Radiosynthesis of $^{99m}\text{Tc}(\text{CO})_3$ -Clinafloxacin Dithiocarbamate and Its Biological Evaluation as a Potential *Staphylococcus aureus* Infection Radiotracer

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Received: 16 June 2011 / Revised: 21 August 2011 / Accepted: 23 August 2011 / Published online: 9 September 2011
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Abstract

Background Clinafloxacin dithiocarbamate (CNND) was radiolabeled with technetium-99m (^{99m}Tc) using $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ and assessed for its radiochemical stability in saline and serum, its in vitro binding with methicillin-resistant *Staphylococcus aureus* (MRSA) and biodistribution in female nude mice (FNM) artificially infected with live and heat-killed MRSA.

Methods In normal saline (NS) the $^{99m}\text{Tc}(\text{CO})_3$ -clinafloxacin dithiocarbamate ($^{99m}\text{Tc}(\text{CO})_3$ -CNND) showed radiochemical stability with a maximum value of $99.10 \pm 0.20\%$ and remained stable up to 4 h ($92.65 \pm 0.18\%$).

Results In human serum at 37°C within 16 h of incubation, 14.85% side products as a result of de-tagging developed. Incubation with MRSA gave saturated binding with a maximum value of $72.75 \pm 1.20\%$. Almost six-fold higher uptake was seen in the infected muscle of the FNM as compared to the inflamed and normal muscle. The $^{99m}\text{Tc}(\text{CO})_3$ -CNND complex showed a normal route of excretion from the body of the FNM model.

Conclusion The higher stability in NS, HS, saturated in vitro binding with a live strain of MRSA and six-fold higher uptake

in the target organ showed the $^{99m}\text{Tc}(\text{CO})_3$ -CNND complex to be a potential MRSA infection radiotracer.

Keywords $^{99m}\text{Tc}(\text{CO})_3$ -Clinafloxacin dithiocarbamate complex · MRSA · Infection radiotracer

Introduction

In the early stages of infectious diseases, the diagnostic roles of the currently used techniques, such as ultrasound technology (UST), computerized tomography (CT) and magnetic resonance imaging (MRI), have proven unsatisfactory. However, nuclear medicine scintigraphy (NMS) has shown promise in the precise and early diagnosis of infection and its discrimination from inflammation. NMS has considerably improved the rate of accurate diagnosis and to a large extent decreased the chances of incorrect identification [1, 2].

Technetium-99m (^{99m}Tc)-labeled radiopharmaceuticals have further potentiated the diagnostic capabilities of NMS in the topical decade specifically for the diagnosis of deep tissue infection and its discrimination from inflammation. Recently, some novel specific infection radiotracers have been developed, including ours, for localization and discrimination of infection from inflammation. The effectiveness of these radiopharmaceuticals has encouraged the development of novel and more specific agents [3–11].

Recently, clinafloxacin (CNN) [7-(3-aminopyrrolidin-1-yl)-8-chloro-1-cyclopropyl-6-fluoro-4-oxoquinoline-3-carboxylic acid] has been described as a fluoroquinolone antibiotic. Figure 1a shows broad-spectrum antibiotic activity against gram-positive and -negative bacteria, aerobes and anaerobes. CNN inhibits bacterial DNA gyrase or topoisomerase IV enzyme, and consequently affects DNA replication and

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Fig. 1 **a** Structure of cinafloxacin (CNN). **b** Derivatized cinafloxacin dithiocarbamate (CNND). **c** Proposed structure of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex

transcription. CNN has been reported to show higher potency against MRSA than ciprofloxacin [12, 13].

To explore CNN's higher rate of antibiotic activity against MRSA, we carried out (1) derivatization of the CNN, (2) radiolabeling with ^{99m}Tc using $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$, (3)

checking of the radiochemical stability and (4) evaluation of in vitro binding. Further, the radiochemical and biological characteristics of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex were compared with those of the recently reported $^{99m}\text{Tc} \text{V}\equiv\text{N-CNND}$ and $^{99m}\text{Tc-CNND}$ complexes [10, 11].

Experimental

Materials

Clinafloxacin (CNN; from Laiwu Hehui Chemical Co., Ltd., Shandong, China), TLC (Merck), and all the other chemicals and solvents were of analytical grade (Sigma). A RP-HPLC (Shimadzu, Japan) well counter and scalar count rate meter (Ludlum, USA), dose calibrator (Capintech, USA) and gamma camera GKS-1000 (GEADE Nuclearmedizine system, Germany) were used.

