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Cumulated Activity Comparison of ^{64}Cu-/^{177}Lu-labeled Anti-EGFR Antibody in Esophageal Squamous Cell Carcinoma Model.

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ABSTRACT

Objective: This work aimed at estimating kinetic parameters, and hence cumulated activity (A_C), of a diagnostic/therapeutic convergence radiopharmaceutical, namely ^{64}Cu-/^{177}Lu-labeled antibody (^{64}Cu-/^{177}Lu-cetuximab), that acts as anti-epidermal growth factor receptor (EGFR).

Methods: In mice bearing esophageal squamous cell carcinoma (ESCC) tumor, to estimate uptake (K), release rate constant (k_R), and hence cumulated activity, a kinetic model analysis was applied to recently published biodistribution data of immuno-PET imaging with ^{64}Cu-cetuximab and of micro-SPECT/CT imaging with ^{177}Lu-cetuximab, including blood and TE-8 tumor.

Results: K, k_R and A_C were estimated to be: 0.0566/0.0593 $g.h^{-1}.g^{-1}$, 0.0150/0.0030 h^{-1} and 2.3×10^{10}/4.1×10^{12} disintegrations (per gram of TE-8 tumor), with injected activity of 3.70/12.95 MBq for ^{64}Cu-/^{177}Lu-cetuximab, respectively.

Conclusions: A model is available for comparing kinetic parameters and cumulated activity of the companion diagnostic/therapeutic ^{64}Cu-/^{177}Lu-cetuximab that may be considered as a step for determining whether one can really use the former to predict dosimetry of the latter.

Keywords: ^{64}Cu-labeled PET tracer; ^{177}Lu-labeled SPECT tracer; EGFR; Kinetic model analysis; Cumulated activity.

INTRODUCTION

Theragnostics strategy relies on non-invasive quantitative immuno-positron emission tomography (immuno-PET) to select patients eligible for radioimmunotherapy. In this framework, Dr Song and colleagues recently investigated a companion diagnostic/therapeutic radiopharmaceutical acting as anti-EGFR antibody that was prepared via identical chelator, 3,6,9,15-tetraazabicyclo[9.3.1]-pentadeca-1(15),11,13-trience-3,6,9,-triacetic acid (PCTA), labeled with ^{64}Cu or ^{177}Lu (^{64}Cu-/^{177}Lu-PCTA-cetuximab) *(1).* This compound was designed for assessing EGFR expression level in ESCC tumors as well as for subsequent radioimmunotherapy. Any advance in this field is of major interest since innovative therapeutic strategies are actually needed in ESCC patients. In ESCC-tumor-bearing mice, the authors reported biodistribution data from immuno-PET imaging with ^{64}Cu-cetuximab and micro single-photon-emission computed tomography (micro-SPECT/CT) imaging with ^{177}Lu-cetuximab, including blood (i.e, the tracer input function: IF) and TE-8 tumor.

We would like to suggest that further information can be derived from Song *et al.*'s results that may prove of interest to comprehensively characterize this novel companion diagnostic/therapeutic radiopharmaceutical. Thus, the aim of this work was to estimate uptake (K), release rate constant (k_R) and, hence, cumulated activity (A_C) that is the number of disintegrations per gram of TE-8 tissue that have occurred from the time of tracer administration (zero) to (theoretically) infinity, after administration of ^{64}Cu-cetuximab and ^{177}Lu-cetuximab for immuno-PET and micro-SPECT/CT imaging, respectively. To this end, a simple model derived from a previously published kinetic model analysis was used *(2,3).* Furthermore, this study addresses the issue of determining whether ^{64}Cu-cetuximab imaging might predict ^{177}Lu-cetuximab A_C, and hence its dosimetry, in real clinical situation.

MATERIALS AND METHODS

A previously published formula was used for estimating cumulated activity (expressed per gram of TE-8 tissue: g^{-1}), including K and k_R *(3)*:

$$A_C = [\ K / (\lambda + k_R)] \times AUC_{IF} \qquad (1)$$

where AUC_{IF} is the area-under-curve of the tracer IF (i.e., total number of disintegrations per gram of blood that have occurred from the time of tracer administration to infinity, in g^{-1}). It can be derived from mean blood data obtained by Song *et al.* in TE-8 tumor model at 2, 24, 48 and 72 h after injection for ^{64}Cu-cetuximab: 20.5, 6.4, 4.4 and 2.5 %ID/g (percentage of injected radioactivity dose per gram of tissue; supplemental Table 1 in reference *(1)*). For ^{177}Lu-cetuximab, mean blood data obtained in TE-8 tumor model at 2, 24, 72 and 120 h after injection were used: 30.2, 12.1, 6.0 and 3.1 %ID/g (supplemental Table 2 in reference *(1)*). First, the decay correction of Song *et al.*'s data was removed, that is, the data were multiplied by $\exp(-\lambda \times t)$ where "λ" is the ^{64}Cu/^{177}Lu physical decay constant (i.e., Ln2/12.7 and Ln2/160 h^{-1}, respectively). Then, they were fitted with a mono-exponential decreasing function (time constant α; in h^{-1}):

