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Detecting inflammation with ^{131}I-labeled ornidazole

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Abstract

The aim of this study was to demonstrate the accumulation of ^{131}I-labeled ornidazole (^{131}I-ORN) in experimental abscesses. ^{131}I-ORN was prepared by electrophilic radioiodination of ORN, using radioiodide in the presence of Iodogen®. An in vivo inflammation model was prepared by intramuscular injection of turpentine into the thigh of rabbits. Four days later ^{131}I-ORN was intravenously administered to rabbits. Serial scintigrams were performed at different periods, using a Sophy DX Gamma Camera. ^{131}I-ORN was visualized at 10 min after injection. ^{131}I-ORN was also administered intraperitoneally to rats with turpentine-induced inflammation, for quantitative biodistribution studies. Counts of selected tissues were taken by a NaI(Tl) scintillation detector (gamma counter) after rats were decapitated. The target-to-non-target muscle ratios were 2.5, 2.6, 2.9 and 1.9 at 1, 3, 5 and 24 h, respectively.

1. Introduction

Inflammatory diseases and infections such as sepsis or abscess in various parts of body can be successfully detected by nuclear medicine imaging with some radiopharmaceuticals. [^{67}Ga] Gallium citrate is most commonly used for this diagnosis, with whole body imaging at 24, 48 and sometimes 72 h after injection of the radiotracer (Brunetti et al., 1994; Rubin and Fischman, 1996). ^{111}In-labelled leukocytes are also used for the detection of inflammatory diseases such as abscesses (Thakur et al., 1977). ^{99}Tc^{m}-citrate has been used for the scintigraphic visualization of inflammatory lesions and pancreatitis (Ercan et al., 1993). Promising new agents for scintigraphic detection of infection and inflammation include labeled liposomes, which have been widely studied for achieving controlled drug delivery and for imaging purposes. Conventional liposomes are rapidly cleared from the circulation by phagocytic cells of the mononuclear phagocyte system (MPS) (Karlowsky and Zhanel, 1992), therefore their use for diagnostic imaging is limited. However, ^{99}Tc^{m} Stealth® liposomes preferentially accumulate in abscesses, leading to very high target-to-non-target ratios (Oyen et al., 1996).

Ornidazole, a 5-nitroimidazole, sensitizes anoxic bacteria to the killing effects of gamma radiation almost as efficiently as 2-nitro derivatives (Jokipii and Jokipii, 1981). Nitroimidazoles, including metronidazole, tinidazole and ornidazole, are low molecular weight antimicrobial compounds with excellent activity against anaerobic microorganisms. Clinical studies have shown nitroimidazoles to be efficacious in the therapy of a

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variety of anaerobic infections including non-traumatic brain abscess, intraabdominal abscesses, pelvic suppuration and necrotizing soft tissue infections (Tally et al., 1981).

In this study, ^{131}I-labeled ornidazole was investigated as a useful radiopharmaceutical to image experimental inflammatory lesions in animals.

2. Materials and methods

Ornidazole (1-chloro-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol) was obtained from Roche (Germany) and Na ^{131}I was provided by the Department of Nuclear Medicine (Ege University, Turkey). All chemicals used were analytical grade, and ornidazole was pharmaceutical grade.

2.1. Radiolabeling

The radiolabeling of ^{131}I-ORN was performed using a procedure reported elsewhere (Asikoglu et al., 1998). Briefly, ornidazole (0.010 g) in ethanol (100 μL) was added into an Iodogen-coated tube. Na^{131}I (50 μL of NaOH solution containing 37–111 MB1/1–3 mCi ^{131}I) was added to this reaction mixture, the tube was capped and incubated at room temperature for 25 min, and then Na_2SO_3 (100 μL; 0.2 N) solution was added to reduce the remaining (non-reacted) iodine. The final product was sterilized by filtration through a 0.22 μm filter. The radiolabeling yield, determined by ITLC (cellulose F, 4:2:1 v/v/v *n*-butanol/water/acetic acid, R_f 0.07–0.17; 2:1:1 v/v/v isopropyl alcohol/*n*-butanol/0.2 N ammonium hydroxide, R_f 0.08–0.25), was always greater than 91%.

