Cumulated Activity Comparison of ^{64}Cu-/^{177}Lu-Labeled Anti–Epidermal Growth Factor Receptor Antibody in Esophageal Squamous Cell Carcinoma Model

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This work aimed at estimating the 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. **Methods:** In mice bearing esophageal squamous cell carcinoma tumors, to estimate uptake (K), release rate constant (k_R), and hence A_C, a kinetic model analysis was applied to recently published biodistribution data of immuno-PET imaging with ^{64}Cu-cetuximab and of small-animal 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 $g \cdot h^{-1} \cdot g^{-1}$, 0.0150/0.0030 h^{-1}, and 2.3×10^{10}/4.1×10^{12} disintegrations (per gram of TE-8 tumor), with an injected activity of 3.70/12.95 MBq, for ^{64}Cu-/^{177}Lu-cetuximab, respectively. **Conclusion:** A model is available for comparing kinetic parameters and A_C 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.

Key Words: ^{64}Cu-labeled PET tracer; ^{177}Lu-labeled SPECT tracer; EGFR; kinetic model analysis; cumulated activity

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Theragnostics strategy relies on noninvasive quantitative immuno-PET to select patients eligible for radioimmunotherapy. In this framework, Song et al. recently investigated a companion diagnostic/therapeutic radiopharmaceutical acting as anti–epidermal growth factor receptor (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 esophageal squamous cell carcinoma (ESCC) tumors as well as for subsequent radioimmunotherapy. Any advance in this field is of major interest because 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 small-animal SPECT/CT imaging with ^{177}Lu-cetuximab, including blood (i.e., the tracer input function [IF]) and TE-8 tumor.

We suggest that further information can be derived from Song et al.'s results, which 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 small-animal 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 a real clinical situation.

MATERIALS AND METHODS

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

$$A_C = [K/(\lambda + k_R)] \times AUC_{IF}, \quad \text{Eq. 1}$$

where AUC_{IF} is the area under the 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}). A_C can be derived from mean blood data obtained by Song et al. in a 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 injected radioactivity dose per gram of tissue; Supplemental Table 1 in Song et al. (*1*)). For ^{177}Lu-cetuximab, mean blood data obtained in a 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 Song et al. (*1*)). First, the decay correction of Song et al.'s data was removed—that is, the data were multiplied by $\exp(-\lambda t)$, where λ is the ^{64}Cu/^{177}Lu physical decay constant (i.e., Ln2/12.7 and Ln2/160 h^{-1}, respectively). Then, data were fitted with a monoexponential decreasing function (time constant α, in h^{-1}):

$$A_b(t) = A_b(t = 0) \times \exp(-\alpha \times t), \quad \text{Eq. 2}$$

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*):

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$$t_{max} = \text{Ln}\ [(\alpha - \lambda)/k_R]/[\alpha - \lambda - k_R], \qquad \text{Eq. 3}$$

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 using a solver (Excel; Microsoft).

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*):

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

Mean tissue data published by Song et al. for $A_{Trap}(t)$ in a 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 Tables 1 and 2 in Song et al. (*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, because 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 (Fig. 1) (*2,3*).

A_C can also be calculated from original data (after decay correction is removed) published by Song et al. for ^{64}Cu-cetuximab and ^{177}Lu-cetuximab in a TE-8 tumor model, respectively (Supplemental Tables 1 and 2 in Song et al. (*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 a TE-8 model (Supplemental Tables 1 and 2 in Song et al. (*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 (Fig. 1, Eq. 2: R = 0.99–0.98; $P < 0.01$–0.02) (*1*). Numeric solving of Equation 3 provided the following estimate of k_R: 0.0150 and 0.0030 h^{-1} for ^{64}Cu- and ^{177}Lu-cetuximab, respectively. From Equation 4, K was estimated to be 0.0566/0.0593 $g{\cdot}h^{-1}{\cdot}g^{-1}$ 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 an 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 theoretic work aimed at providing further quantitative information, including A_C, 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 close: K = 0.0566/0.0593 $g{\cdot}h^{-1}{\cdot}g^{-1}$, respectively. In other words, labeling 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 using peak time values assessed with a 24- to 48-h time of resolution for ^{64}Cu-/^{177}Lu-cetuximab, respectively (Eq. 3). This large time of resolution very likely introduces some uncertainty measurement for k_R, especially because it is derived from a logarithmic equation (Eq. 3).

A_C for ^{177}Lu-cetuximab was found to be much greater than that for ^{64}Cu-cetuximab: 4.1×10^{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 monoexponential decreasing function (Fig. 1B; R = 0.98; $P < 0.02$) is particularly relevant for assessing its dosimetry

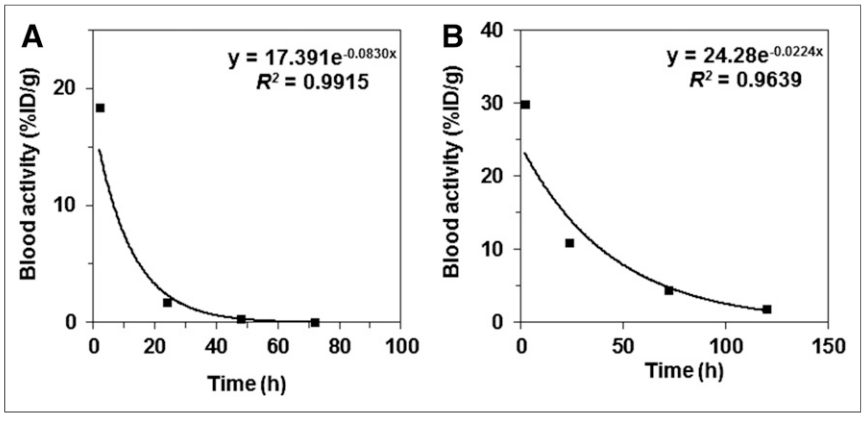

FIGURE 1. Decreasing monoexponential fitting of IF: ^{64}Cu-cetuximab ($P < 0.01$) (A) and ^{177}Lu-cetuximab ($P < 0.02$) (B).

(Eq. 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 with 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 situations, 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 (Eqs. 1, 3, and 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 (Eq. 3). Indeed, even if the ^{64}Cu-cetuximab IF is known in each individual (i.e., the value of α in Eq. 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, 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 close, and their release rate constants are low in comparison with the uptake rate constants. Moreover, because of a longer physical half-life of ^{177}Lu than ^{64}Cu, a longer IF life time of ^{177}Lu-cetuximab than ^{64}Cu-cetuximab, and a greater injected activity of ^{177}Lu-cetuximab than ^{64}Cu-cetuximab (12.95 vs. 3.70 MBq in Song et al.'s experiments), A_C 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 situations. This major issue requires additional experiments in preclinical models, of which results should be then tested in humans.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

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