Methods

Derivatization of Clinafloxacin

Clinafloxacin (CNN) was derivatized according to the reported method [6, 7, 11]. Briefly, 0.001 mol of CNN and 1.2 mg of sodium hydroxide were mixed with 11 ml of tetrahydrofuran (THF). The mixture was placed in an ice bath for 15 min followed by the addition of 1 ml of carbon disulfide. The mixture was again placed in an ice bath for 4 h followed by overnight stirring at room temperature. The CNND was re-crystallized from methanol/diethyl ether and characterized by FT-IR, ^1H NMR, ^{13}C NMR, ESI-MS and elemental analysis.

FT-IR/ ν : 3,400–3,250 cm^{-1} (b.s OH), 1,680 cm^{-1} (C=O), 1,620 cm^{-1} (C=N). ^1H NMR (400 MHz, DMSO) δ 9.9 (s, OH), δ 7.93 (d, $J=12.8$ Hz, 1H, $-\text{HC}=\text{C}-\text{COOH}$), δ 7.12 (s, 1H, Ar-H), δ 3.48 (dd, $J=12.8$ Hz, 2.8 Hz, 1H, $\text{CH}_2-\text{C}=\text{COOH}$), δ 0.90 (td, 11.0 Hz, 3.2 Hz, 1H $-\text{CH}_2-\text{CH}-\text{CH}$), δ 0.42 (dt, 4H, $\text{CH}_2-\text{CH}_2-\text{CH}-$), δ 2.98 (d, $J=7.5$ Hz, 2H, N- CH_2), δ 2.86 (t, $J=7.2$, 2H, N- CH_2-CH_2), δ 1.8 (dt, 9.8 Hz, 2.2 Hz, 2H, $\text{CH}-\text{CH}_2-\text{CH}_2$), δ 1.6 (td, 9.8 Hz, 7.5 Hz, 1H, =N- $\text{CH}-\text{CH}_2$). ^{13}C NMR (400 MHz, DMSO): δ 164.3 (NaS-C=N-), δ 178.2 (Ar-CO-C=C), δ 158.0 ($\text{CH}-\text{CH}=\text{C}-\text{COOH}$), δ 155.2 (F-C=C(Ar)), δ 137.8 (N-C=C(Ar)), δ 138.0 (C=C(Ar)), δ 130.1 (Cl-C=C(Ar)), δ 125 (C=C(Ar)), δ 114.5 (C=C(Ar)), δ 170 ($-\text{COOH}$), 131.0 ($\text{CO}-\text{C}=\text{C}-\text{COOH}$), δ 61.5 (N- CH_2), δ 58.8 (=N- CH), δ 50.0 (N- CH_2-CH_2-), δ 47.5 (Ar- $\text{CH}-\text{CH}$ (Cyclic)), δ 27.6 ($-\text{CH}-\text{CH}_2-\text{CH}_2$), δ 16.2 ($\text{CH}_2-\text{CH}-\text{CH}$ (Cyclic)), δ 5.3 ($\text{CH}_2-\text{CH}-\text{CH}_2$). EIMS: $M/Z=484$, ESI: 485.0180 (M+H). Elemental analysis: % C, 47.06; % H, 3.33; % Cl, 7.31; % F, 3.92; %N, 5.78; % Na, 9.48.

Synthesis of $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-CNND}$ Complex

A total of 74 MBq (0.5 ml) of sodium pertechnetate was mixed with 0.1 M HCl (for adjustment of pH to around 10) and injected into the Isolink kit followed by incubation at room temperature for 10 min. Thereafter, 2 mg of clinafloxacin dithiocarbamate (CNND) dissolved in water (0.5 ml) was injected, followed by incubation at room

temperature for 10 min. The suitability of the $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-CNND}$ complex was evaluated by comparing its stability profile in saline and serum, and its in vitro binding behavior with bacteria and in vivo biodistribution in an animal model with the recently reported $^{99\text{m}}\text{Tc-CNND}$ and $^{99\text{m}}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complexes [10, 11].

Partition Coefficient (P)

The $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-CNND}$ complex, octanol and phosphate buffer were vortexed in equal amounts for 10 min at room temperature. The reaction mixture was centrifuged at 3,000 rpm for 15 min. Thereafter, aliquots at different intervals were withdrawn and counted for radioactivity using a single well counter fitted with a scalar count rate meter (Ludlum) using the formula: $P = \text{CPM in octanol} - \text{CPM in background} / \text{CPM in buffer} - \text{CPM in background}$. The P value of the $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-CNND}$ complex was compared with the recently reported $^{99\text{m}}\text{Tc-CNND}$ and $^{99\text{m}}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complexes [10, 11].

