Am J Nucl Med Mol Imaging 2023;13(6):295-299
www.ajnmmi.us /ISSN:2160-8407/ajnmmi0154001

Editorial

Dive into the details of radionuclide antibody conjugates: what role do EPR effects and LETs of different radionuclides play?

Sixuan Cheng[1,2], Dawei Jiang[1,2], Mengting Li[1,2]

[1]Department of Nuclear Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei, China; [2]Hubei Key Laboratory of Molecular Imaging, Wuhan 430022, Hubei, China

Received October 19, 2023; Accepted December 20, 2023; Epub December 25, 2023; Published December 30, 2023

Abstract: Radionuclide antibody conjugate (RAC) is a promising diagnostic and therapeutic tool. It combines radionuclides and antibodies by connecting arms and chelating agents, offering precise targeting and potent killing of tumor cells. However, further development and optimization of this radiopharmaceutical is needed to enhance the ultimate substantive efficacy for clinical translation. In this issue of AJNMMI, Strand et al. evaluated the enhanced permeability effect and different linear energy transfer (LET) of radionuclides in a prostate cancer xenograft model. The results showed that specific targeting might negatively influence normal organ uptake when targeting secreted antigens and different LETs of radionuclides might have diverse effects on receptor expression and cell proliferation in tumors. The findings provide new thinking for the development of antibody-based radiopharmaceuticals.

Keywords: Radionuclide antibody conjugates (RAC), enhanced permeability and retention (EPR), linear energy transfer (LET), tumor therapy, nuclear medicine

For tumor molecularly targeted therapy, monoclonal antibodies are widely recognized, and radionuclides can enhance their anti-tumor properties when combined with them. Radionuclide antibody conjugates (RACs), the ingenious fusion of radionuclides and antibodies through connecting arms and chelating agents, achieve the dual advantages of precise targeting and potent killing of tumor cells (**Figure 1**). These remarkable advancements have demonstrated definite effectiveness, safety, and acceptable toxicity, leading to prolonged survival in patients with advanced cancer [1]. They exhibit the ability to concentrate the energy released by radionuclides within several times the diameter of cancer cells. RACs combine radionuclides emitting alpha or beta rays with specific monoclonal antibodies to efficiently exterminate tumor cells, thus minimizing damage to surrounding normal tissues. However, the complex microenvironment in vivo as well as different preparation protocols for RACs may affect the final therapeutic outcome. We read with interest the article written by Joanna Strand and colleagues published in this issue of the AJNMMI [2]. In this study, Strand et al. investigated the impact of enhanced permeability and retention (EPR) effects in tumors and the influence of different linear energy transfer (LET) on substantial treatment outcomes.

Previous studies have found that radionuclide-labeled free prostate-specific antigen (fPSA)-targeted antibodies have high uptake and slow clearance rates from the liver [3]. The accumulation of antibodies in the liver is associated with antibody catabolism and FcRn-mediated recirculation [4]. Interestingly, in the present study, compared to the fPSA-targeted antibody, a nonspecific antibody that had the same scaffold and retained FcRn binding as the fPSA-targeted antibody had approximately 3 times and 2 times lower liver uptake at 24 h and 72 h. There is a possibility that RACs and fPSA form soluble immune complexes can increase liver uptake [5, 6]. Several researchers have demonstrated that the binding of monoclonal antibodies with other small molecules affects their tis-

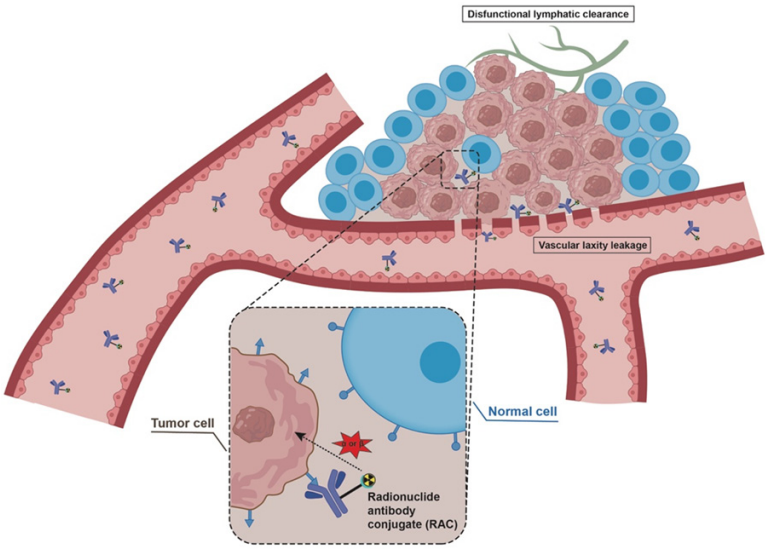

Figure 1. Schematic figure showing the Radionuclide antibody conjugate (RAC) in action. RACs are usually administered intravenously and circulate through the bloodstream, targeting tumor surface antigens and binding with them in the presence of antibodies. Meanwhile, radionuclides emit ionizing radiation to kill surrounding cells. Due to vascular laxity leakage and dysfunctional lymphatic clearance of enhanced permeability and retention (EPR) effects, RACs are more likely to accumulate in the tumor microenvironment.

