ABSTRACT

In this study, we addressed the biological activity and pharmacological features of selected HPMA copolymer-based drug conjugates. We determined their cytostatic activity *in vitro* as well as toxicity *in vivo* and therapeutic effcicacy in mouse tumor models. Assessment of maximum tolerated dose (MTD) of two structurally different HPMA copolymer-based conjugates bearing doxorubicin (DOX) attached via pH-sensitive hydrazon bond (HPMA-DOX^{HYD}) showed that high molecular weight non-degradable star HPMA-DOX^{HYD} conjugate possesses relatively low MTD ~22.5 mg DOX/kg, while linear HPMA-DOX^{HYD} has MTD ~85 mg DOX/kg. Thus, MTD of linear conjugate is 3.7 times higher than that of the star conjugate. Subsequently, we reported that linear conjugate proved to be more efficient in case of treatment of solid tumor EL4 lymphoma and star conjugate to be superior in case of BCL1 leukemia treatment. We also compared biological activity of star and linear HPMA copolymer-based conjugates bearing docetaxel (DTX) attached via pH-sensitive hydrazon bond (HPMA-DTX^{HYD}). MTD of star conjugate (~160 mg DTX/kg) was proved to be 4 times higher than MTD od free DTX (40 mg/kg). We were not able to determine MTD of linear conjugate as it exceeded 200 mg DTX/kg (the highest soluble dose we were able to administer as a bolus). Anti-tumor activity of both conjugates was tested in EL4 lymphoma and they proved to be superior to free DTX given at the same dose, with star conjugate to be more potent than the linear one.

Further, we have investigated binding and therapeutic activity of targeted conjugate composed of HPMA copolymer bearing pirarubicin and recombinant scFv fragment derived from BCL1 leukemia-specific B1 mAb non-covalently attached to conjugate via coiled-coil interaction of two complementary peptides $(VAALKEK)_4$/$(VAALEKE)_4$ or IAALKSKIAALKSE-$(IAALKSK)_2$/$(IAALESE)_2$-IAALESKIAALESE (abbreviated KEK/EKE or KSK/ESE, respectively). We proved that targeted conjugate exerts higher anti-tumor efficacy than non-targeted conjugate or free pirarubicin. Moreover, we compared two different pairs of complementary peptides and we showed that conjugate containing KSK and ESE peptides exerts 4 times better binding activity and 2 times higher cytotoxicity *in vitro* compared to conjugate containing KEK and EKE peptides.

In conclusion, our findings shed a light on relationship of HPMA copolymer-based drug conjugates structure and their biological and pharmacological activities. These findings might be useful in design of novel anti-cancer HMW therapeutics not only those based on HPMA copolymer.