Characterization of $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-CNND}$ Complex

HPLC was used for the characterization of the $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-CNND}$ complex in normal saline using water:methanol (W:M) as the mobile phase and C-18 column as the stationary phase. To the Shimadzu HPLC unit fitted with a UV detector (operating at 254 nm), flow scintillation analyzer, binary pump, an online degasser and C-18 (Brounlee, 150 $\times$ 4.6 mm) column, 10 μl of the freshly prepared $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-CNND}$ complex was injected, followed by elution with water:methanol mobile phase for 15 min at a flow rate of 1 ml/min. The detailed water:methanol gradient conditions were 100% water in 0–2 min, 60% water in 3–5 min, 55% water in 6–8 min, 25% water in 9–10 min, 100% methanol in 11–12 min and 50% water in 13–15 min. The radio-elutes were collected in separate vials at different intervals and counted for activity in each vial (containing radio-elutes) using a single well counter fitted with a scalar count rate meter. The HPLC profile of the $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-CNND}$ complex was compared with the $^{99\text{m}}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complex [11].

Stability in Serum

The TLC technique was used to investigate the stability of the $^{99\text{m}}\text{Tc}(\text{CO})_3\text{-CNND}$ complex (0.2 ml, 37 MBq) in human serum (1.8 ml) at 37°C for 16 h. During incubation for the evaluation of stability, aliquots were withdrawn and spotted on the TLC strips. After drying, the strips were developed in a mobile phase [$\text{CH}_2\text{Cl}_2:\text{CH}_3\text{OH}$ (9:1) (v/v)]. Then the developed strips were equally divided into two parts, and the activity in each part of the strip was counted using a single well counter fitted with a scalar count rate

meter. The stability behavior of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex was compared with the reported $^{99m}\text{Tc-CNN}$ and $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complexes [10, 11].

Binding with Methicillin-Resistant *Staphylococcus aureus*

Binding with methicillin-resistant *Staphylococcus aureus* (MRSA) was assessed using the reported method [14]. Briefly, 0.1 ml of the freshly prepared $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex was mixed with sodium phosphate buffer in a sterilized test tube followed by addition of 0.8 ml of 0.01 M acetic acid (50%, v/v) containing approximately 1×10^8 colony-forming units (CFU) of MRSA at 4°C. The reaction mixture was incubated for 1 h, and the pH was set to 5. Thereafter, the mixture was centrifuged at 3,000 rpm for 15 min. Subsequently, the supernatant was removed, and the mixture was dissolved in 2 ml sodium phosphate buffer. Then, the mixture was centrifuged at 3,000 rpm for 15 min, followed by the removal of the supernatant and measurement of the radioactivity in the MRSA using a single well counter fitted with a scalar count rate meter. In vitro binding affinity of $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex with bacteria was compared with the reported $^{99m}\text{Tc-CNN}$ and $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complexes [10, 11].

Biodistribution in Female Nude Mice

In vivo percent distribution of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex was studied in female nude mice (FNM) artificially infected with live and heat-killed MRSA. Ten healthy FNM (weight: 30–35 mg) were selected and equally divided into two groups (A and B) followed by intramuscular injection (IM) of sterile turpentine oil to the left thigh. Thereafter, 0.2 ml of the live MRSA was IM injected into the right thigh of group A and heat-killed into group B. After 18 h, 0.2 ml of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex was intravenously (IV) injected into all the FNM in group A and B. The FNM in group A and B were then killed in accordance with the rules of the Nuclear Medicine Research Laboratory (NMRL), University of Peshawar (Part I and II). For determination of percent uptake, 1 g each of the blood, liver, spleen, stomach, intestine, kidney, infected muscle, inflamed and normal muscle of the group A and B FNM was accurately obtained and counted for activity in a single well counter fitted with a scalar count rate meter.

Results and Discussion

Radio-Geometry of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ Complex

Clinfloxacin (CNN) (Fig. 1a) was converted to its dithiocarbamate derivative (CNND) (Fig. 1b) and then

complexed with $^{99m}\text{Tc}(\text{CO})_3$ to give $(^{99m}\text{Tc}(\text{CO})_3\text{CNND})$ (Fig. 1c) by the method reported earlier. The structure of the $^{99m}\text{Tc}(\text{CO})_3\text{CNND}$ complex (Fig. 1c) can be predicted by comparison with the reported structure of the ^{99m}TcN core [15].

