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IN VIVO STUDY OF RADIOLABELED FLAVONOID 99mTc-QUERCETIN AS CANCER RADIOTRACER ON NORMAL BALB/C MICE

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Abstract
[OBJECTIVE] Cancer is a major health problem and it is estimated that more than 10 million new cases of cancer diagnosed worldwide with more than 4 million deaths, annually. Chemotherapy is still the primary choice in cancer modality that uses chemotherapeutic drugs to eradicate and inhibit the growth of cancer cells. This treatment has excellent reliability and effective to kill cancer cell, but its cost is high. Therefore, patient tends to seek alternative treatment such as consuming traditional herbal medicine. Abundant presence of flavonoid in natural product used as traditional herbal medicines that have an interesting bioactivities needs to be studied. Quercetin (3,3′,4′,5,7-pentahydroxyl-flavone) is a flavonoid compound found in many fruits and vegetables that have antioxidant activity. As an antioxidant compound, quercetin will protect the body from free radical that can increase the risk of disease. However, as traditional herbal medicine, its effectiveness is not yet been fully established due to the lack of scientific information. Many in-vitro studies have proven the effectiveness of quercetin as an anticancer compound, but the data from in-vivo study is still limited. In recent years, several radioisotopes have been utilized for biodistribution studies of biologically important natural product because nuclear medicine techniques provide some advantages over conventionally used methods in term of detection sensitivity and availability. This study was conducted as a preliminary study to understand biodistribution pattern of 99mTc-quercetin on normal balb/c mice. In vivo data from this study would provide meaningful biological and pharmacological information of 99mTc-quercetin for understanding its effectiveness as for development of quercetin as anticancer agent.
[METHODS] 99mTc-quercetin was prepared by addition of 30 µl (1 mg/ 1 mL) of solution SnCl2.2H2O into a glass vial containing 320 µL quercetin solution (0,5 mg/320 µL) and 200 µL 0.2 M phosphate buffer pH 7.5. pH of the mixture was adjusted to 7.5 by addition of NaOH 0,1 N and final volume of the mixture was carried out into 600 uL by addition of bidistilled water. Thereafter, 400µL (± 0,5 mCi) of freshly eluted 99mTcO4- was added into the vial and incubated within 5 minutes in room temperature.
Radiochemical yield of 99mTc-quercetin was determined using thin layer chromatography using TLC-SG F254 strips with two solvent systems to distinguish and quantify the amounts of radioactive contaminants (free 99mTcO4, 99mTcO2). Chromatography system of TLC-SG F254 / acetone was used to separate impurities of 99mTcO2, while TLC-SG F254/NaCl 0.9 % was used to separate free 99mTcO4. Radioactivity on chromatograms strips was measured using TLC-scanner (AR-2000, BIOSCAN).
Animal studies were conducted in accordance with our institutional guidelines and were approved by Ethics Committee for Care and Use of Experimental Animal - National Nuclear Energy Agency. Biodistribution studies were performed by intravenous administration of a 0.1 mL 99mTc-quercetin (2.6 µCi/100 µL) to 5-week-balb/C mice (BIOFARMA). Groups of three mice were used for the experiments. Organs of interest were removed, weighed, and the radioactivity was determined with an automatic-well γ counter (2470 Wizard, PERKIN ELMER) at 15 minutes, 1, 3, and 24 h post-injection. Urine and feces were collected for 24 h post injection, and the radioactivity counts were determined

[RESULTS AND DISCUSSION]: Labeling efficiency of the 99mTc-quercetin was assessed by thin layer paper chromatography. In TLC-SG F254 using saline as the solvent, free 99mTc moved with the solvent front, while 99mTc-quercetin and 99mTcO2 remained at the spotting point. 99mTcO2 was determined by using TLC-SG F254 / acetone as the mobile phase where the 99mTcO2 at the point of spotting while free 99mTc and 99mTc-quercetin moved with the solvent front. The radiochromatogram of 99mTc-rutin was presented in Fig 1. and 2. 99mTc-quercetin had labelling efficiency > 90 % and can be used to carry out in vivo test.

Figure 1. Chromatogram profile of 99mTc-quercetin and TcO4- with TLC-SG F254 /saline

Figure 2. Chromatogram profile of 99mTc-quercetin and TcO2 with TLC-SG F254 /acetone

Figure 3. Biodistribution study of 99mTc-quercetin

Biodistribution study in normal mice showed that the radioactivity levels in the stomach were below 1%ID up to 24 h post-injection, indicating that 99mTc-quercetin was stable in vivo. The uptake of 99mTc-quercetin in kidney in 15 minutes was 6.13±1.05 %ID/g and remain 3.31±0.29 %ID/g at 24 hour post injection. After 15 minutes, 1 hour, 3 hour and 24 hour post injection the radioactivity levels on blood was 3.39±0.21 %ID/g, 1.81±0.54 %ID/g 1.59±0.30 %ID/g and 0.46±0.07 %ID/g respectively. These results suggested that 99mTc-quercetin had fast rate of plasma clearance after administration. The biodistribution study of 99mTc-quercetin also demonstrated that high radioactivity accumulation was found on liver at all post injection time points, indicating that 99mTc-quercetin was lipophilic compound. Moreover the radioactivity was observed in intestine for 15 minutes, 1 hour, and 3 hours that is 1.63±1.96 %ID/g, 1.53±0.33 %ID/g, and 1.44±0.53 %ID/g then the uptake value was decreased after 24 hours to 0.54±0.20 %ID/g. This study also showed that 99mTc-quercetin was excreted through urinary and fecal excretion. Those result in the current study demonstrated that intravenously injected of 99mTc-quercetin was metabolized in the liver and moved to intestine via the bile duct.
[CONCLUSION] This study gave preliminary biodistribution data of 99mTc-quercetin in normal mice. Further studies on target accumulation of 99mTc-quercetin in animal model with cancer would provide a good basis for developing radiolabeled flavonoid as radiotracer to understand the mechanism of quercetin as anticancer.
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