$$A_b(t) = A_b(t=0) \times \exp(-\alpha \times t) \qquad (2)$$

where $A_b(t=0)$ is expressed in %ID/g. In Equation 1 AUC_{IF} is simply $A_b(t=0)/\alpha$.

The constant k_R (h^{-1}) appearing in Equation 1, can be estimated from the following formula that applies to both PET and SPECT tracer *(2)*:

$$t_{max} = \mathrm{Ln}\ [(\alpha - \lambda)/k_R] / [\alpha - \lambda - k_R] \qquad (3)$$

where t_{max} is the uptake peak of the TE-8 tumor time-activity-curve, as published by Song *et al.* (i.e., involving decay correction): t_{max} = 48 and 120 h for ^{64}Cu-cetuximab and ^{77}Lu-cetuximab, respectively *(1)*. Equation 3 can be solved for k_R by using a solver (Microsoft Excel).

The constant K ($g.h^{-1}.g^{-1}$) appearing in Equation 1, can be estimated from the following formula involving trapped tracer activity in TE-8 tumor, $A_{Trap}(t)$ *(2)*:

$$A_{Trap}(t) = K \times A_b(t=0) \ [\exp(-\alpha \times t) - \exp(-(\lambda + k_R) \times t)] / [\lambda + k_R - \alpha] \quad (4)$$

Mean tissue data published by Song *et al*. for $A_{Trap}(t)$ in TE-8 tumor were used: 17.5 and 55.7 %ID/g at t = 48 and 120 h for ^{64}Cu-cetuximab and ^{177}Lu-cetuximab, respectively (supplemental Table 1 and 2 in reference *(1)*). The decay correction of these data was removed, that is, they were multiplied by $\exp(-\lambda \times t)$ where "λ" is the ^{64}Cu/^{177}Lu physical decay constant. Note that Equation 4 does not involve free tracer in blood and interstitial volume, since the part of free tracer becomes negligible in comparison to trapped tracer at late imaging. Indeed, the value of $F \times A_b(t)$ (with $F << 1$; no unit) is much lower than that of $A_{Trap}(t)$ at t = 48 and 120 h for ^{64}Cu-cetuximab and ^{177}Lu-cetuximab, respectively (Figure 1)*(2,3)*.

Cumulated activity can also be calculated from original data (after removing decay correction) published by Song *et al*. for ^{64}Cu-cetuximab and ^{177}Lu-cetuximab in TE-8 tumor model, respectively (supplemental Table 1 and 2 in reference *(1)*). A crude estimate of A_C can be obtained by trapezoidal integration and assuming a simple radioactive decay after the last data point.

RESULTS

From Song *et al*.'s data in TE-8 model (supplemental Table 1 and 2 in reference *(1)*), α (uncorrected for physical decay) was estimated to be 0.0830 h^{-1} for ^{64}Cu-cetuximab and 0.0224 h^{-1} for ^{177}Lu-cetuximab (Figure 1, Equation 2: R = 0.99–0.98; P<0.01–0.02)*(1)*. Numerical solvation of Equation 3 provided the following estimate of k_R: 0.0150/0.0030 h^{-1} for ^{64}Cu-/^{177}Lu-cetuximab, respectively. From Equation 4, K was estimated to be 0.0566/0.0593 $g.h^{-1}.g^{-1}$ for

^{64}Cu-/^{177}Lu-cetuximab, respectively. Then, from Equation 1, A_C was estimated to be 2.3×10^{10} and 4.1×10^{12} disintegrations per gram of TE-8 tumor, with injected activity of 3.70 and 12.95 MBq, and $K/(\lambda+k_R)$ ratio of 0.8 and 8.1, for ^{64}Cu-cetuximab and ^{177}Lu-cetuximab, respectively.

For comparison, A_C obtained by trapezoidal integration of Song et al.'s TE-8 tumor data and assuming a simple radioactive decay after the last data point, was estimated to be 2.5×10^{10} and 5.3×10^{12} disintegrations per gram of TE-8 tumor, for ^{64}Cu-cetuximab and ^{177}Lu-cetuximab, respectively.