sue distribution, with an increased distribution in the liver and a diminished distribution in normal and tumor tissues [7, 8]. Although fPSA has a small molecular mass compared to complex prostate-specific antigen (cPSA) (28.4 kDa vs. 90 kDa) and is cleared rapidly (t1/2 of 12-18 hours) mainly by the kidneys, studies have also shown that the liver plays a role in the elimination of fPSA [9]. These results are particularly meaningful since nonspecific uptake from the liver has a significant impact on the detection of tumor liver metastasis. In addition, hepatic radio toxicity may also result from slow liver clearance when using alfa or beta-emitting therapeutic radionuclides [10, 11]. The pursuit of attaining diminution in nonspecific background uptake by the liver in antibody-based studies has emerged as a formidable challenge, engaging researchers in an ardent struggle to overcome it. As a result, free antigens such as fPSA may pose limitations when it comes to the diagnosis and treatment of antibodies labeled with radioactive isotopes. In recent years, molecular imaging probes targeting prostate-specific membrane antigen (PSMA) have gained increased attention.

Comparing the tumor uptake of fPSA-targeted and nonspecific antibodies, the latter accounted for half of the former's tumor uptake at 24 h and 72 h, which may result from the background activity of the blood pool and the potential accumulation of the EPR effect. The EPR effect is predominantly observed in solid tumors, where macromolecules, including antibodies, accumulate in the tumor as a result of vascular laxity leakage and dysfunctional lymphatic clearance [12]. Both the EPR effect and nonspecific uptake are off-target mechanisms. Drugs have always been expected to act only on targets relevant to treating disease and not to interact with targets that are not part of the drug design. In some cases, however, ERP effects may provide a benefit to patients who wish to improve the uptake of therapeutic drugs into tumors. A new trend in improving tumor delivery is to explore ways of enhancing the EPR effect from the perspective of the drug itself and the tumor microenvironment. Intravenous drugs have to pass through the blood circulation before reaching the tumor site, and then diffuse through the capillary wall to the tumor tissue and penetrate into the deeper layers of the tumor. In order to achieve a better EPR effect, the drugs need to be stable in plasma and have a long circulation time to increase the chances of aggregation in tumor tissue while continuously passing through the tumor vasculature and being able to penetrate into the deeper layers of tumor [13]. Among these factors, long circulation and tumor penetration have the greatest impact on EPR, and both are governed by different or even contradictory drug requirements. Long circulation in the bloodstream requires antibody-related drugs to have a large molecular mass, FcRn-binding modifications, and negative charges [14]. After diffusion from capillaries to tumor sites, it is desirable to have smaller molecular mass and positively charged surfaces in order to penetrate deeper into the tumor and be effectively taken up by tumor cells [15]. In the process of antibody-based drug development, it is necessary to compre-

hensively consider the influence of various factors on the rational design of drugs in order to better realize the EPR effect. In addition to drug factors, the pathological factors, such as tumor size and type, tumor perfusion and vascular permeability, and tumor extracellular matrix can also affect the EPR effect to a large extent [16, 17]. Studies have shown that increasing NO production, utilizing cellular inflammatory factors, and modulating the extracellular matrix by combining drugs that modulate the tumor microenvironment prior to or at the time of treatment can increase the EPR effect to further achieve antitumor efficacy [18-20]. In addition, the EPR effect has been further improved by some physical therapies such as photodynamic therapy, photothermal therapy, and ultrasound therapy in the tumor local area, which significantly improves the effect of tumor treatment [21-23]. The EPR effect has become one of the main theoretical bases for targeted delivery of antitumor drugs since it was reported by Maeda et al. in 1986 [24]. However, in recent years, it has been gradually recognized that the EPR effect has limitations, and the successful translation rate of anticancer drugs designed on the basis of the EPR effect from preclinical studies to clinical use is only 6% [25]. Various factors contribute to this problem, including the heterogeneity of tumors and a lack of understanding of the complexity of the tumor microenvironment. Therefore, many scholars advocate that the EPR effects of patients can be evaluated before using drugs mediated by EPR effects, and the appropriate drugs can be given to patients with significant EPR effects. In addition to the therapeutic effect, radionuclide-labeled drugs have the potential to be used as positive reagents for EPR in the evaluation of patients, the details of which need to be followed up with further in-depth studies.

