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Technetium-99m labelled antimicrobial peptides discriminate between bacterial infections and sterile inflammations

Dear Sir,

We read with interest the above article by Welling et al. We wish to make a few points, the first two of which relate to their criticism of radiolabelled ciprofloxacin (Infecton) imaging, which lacks substantial proof.

1. Reference 11 is *wrongly cited* – it does not appear in that particular issue of the *American Journal of Medicine*.

The authors display a lack of understanding of the mechanism of action of antibiotics. *Selective toxicity* is a fundamental principle that underpins the therapeutic use of antibiotics in human medicine and ciprofloxacin is no exception to this general rule. Quinolones, including ciprofloxacin, selectively inhibit bacterial DNA synthesis by acting on the enzyme DNA gyrase (a type II topoisomerase, consisting of four subunits), which mediates the negative supercoiling of double-stranded DNA. Although human cells possess a topoisomerase II, unlike bacterial DNA gyrase, the mammalian enzyme is composed of two subunits (and not four) and more importantly is devoid of negative supercoiling activity. Therefore, it is not surprising that the human enzyme is not susceptible to inhibition by quinolones, these antibiotics being 100–1,000 times less potent against purified mammalian topoisomerase II than bacterial DNA gyrase [1, 2, 3].

2. Reference 12 is not really relevant as it deals with the problem of selection of ciprofloxacin-resistant organisms with long-term (3 months) use of this antibiotic orally to treat chronic leg ulcers. More valid would have been citation of publications dealing with ciprofloxacin-resistant organisms emerging after single dose use (in treatment or prophylaxis). Selection of resistant organisms is unlikely to be a significant problem when ciprofloxacin is used in such a tiny amount as is present in Infecton – 2 mg, which is 1/200th of the normal intravenous therapeutic dose of ciprofloxacin. Indeed, we did not encounter such a problem in a large multi-centre trial of Infecton imaging involving 879 patients in eight countries. This study [4] confirmed our previous findings [5, 6, 7] that Infecton imaging is a safe and highly specific (94% in microbiologically proven cases) technique for detecting a wide range of bacterial infections in patients, including tuberculosis

3. The authors' statement that they have tested radiolabelled antimicrobial peptides against "various" bacteria/bacterial infections is misleading, when in fact they have only used three bacterial strains – two strains of *Staphylococcus aureus* (one of which was MRSA), and one of *Klebsiella pneumoniae*. Extrapolation of their data to cover infections due to other bacterial species should, therefore, be viewed with caution.

4. The statement (first paragraph, page 293) that "...antimicrobial peptides remain the first choice for the development of new radiopharmaceuticals for infection imaging" is rather bold, unproven, premature and a matter of opinion. There are still many unanswered questions about these important molecules, including specificity for bacteria, pharmacokinetics and toxicity [8].

Infection imaging is an important area for research and development, and should ultimately enhance patient care. Healthy competition is therefore to be welcomed. Welling et al.'s habit of trying to undermine techniques that have not been developed by themselves, without providing good evidence and incorrectly citing references, goes against the spirit of good scientific research. Similar concern has already been raised about their recent article in *The Journal of Nuclear Medicine* [9].

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Dear Sir

We would like to reply to the specific concerns raised by Das et al. [1] about our recent paper in the *European Journal of Nuclear Medicine* [2]. A similar discussion on the characteristics of ^{99m}Tc-labelled ciprofloxacin and antimicrobial peptides for infection detection has been published elsewhere [3].

Concerns about ^{99m}Tc-labelled ciprofloxacin for infection detection

In our paper [2] we indicated that binding of radiolabelled ciprofloxacin to DNA of both bacteria and host cells and the emergence of antibiotic-resistant bacteria are possible drawbacks of use of this agent for infection detection [4]. These concerns are disputed by Das et al. [1]. Before responding to their criticism we need to clarify the confusion regarding reference [11] in our paper. This publication appeared in the supplementary issue of volume 87 of the *American Journal of Medicine* in 1989 [5]. If, however, Das et al. had been really interested in this publication, a single search in the index of that journal would have solved this problem. We apologise for not correctly mentioning this fact in our paper.

The following paragraph is in response to the suggestion of Das et al. that we are unaware of the mechanism of action of quinolones on bacteria. We want to pursue this issue because it provides an explanation for the main limitation of radiolabelled ciprofloxacin for infection detection, i.e. its inability to discriminate between infections and sterile inflammatory processes. We do agree with Das et al. that quinolones, including ciprofloxacin, inhibit DNA gyrases and, with less sensitivity, mammalian topoisomerase II. These enzymes introduce continuously negative supercoils into bacterial DNA, thus permitting DNA replication, transcription, repair and chromosomal segregation. The quinolone is able to insert itself within the gyrase-DNA pocket and binds both components through a number of attractive forces. Hydrogen bonds between the quinolone and the nucleotide bases of the DNA strand appear to be a strong contributor and most likely lipophilic, and Van der Waals interactions further stabilise the complex. It is proposed that the C-7 substituent of the quinolone molecule may be essential in direct quinolone-gyrase associations [6]. Additionally, the N-1 substituent of the molecule may contribute to a drug-drug self-association in which quinolone molecules adopt a stacked configuration within the DNA strands. This elegant model has been suggested by Shen et al. to explain the interaction of quinolone with bacterial as well as mammalian DNA [7]. In agreement with these data, experimental evidence has been published showing that radiolabelled ciprofloxacin prepared according to the instructions published by the group of Britton et al. [8] also interacts with DNA of both bacteria and mammalian cells [3]. Furthermore, several research groups, including ours, recently reported that ^{99m}Tc-labelled ciprofloxacin significantly accumulates at sites of sterile inflammatory processes in laboratory animals and patients [9, 10]. In addition, to our knowledge, results of preclinical studies with ^{99m}Tc-ciprofloxacin or an adequate radiochemical analysis of this agent have never been published. We think that such data, rather than data from a large number of patients who were selected on the basis of a microbiologically proven infection, are required before a tracer that distinguishes infections from sterile inflammations can be considered for use in humans. Based on the above-mentioned considerations, we believe that the claim of Britton et al. [8] that bacterial infections are specifically detected by ^{99m}Tc-labelled ciprofloxacin is premature. In addition, stability of the ^{99m}Tc-labelled ciprofloxacin complex has been proven for up to 8 h after labelling [8]; however, this group has evaluated many scintigrams of patients at 24 h after injection [11, 12, 13].

We agree that emergence of drug-resistant bacteria due to application of a single-dose injection with ciprofloxacin as the tracer is a minor concern. It should be realised, however, that repeated injections of this tracer are necessary when one wants to monitor the efficacy of antimicrobial treatment. Currently, quinolones, including ciprofloxacin, are often the treatment of choice in cases of infections with (antibiotic-resistant) Gram-negative bacteria. However, the increasing incidence of bacterial strains with decreasing susceptibility to these agents is a matter of concern. Since resistance to ciprofloxacin occurred in bacteria grown in the presence of sub-inhibitory concentrations of the drug [4], we would not advocate the use of ciprofloxacin for diagnos-

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