EVALUATION OF TWO ALBUMIN-TARGETING MOIETIES AS *IN SITU* BINDERS IN COMPARISON TO MALEIMIDE

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Aldoxorubicin represents the first clinically investigated anticancer drug that binds, upon intravenous administration, covalently to endogenous human serum albumin (HSA), acting as a nanocarrier for tumor-targeted delivery.[1] This approach relies on the introduction of a maleimide moiety to allow *in situ* binding to the free thiol of HSA, rather than the *ex vivo* synthesis of an albumin conjugate. In 2013, this concept was first transferred to metal-based anticancer agents through the introduction of maleimides as axial ligands of an oxaliplatin(IV) complex.[2] Mayr et al. further showed that such maleimide-bearing platinum(IV) derivatives yielded highly superior results in murine *in vivo* models compared to the clinically approved oxaliplatin(II) parent compound.[3] Yet, even in spite of conceptual clinical success, maleimides are subject to three major drawbacks, namely hydrolysis at physiological pH, dissociation from HSA *via* retro-Michael and thiol exchange reactions with other available thiols.

As such, this work focuses on the investigation of two albumin-targeting moieties to determine their viability as alternatives to maleimides in the *in situ*-binding approach. Therefore, either a Cys34 (methylsulfonyl oxadiazole) or a Lys64 (TAK-242 derivative) HSA-binding moiety, both developed in the group of C. F. Barbas,[4],[5] were introduced as axial ligands of oxaliplatin(IV) and tested in reference to maleimide. Our results showed that both binders exhibit superior hydrolytic stability, as determined by HPLC. Interestingly, an organ distribution study in mice, evaluated with Pt-ICP-MS, showed that the highest Pt-content in both tumor and blood serum after 24 h was found in the oxadiazole-treated animals, whereas the lowest values were attributed to the TAK-treated group. This was explained by SEC-ICP-MS measurements, revealing fast binding of the oxadiazole to albumin, comparable to maleimide, whereas the TAK-derivative exhibited very slow binding kinetics. In good agreement, *in vivo* data of CT-26-bearing Balb/c mice showed the strongest tumor regression and overall survival benefit for the oxadiazole-treated group. We therefore conclude that methylsulfonyl oxadiazole constitutes a viable alternative to maleimide for the application of endogenous albumin-binding therapeutics.

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