Another interesting point is that therapy studies of Lutetium-177 ([^{177}Lu]Lu) and Indium-111 ([^{111}In]In)-labeled-IgG performed in BALB/c mice showed effective but dissimilar results. Immunohistochemical analysis demonstrated a significant reduction in cell proliferation and an increase in cell death of PSA expressing cells after treatment of LNCaP xenografts with [^{111}In]In-labeled-IgG, compared to [^{177}Lu]Lu-labeled-IgG treated mice. However, [^{111}In]In emits gamma rays and its labeled compounds are used clinically mostly for diagnosis, localization, staging, and prognostic assessment. While [^{177}Lu]Lu emits beta rays with a range of about 1.5 millimeters in tissue and is a therapeutic nuclide that attracts considerate attention to the field of nuclear medicine. This phenomenon of differences in PSA expression between [^{177}Lu]Lu-IgG and [^{111}In]In-IgG treated xenografts may be attributed to the complicated process of complex internalization. The different ionic radii and coordination numbers of In^{3+} (81 picometers) and Lu^{3+} (86 picometers) may affect the interaction between the targeting molecule and the radioimmunoconjugate, the intricacies require further in-depth study. The power of RACs lies in the variety and flexibility of combinations, and their functional classification is closely related to the type of radionuclides. Because of different half-lives, ranges, and modes of exposure, they can be used for diverse diagnostic and therapeutic applications [26-29]. Presently, there is an imperative need for conducting comprehensive and meticulous investigations into the impact of antibody-conjugated intermediates coupled with different radionuclides, specifically emphasizing their influence on advanced structural modifications and biological activities of the antibodies.

Since 1951, when the U.S. Food and Drug Administration first approved Na[^{131}I]I for the treatment of thyroid disease, the realm of radiopharmaceuticals has persistently undergone development, unfurling novel dimensions and unveiling captivating frontiers. Nowadays, the research and development in the field of targeted radionuclide therapy (TRT) has entered an accelerating trajectory [30]. Several targets have garnered international attention and achieved noteworthy advancements. For example, the somatostatin receptor (SSTR) stands prominent for its application in the diagnosis and treatment of neuroendocrine tumors, and the prostate-specific membrane antigen (PSMA) takes center stage in the field of prostate cancer [31]. Notably, these targets have already witnessed the advent of diagnostic and therapeutic products in the marketplace, albeit predominantly based on small molecules or peptides coupled with radionuclides. A number of challenges are encountered in the development and evaluation of TRT-based pharmaceutical products. Although RACs have high efficacy and affinity, they also have the demerit of high immunogenicity. Furthermore, the parameters explored in this article, including the non-

specific uptake and EPR effect, as well as different LET results arising from the utilization of distinct radionuclides to label antibodies, all have various impacts on final therapeutic efficacy. Thus, it is necessary to duly consider these factors in the development of antibody-based therapeutic radiopharmaceuticals.

In conclusion, this Article shows very interesting results that the EPR effect along with different LET can significantly influence the action of therapeutic radiopharmaceuticals, which has opened the door to further in-depth research in the field of TRT drugs, encompassing RACs. These findings serve as a catalyst for both the initial stage of development and subsequent evaluation and translation endeavors, which, undeniably, persist as the most formidable challenge up to now. It is believed that in the forthcoming years, the domain of TRT drug development and its application in anti-tumor therapy shall bear witness to remarkable leaps and bounds. This will bestow great comfort to individuals struggling with tumors.

Acknowledgements

The authors are grateful for the support from the National Key Research and Development Program of China (No. 2022YFB3808200), and the National Natural Science Foundation of China (Nos. 22277031 and 82202233).

Disclosure of conflict of interest

None.

Address correspondence to: Dawei Jiang and Mengting Li, Department of Nuclear Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1277 Jiefang Ave., Wuhan 430022, Hubei, China. E-mail: daweijiang@hust.edu.cn (DWJ); limengtingtjmu@163.com (MTL)

References

[1] Ehlerding EB, Lacognata S, Jiang D, Ferreira CA, Goel S, Hernandez R, Jeffery JJ, Theuer CP and Cai W. Targeting angiogenesis for radioimmunotherapy with a 177Lu-labeled antibody. Eur J Nucl Med Mol Imaging 2018; 45: 123-131.

