Winter et al. reported similar findings using planar and SPECT imaging of 48 patients to evaluate postoperative spinal infection. This study showed highly specific imaging in patients who had undergone a spinal operation more than 6 months previously [100]. Singh et al. reported a case that indicated better specificity of ^{99m}Tc-ciprofloxacin than the bone-seeking agent ^{99m}Tc-MDP at the point of a fixed screw following its removal [101].

^{99m}Tc-ciprofloxacin also showed good results with tubercular bone infection imaging ($n = 14$; specificity 71%) [102], cholecystitis (specificity 75%) [81], pelvic inflammatory disease ($n = 2$, case report) [103], pulmonary tuberculosis ($n = 21$; specificity 90.9%) [60], osteoarticular tuberculosis ($n = 25$; 100% positive response) [104] and pediatric osteomyelitis ($n = 94$; specificity 100%). ^{99m}Tc-ciprofloxacin was also implemented in assessing disease activity [105, 106]. Apart from the literature reported on patient study, a variety of infections induced in animal models were also applied (described above) to sort out the spectrum-of-infection-imaging potential and specificity of ^{99m}Tc-ciprofloxacin. The results indicated target to non-target ratios ranging from 3.23 ± 0.05 to 5.425 ± 0.17 [60].

Future perspectives

^{99m}Tc-ciprofloxacin, over the period of 10 years (1995–2005), showed potential for infection imaging, but in recent reports it is considered to be a controversial tracer agent. The molecular basis of the absence of specificity has not been addressed, nor has the relationship of ^{99m}Tc-ciprofloxacin structure to activity. Indeed, preclinical experience with this agent needs to be considered in much more detail to define the mechanism of action, which will be needed to design more specific derivatives, their labeling with ^{99m}Tc and detailed screening correspond to infection imaging specificity. One immediate approach is to perform a series labeling and imaging experiments with other existed antibiotics and quinolone derivatives starting from sitafloxacin [88, 107].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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