2.2. Imaging studies

Three male rabbits (2–3 kg) were used. A sterile inflammation was induced by injection of turpentine (0.5 mL) into the right thigh muscle. Four days later ^{131}I-ORN (400 μCi) was injected intravenously (I.V.) via an earlobe vein of the rabbit. Serial scintigrams were performed at 1, 2, 3, 4, 5, 10, 15, 30, 45 and 60 min, and 3 and 14 h after injection.

2.3. Biodistribution studies

Twelve male rats were used for quantitative biodistribution studies. Sterile inflammation was induced by injection of turpentine (0.15 mL) into the right thigh muscle of ether-anaesthetized rats. Four days later, ^{131}I-ORN (400 μCi) was injected intraperitoneally (I.P.). Three rats of each group were sacrificed by decapitation under ether anesthesia at 1, 3, 5 and 24 h after injection, respectively. Blood samples were collected at the time of decapitation. Both thighs (right thigh muscle as target, left thigh muscle as control) and organs (lungs, heart, liver, spleen and kidneys) were dissected, weighed and their radioactivity measured using a well-type NaI (Tl) scintillation detector and a multi-channel analyzer set for 364 keV γ photons. Results were expressed as % of the injected dose per gram tissue (%ID/g).

3. Results and discussion

Whole-body images (not shown) of ^{131}I-ORN in rabbits at different times after I.V. injection depicted rapid distribution throughout the body, and uptake in the inflamed area was observed within the first 10 min. Biodistribution data for ^{131}I-ORN in rats following I.P. administration are shown in Table 1. There were marked increases in uptake of radioactivity in target tissue and organs within 3 h. The target-to-non-target ratios were 2.5, 2.6, 2.9 and 1.9, at 1, 3, 5 and 24 h, respectively. Radionuclide imaging with ^{67}Ga citrate and radiolabeled leukocytes or ^{111}In-labeled nonspecific polyclonal IgG has been relatively successful for the detection of early inflammation. These techniques have required 18–24 h between injection and imaging to detect sites of inflammation (Rubin and Fischman, 1996). Our study showed that the accumulation of ^{131}I-ORN was rapid, whereas another study showed that uptake rates of ^{67}Ga in abscesses increased with time after injection of turpentine and reached a plateau within 5–7 days. Ten minutes, 24 and 72 h ^{67}Ga uptakes in abscesses were 0.92, 3.3 and 5.6%ID/g, respectively (Nitta et al., 1983). One, 3, 5 and 24 h following injection of ^{131}I-ORN in inflamed thighs, accumulated radioactivity accounted for 2.1 ± 0.5, 3.1 ± 0.7, 2.4 ± 0.9, and 0.7 ± 0.4%ID/g, respectively. There are other radiopharmaceuticals that show high target-to-non-target ratios. For example, ^{99m}Tc-liposomes accumulated in infectious and inflammatory

Table 1
Biodistribution results from ^{131}I-ORN in rats ($n = 3$)

	%ID/g of tissue (mean ± S.D.)			
Organ	1 h	3 h	5 h	24 h
Inflamed thigh	2.1 ± 0.5	3.1 ± 0.7	2.4 ± 0.9	0.7 ± 0.4
Control thigh	0.8 ± 0.3	1.2 ± 0.3	0.8 ± 0.4	0.3 ± 0.2
Lungs	2.4 ± 0.1	3.4 ± 0.6	3.1 ± 0.4	1.0 ± 0.1
Heart	1.6 ± 0.2	2.0 ± 0.1	1.9 ± 0.5	0.3 ± 0.0
Liver	1.9 ± 0.4	3.4 ± 0.2	2.9 ± 0.4	0.8 ± 0.1
Spleen	2.0 ± 0.2	2.1 ± 0.2	2.5 ± 0.3	0.8 ± 0.1
Kidneys	2.6 ± 0.6	4.4 ± 0.2	3.1 ± 0.7	0.8 ± 0.1
Blood	4.5 ± 1.0	4.3 ± 0.2	3.8 ± 0.0	1.0 ± 0.1

muscle foci over 24 h (0.59%ID/g for *S. aureus*; 1.18%ID/g for turpentine) and abscess-to-muscle ratios increased to 24.0, 41.7 and 44.5 for the respective models at 24 h postinjection (Oyen et al., 1996). However, ^{131}I-ORN has some advantages over other agents: it is inexpensive, its preparation is simple, its labeling efficiency is high, and its accumulation at the target site is rapid. The lesion accumulation data reported in this study support the conclusion that ^{131}I-ORN is a potential inflammation-seeking agent that worthy further evaluation.

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