[2] Vilhelmsson Timmermand O, Safi M, Holmqvist B and Strand J. Evaluation of enhanced permeability effect and different linear energy transfer of radionuclides in a prostate cancer xenograft model. Am J Nucl Med Mol Imaging 2023; 13: 147-155.

[3] Veach DR, Storey CM, Lückerath K, Braun K, von Bodman C, Lamminmäki U, Kalidindi T, Strand SE, Strand J, Altai M, Damoiseaux R, Zanzonico P, Benabdallah N, Pankov D, Scher HI, Scardino P, Larson SM, Lilja H, McDevitt MR, Thorek DLJ and Ulmert D. PSA-targeted alpha-, beta-, and positron-emitting immunotheranostics in murine prostate cancer models and nonhuman primates. Clin Cancer Res 2021; 27: 2050-2060.

[4] Ghetie V and Ward ES. Transcytosis and catabolism of antibody. Immunol Res 2002; 25: 97-113.

[5] Agha AH, Schechter E, Roy JB and Culkin DJ. Prostate specific antigen is metabolized in the liver. J Urol 1996; 155: 1332-1335.

[6] Johansson AG, Løvdal T, Magnusson KE, Berg T and Skogh T. Liver cell uptake and degradation of soluble immunoglobulin G immune complexes in vivo and in vitro in rats. Hepatology 1996; 24: 169-175.

[7] Boswell CA, Mundo EE, Zhang C, Bumbaca D, Valle NR, Kozak KR, Fourie A, Chuh J, Koppada N, Saad O, Gill H, Shen BQ, Rubinfeld B, Tibbitts J, Kaur S, Theil FP, Fielder PJ, Khawli LA and Lin K. Impact of drug conjugation on pharmacokinetics and tissue distribution of anti-STEAP1 antibody-drug conjugates in rats. Bioconjug Chem 2011; 22: 1994-2004.

[8] Scott AM, Tebbutt N, Lee FT, Cavicchiolo T, Liu Z, Gill S, Poon AM, Hopkins W, Smyth FE, Murone C, MacGregor D, Papenfuss AT, Chappell B, Saunder TH, Brechbiel MW, Davis ID, Murphy R, Chong G, Hoffman EW and Old LJ. A phase I biodistribution and pharmacokinetic trial of humanized monoclonal antibody Hu3s193 in patients with advanced epithelial cancers that express the Lewis-Y antigen. Clin Cancer Res 2007; 13: 3286-3292.

[9] Kilic S, Yalcinkaya S, Guntekin E, Kukul E, Deger N and Sevuk M. Determination of the site of metabolism of total, free, and complexed prostate-specific antigen. Urology 1998; 52: 470-473.

[10] Ehlerding EB, Ferreira CA, Aluicio-Sarduy E, Jiang D, Lee HJ, Theuer CP, Engle JW and Cai W. 86/90Y-based theranostics targeting angiogenesis in a murine breast cancer model. Mol Pharm 2018; 15: 2606-2613.

[11] Ferreira CA, Ehlerding EB, Rosenkrans ZT, Jiang D, Sun T, Aluicio-Sarduy E, Engle JW, Ni D and Cai W. 86/90Y-labeled monoclonal antibody targeting tissue factor for pancreatic cancer theranostics. Mol Pharm 2020; 17: 1697-1705.