Although technetium can have a number of oxidation states, the +V state seems to be the most common in TcN complexes. The two sets of molecular orbitals (MO) will be the highest occupied non-bonding MO (HOMO), and the lowest two unoccupied ones degenerate π^* antibonding orbitals (LUMO). The arrangement of these will give a square pyramidal structure of the TcN complexes. The crystal structural studies on TcN complexes revealed that when the four atoms involved in coordination are π -donor Lewis bases, then a square pyramidal (sp) structure is preferred [16, 17]. Based on the ^{99m}TcN comparison, the structure of $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ (Fig. 1c) with tetradentate (two bidentate sites) ligand will have a square bipyramidal structure with a $^{99m}\text{Tc}(\text{CO})_3$:ligand ratio of 2:1.

Characterization and Purity

The coalesced HPLC profile of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ and $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complexes is shown in Fig. 2. The red radiochromatogram trace characterized $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ and the blue $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complex. Two distinctly variable peaks at 5.5- and 13.8-min retention were observed in the red trace. The peak observed at 13.8-min retention expressed the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex, while in the blue trace also two distinctly variable peaks at 5.1 and 12.9 min were observed. The signal at 5.1-min retention represented the intermediate fraction ($[^{99m}\text{Tc}^{\text{V}}\equiv\text{N}]^{2+}$) and at 12.9 min the $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complex (Fig. 3).

Fig. 2 HPLC radiochromatogram of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ and $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complexes. [The red trace represents $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex (I) and the blue $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complex (II)]

Fig. 3 Stability of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$, $^{99m}\text{TcN-CNND}$ and $^{99m}\text{Tc-CNN}$ complexes in normal saline

In NS the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex showed normal stable behavior with a maximum radiochemical purity of $99.10 \pm 0.20\%$ after 30 min of reconstitution. A decrease in the radiochemical purity was noted with time and reached $92.65 \pm 0.18\%$ within 4 h after reconstitution. The radiochemical purity of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex prepared using the $[\text{^{99m}Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ technique was found to be higher than the complex prepared directly and through the $[\text{TcN}]$ core [10, 11]. The stability of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$, $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ and $^{99m}\text{Tc-CNN}$ complex in NS at 30, 60, 90, 120 and 240 min after reconstitution is shown in Fig. 4.

Partition Coefficient (P)

The participation coefficient (P) value of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex was measured as 0.46 ± 0.03 . In comparison it was observed that the P value of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex was higher than that of the $^{99m}\text{Tc-CNN}$ (-1.04 ± 0.01) complex [10] and lower than that of the $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ complex (1.09 ± 0.02) [11]. These P values suggested that the $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ and $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complexes were lipophilic, whereas the $^{99m}\text{Tc-CNN}$ complex was hydrophilic. It was observed that labeling through the $[\text{^{99m}Tc}(\text{CO})_3]^+$ core lowered the lipophilicity of the complex as compared to $[\text{^{99m}Tc}\equiv\text{N}]^{2+}$ analogue.

Stability in Serum

A stable profile of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex in human serum at 37°C up to 16 h was observed. The ^{99m}Tc

Fig. 4 Stability of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$, $^{99m}\text{TcN-CNND}$ and $^{99m}\text{Tc-CNN}$ complex in serum at 37°C up to 16 h

($\text{CO})_3\text{-CNND}$ complex was more than 90% stable up to 240 min of incubation. However, the stability decreased, and the appearance of unknown fractions increased with time and reached 14.85% within 16 h. The $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex showed higher stability in serum as compared to the $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ and $^{99m}\text{Tc-CNN}$ complexes. The unwanted side product level in $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ and $^{99m}\text{Tc-CNN}$ complexes was 15.35% and 18.15%, respectively [10, 11]. The stability of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$, $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ and $^{99m}\text{Tc-CNN}$ complexes in human serum at 37°C for up to 16 h of incubation is shown in Fig. 4.

Binding with Methicillin-Resistant *Staphylococcus aureus*

The $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$, $^{99m}\text{Tc}^{\text{V}}\equiv\text{N-CNND}$ and $^{99m}\text{Tc-CNN}$ complexes showed saturated in vitro binding with MRSA, as shown in Fig. 5. However, a higher rate of in vitro binding was observed with the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex. The maximum binding of MRSA with $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ was $72.75 \pm 1.20\%$.