DISCUSSION

This theoretical work aimed at providing further quantitative information, including cumulated activity, regarding the companion diagnostic/therapeutic ^{64}Cu-/^{177}Lu-cetuximab from recently published biodistribution data in ESCC-tumor-bearing mice. A simple model derived from a published kinetic model analysis was used, allowing us to obtain estimates of K, k_R and, hence, A_C for ^{64}Cu-cetuximab and ^{177}Lu-cetuximab *(2,3)*.

The uptake rate constants of ^{64}Cu-/^{177}Lu-cetuximab were found to be very close: K = 0.0566/0.0593 $g.h^{-1}.g^{-1}$, respectively. In other words, labelling cetuximab with either ^{64}Cu or ^{177}Lu does not influence its trapping in TE-8 tumors. K actually represents the probability that a ^{64}Cu-/^{177}Lu-cetuximab molecule is trapped in the tissue of interest as the result of an antibody-antigen linking. It does not give any information about its further fate, such as internalization. The release rate constants of ^{64}Cu-/^{177}Lu-cetuximab were found to be low in comparison to K: k_R = 0.0150/0.0030 h^{-1}, respectively. k_R actually represents the probability that a ^{64}Cu-^{177}Lu-cetuximab molecule trapped in the tissue of interest is released from its target and returns back to blood. This probability should, additionally, take into account a possible internalization of the

antibody-antigen complex that lowers it. Furthermore, we suggest that the 5-fold difference in k_R reported for ^{64}Cu-cetuximab versus ^{177}Lu-cetuximab may be related to the fact that k_R was estimated by using peak-time values assessed with a 24/48-hour time of resolution for ^{64}Cu-/^{177}Lu-cetuximab, respectively (Equation 3). This large time of resolution very likely introduces some uncertainty measurement for k_R, especially since it is derived from a logarithmic equation (Equation 3).

Cumulated activity for ^{177}Lu-cetuximab was found to be much greater than that for ^{64}Cu-cetuximab: $A_C = 4.1 \times 10^{12}$ versus 2.3×10^{10} disintegrations per gram of TE-8 tumor. This result is strengthened by the crude estimates for A_C provided by trapezoidal integration of Song et *al.*'s original data and assuming a simple radioactive decay after the last data point: $A_C = 5.3 \times 10^{12}$ versus 2.5×10^{10} disintegrations per gram of TE-8 tumor, for ^{177}Lu-cetuximab versus ^{64}Cu-cetuximab, respectively. Note that the injected activity was greater for ^{177}Lu-cetuximab than for ^{64}Cu-cetuximab: 12.95 versus 3.70 MBq. However, the difference in A_C may also be explained from Equation 1 showing that, for close values of K and k_R, the lower the value of α and λ the greater that of A_C. It does emphasize the efficiency of radioimmunotherapy with ^{177}Lu-cetuximab investigated in ESCC-tumor-bearing mice, and we suggest that the reliable IF fitting as a mono-exponential decreasing function (Figure 1b; $R = 0.98$; $P<0.02$) is particularly relevant for assessing its dosimetry (Equation 1). Furthermore, one may argue that Equation 1 does not take into account the part of free tracer in blood and interstitial volume (F) in the A_C calculation *(3)*. However, we suggest that this part, and hence the related A_C underestimation, is negligible: F is indeed mandatorily much lower than 1, which has to be compared to the ratio $K/(\lambda+k_R)$ whose value is 8.1 for ^{177}Lu-cetuximab.

Regarding the issue of determining whether ^{64}Cu-cetuximab imaging might predict ^{177}Lu-cetuximab A_C, and hence its dosimetry, in real clinical situation, the current study showed that A_C for ^{64}Cu-cetuximab may be obtained from the computed value of k_R at uptake peak and the corresponding uptake value (Equations 1, 3, 4). Thus, theoretically, if an average A_C ratio between ^{64}Cu-cetuximab and ^{177}Lu-cetuximab has been obtained from previous experiments (with arbitrary injected activities), an estimate for ^{177}Lu-cetuximab A_C may be obtained from a single quantitative imaging session with ^{64}Cu-cetuximab. However, a main concern about this line of argument must be underlined, which is related to performing PET imaging at uptake peak of ^{64}Cu-cetuximab (Equation 3). Indeed, even if the ^{64}Cu-cetuximab IF is known in each individual (i.e., the value of α in Equation 3), the relevant time delay between ^{64}Cu-cetuximab injection and PET acquisition cannot be predicted in each individual, because, precisely, k_R is unknown. Therefore, we suggest that additional experiments, involving lower times of resolution than those reported by Song *et al.* are required for knowledge of the k_R range in a large series of individuals that can also provide the range of the A_C ratio between ^{64}Cu-cetuximab and ^{177}Lu-cetuximab (for arbitrary injected activities). If k_R is found to vary within narrow limits for ^{64}Cu-cetuximab, the uptake-peak timing might be approximately predicted in each individual and, even, an average k_R value might be used for ^{64}Cu-cetuximab A_C calculation. Furthermore, the A_C ratio between ^{64}Cu-cetuximab and ^{177}Lu-cetuximab should also vary within narrow limits for deriving ^{177}Lu-cetuximab A_C. In other words, additional experiments are required to determine whether the measurement uncertainty of the ^{177}Lu-cetuximab A_C is acceptable or not. Finally, let us note that, whatever results obtained in a preclinical model, some adjustment is required in humans.