[12] Wester HJ and Kessler H. Molecular targeting with peptides or peptide-polymer conjugates: just a question of size? J Nucl Med 2005; 46: 1940-1945.
[13] Kang H, Rho S, Stiles WR, Hu S, Baek Y, Hwang DW, Kashiwagi S, Kim MS and Choi HS. Size-dependent EPR effect of polymeric nanoparticles on tumor targeting. Adv Healthc Mater 2020; 9: e1901223.
[14] Pattipeiluhu R, Arias-Alpizar G, Basha G, Chan KYT, Bussmann J, Sharp TH, Moradi MA, Sommerdijk N, Harris EN, Cullis PR, Kros A, Witzigmann D and Campbell F. Anionic lipid nanoparticles preferentially deliver mRNA to the hepatic reticuloendothelial system. Adv Mater 2022; 34: e2201095.
[15] Yang ZZ, Li JQ, Wang ZZ, Dong DW and Qi XR. Tumor-targeting dual peptides-modified cationic liposomes for delivery of siRNA and docetaxel to gliomas. Biomaterials 2014; 35: 5226-5239.
[16] Zi Y, Yang K, He J, Wu Z, Liu J and Zhang W. Strategies to enhance drug delivery to solid tumors by harnessing the EPR effects and alternative targeting mechanisms. Adv Drug Deliv Rev 2022; 188: 114449.
[17] Natfji AA, Ravishankar D, Osborn HMI and Greco F. Parameters affecting the enhanced permeability and retention effect: the need for patient selection. J Pharm Sci 2017; 106: 3179-3187.
[18] Wan MM, Chen H, Da Wang Z, Liu ZY, Yu YQ, Li L, Miao ZY, Wang XW, Wang Q, Mao C, Shen J and Wei J. Nitric oxide-driven nanomotor for deep tissue penetration and multidrug resistance reversal in cancer therapy. Adv Sci (Weinh) 2020; 8: 2002525.
[19] Murata T, Lin MI, Aritake K, Matsumoto S, Narumiya S, Ozaki H, Urade Y, Hori M and Sessa WC. Role of prostaglandin D2 receptor DP as a suppressor of tumor hyperpermeability and angiogenesis in vivo. Proc Natl Acad Sci U S A 2008; 105: 20009-20014.
[20] Seki T, Saida Y, Kishimoto S, Lee J, Otowa Y, Yamamoto K, Chandramouli GV, Devasahayam N, Mitchell JB, Krishna MC and Brender JR. PEGPH20, a PEGylated human hyaluronidase, induces radiosensitization by reoxygenation in pancreatic cancer xenografts. A molecular imaging study. Neoplasia 2022; 30: 100793.
[21] Overchuk M, Harmatys KM, Sindhwani S, Rajora MA, Koebel A, Charron DM, Syed AM, Chen J, Pomper MG, Wilson BC, Chan WCW and Zheng G. Subtherapeutic photodynamic treatment facilitates tumor nanomedicine delivery and overcomes desmoplasia. Nano Lett 2021; 21: 344-352.
[22] Zuo C, Zou Y, Gao G, Sun L, Yu B, Guo Y, Wang X and Han M. Photothermal combined with intratumoral injection of annonaceous acetogenin nanoparticles for breast cancer therapy. Colloids Surf B Biointerfaces 2022; 213: 112426.
[23] Theek B, Baues M, Ojha T, Möckel D, Veettil SK, Steitz J, van Bloois L, Storm G, Kiessling F and Lammers T. Sonoporation enhances liposome accumulation and penetration in tumors with low EPR. J Control Release 2016; 231: 77-85.
[24] Matsumura Y and Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 1986; 46: 6387-6392.
[25] Germain M, Caputo F, Metcalfe S, Tosi G, Spring K, Åslund AKO, Pottier A, Schiffelers R, Ceccaldi A and Schmid R. Delivering the power of nanomedicine to patients today. J Control Release 2020; 326: 164-171.
[26] Ehlerding EB, Lee HJ, Barnhart TE, Jiang D, Kang L, McNeel DG, Engle JW and Cai W. Noninvasive imaging and quantification of radiotherapy-induced PD-L1 upregulation with 89Zr-Df-Atezolizumab. Bioconjug Chem 2019; 30: 1434-1441.
[27] Jiang D, Im HJ, Sun H, Valdovinos HF, England CG, Ehlerding EB, Nickles RJ, Lee DS, Cho SY, Huang P and Cai W. Radiolabeled pertuzumab for imaging of human epidermal growth factor receptor 2 expression in ovarian cancer. Eur J Nucl Med Mol Imaging 2017; 44: 1296-1305.
[28] Kang L, Jiang D, England CG, Barnhart TE, Yu B, Rosenkrans ZT, Wang R, Engle JW, Xu X, Huang P and Cai W. ImmunoPET imaging of CD38 in murine lymphoma models using 89Zr-labeled daratumumab. Eur J Nucl Med Mol Imaging 2018; 45: 1372-1381.
[29] Kang L, Jiang D, Ehlerding EB, Barnhart TE, Ni D, Engle JW, Wang R, Huang P, Xu X and Cai W. Noninvasive trafficking of brentuximab vedotin and PET imaging of CD30 in lung cancer murine models. Mol Pharm 2018; 15: 1627-1634.
[30] Artigas C, Mileva M, Flamen P and Karfis I. Targeted radionuclide therapy: an emerging field in solid tumours. Curr Opin Oncol 2021; 33: 493-499.
[31] Wei W, Rosenkrans ZT, Liu J, Huang G, Luo QY and Cai W. ImmunoPET: concept, design, and applications. Chem Rev 2020; 120: 3787-3851.