Biodistribution in Female Nude Mice

In vivo %ID/g of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex in female nude mice (FNM) artificially infected with live and heat-killed MRSA is given in Table 1. The %ID/g of the $^{99m}\text{Tc}(\text{CO})_3\text{-CNND}$ complex in the blood, liver, spleen, stomach and intestines showed a decreasing pattern in which the values were initially found to be high, but diminished with time. In kidneys, the opposite behavior was seen wherein the uptake was increased with 120 of p.i. In

Fig. 5 In vitro binding of *Staphylococcus aureus* with complex I, II and III. [I: ^{99m}Tc -CNN, II: ^{99m}Tc N-CNND and III: $^{99m}\text{Tc}(\text{CO})_3$ -CNND]

Fig. 6 Ratio-wise distribution in infected, inflamed and normal muscle

the infected muscle of the FNM of group A, an almost six-fold higher uptake was seen as compared to the inflamed and normal muscles. No substantial distinction in the uptake of the $^{99m}\text{Tc}(\text{CO})_3$ -CNND complex in the blood, liver, spleen, stomach, intestines and kidneys of the FNM in group B was seen. In addition, no significant difference in the uptake profile of the $^{99m}\text{Tc}^{\text{V}}\equiv\text{N}$ -CNND and ^{99m}Tc -CNN complex was observed. Ratio-wise distribution of the $^{99m}\text{Tc}(\text{CO})_3$ -CNND, $^{99m}\text{Tc}^{\text{V}}\equiv\text{N}$ -CNND and ^{99m}Tc -CNN complexes in the infected, inflamed and normal muscles of the FNM is shown in Fig. 6. Similarly, like the $^{99m}\text{Tc}^{\text{V}}\equiv\text{N}$ -CNND and ^{99m}Tc -CNN complexes, the $^{99m}\text{Tc}(\text{CO})_3$ -CNND also showed a normal route of excretion.

Conclusion

Clinafloxacin dithiocarbamate (CNND) radiolabeling with ^{99m}Tc was assessed using the $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ technique and evaluated in terms of radiochemical stability in saline, serum, in-vitro binding with methicillin-resistant *Staphylococcus aureus* (MRSA) and biodistribution in female nude mice (FNM) artificially infected with live and heat-killed MRSA. The higher stability in normal saline, human serum, saturated in vitro binding with a live strain of MRSA and six-fold higher uptake in the target organ showed the $^{99m}\text{Tc}(\text{CO})_3$ -CNND complex to be a potential MRSA infection radiotracer.

Table 1 Biodistribution of the $^{99m}\text{Tc}(\text{CO})_3$ -CNND complex in female nude mice model (FNM)

Organs/tissues (g)	Percent absorption of the $^{99m}\text{Tc}(\text{CO})_3$ -CNND at different time (min.)							
	Group A				Group B			
	30	60	90	120	30	60	90	120
Infected muscle	6.50±0.44	10.65±0.38	15.10±0.38	12.45±0.40	3.00±0.36	3.50±0.44	3.00±0.34	3.00±0.40
Inflamed muscle	3.00±0.00	3.50±0.38	3.50±0.42	3.00±0.40	3.00±0.40	4.00±0.42	3.50±0.38	3.00±0.38
Normal muscle	3.00±0.34	2.50±0.44	2.50±0.32	2.50±0.38	3.00±0.40	3.00±0.36	3.00±0.44	2.50±0.32
Blood	19.00±0.44	11.45±0.32	10.20±0.40	4.50±0.00	19.20±0.30	10.70±0.44	9.85±0.38	4.30±0.40
Liver	18.85±0.30	12.45±0.44	10.00±0.34	5.50±0.44	18.90±0.42	12.00±0.34	9.90±0.42	5.00±0.34
Spleen	10.55±0.42	8.00±0.44	6.90±0.30	4.95±0.42	10.20±0.34	8.45±0.40	6.55±0.38	5.00±0.32
Kidney	8.55±0.40	15.40±0.36	18.65±0.34	23.75±0.36	9.10±0.44	16.00±0.32	19.15±0.42	24.00±0.44
Stomach and intestines	8.75±0.32	7.10±0.44	5.95±0.00	4.40±0.00	8.40±0.34	7.35±0.00	5.50±0.44	4.50±0.34

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