CONCLUSION

The study of Song *et al.* showed that the companion diagnostic/therapeutic radiopharmaceutical, namely ^{64}Cu-/^{177}Lu-cetuximab, may be useful as a diagnostic tool for patient selection as well as a potent radioimmunotherapy agent *(1)*. As further evidence, although complexation and catabolism of copper and lutetium may be quite different, the current study showed that the uptake rate constants of ^{64}Cu-cetuximab and ^{177}Lu-cetuximab are very close, and their release rate constants are low in comparison with the formers. Moreover, owing to (i) a longer physical half-life of ^{177}Lu compared to that of ^{64}Cu, (ii) a longer IF life time of ^{177}Lu-cetuximab compared to that of ^{64}Cu-cetuximab, and (iii) a greater injected activity of ^{177}Lu-cetuximab compared to that of ^{64}Cu-cetuximab (12.95 versus 3.70 MBq in Song *et al.*'s experiments), cumulated activity of ^{177}Lu-cetuximab was found to be much greater (2 orders of magnitude in the current framework) than that of ^{64}Cu-cetuximab. However, the current study may be considered as a step for determining whether ^{64}Cu-cetuximab imaging might reliably predict dosimetry with ^{177}Lu-cetuximab in real clinical situation. This major issue requires additional experiments in preclinical models, of which results should be then tested in humans.

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FIGURE LEGENDS

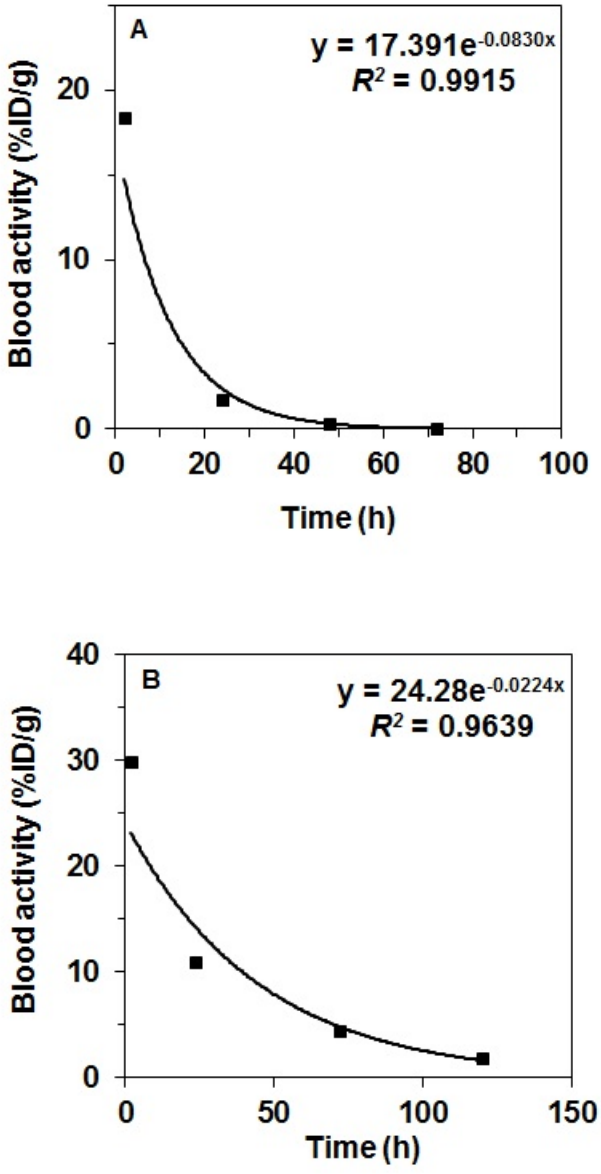

Figure 1. Decreasing mono-exponential fitting of the input function: **A)** ^{64}Cu-cetuximab (P<0.01); **B)** ^{177}Lu-cetuximab (P <0.02).