Cellular uptake mechanism of kojic acod modified o-carborane as a boron drug toward melanoma-targeting BNCT

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Highly metastatic melanoma (melanoma) is one of the serious targets of boron neutron capture therapy (BNCT). We have reported a water soluble hydroxypropyl-β-cyclodextrin complex (CKA/HP-β-CD) of kojic acid modified o-carborane (CKA) as a potent and novel boron compound for melanoma selective BNCT drug. Evaluation of *in vitro* uptake of CKA/HP-β-CD complex revealed that much higher uptake of boron by murine melanoma B16BL6 cells was achieved than that of murine colon 26 cells and murine myoblast C2C12 cells. The uptake of CKA was suppressed one-fourth in the presence of not only excess kojic acid but also glucose in the medium. *In vivo* tumor-accumulation was confirmed with CKA/HP-β-CD complex. After one hour of administration, highest concentration and tumor/normal ratio were observed with melanoma-bearing mice. The higher antitumor effect of BNCT by using CKA/HP-β-CD was also confirmed toward melanoma-bearing mice compared with p-boryl phenylalanine (BPA). I this report, mechanism of high efficient internalization of CKA/HP-β-CD complex into melanoma cells is evaluated .

B16BL6 cells were maintained in RPMI containing 10% FBS and 1% penicillin/streptomycin. B16BL6 cells seeded on the 6 well plate at the concentration of 2.0×10^5 cells/well. The cells were exposed to CKA/HP-β-CD complex (10 ppm) and the cells were corrected at each time point (12 and 24 hr). The involvement of glucose transporter 1 (GLUT1) on the uptake of CKA is confirmed with not only a small-molecule inhibitor of GLUT1 [1] ,but also RNA interference-mediated reduction in GLUT1. The isolated cells were digested by using aqua regia with heating (95 ^{o}C, 30 min; 115 ^{o}C, 60 min). After centrifugation, the cellular uptake amounts were quantified by ICP-AES.

Clear suppression of CKA uptake by melanoma cells were observed in the presence of WZB117 inhibitor and siRNA for GLUT1. Furthermore, CKA uptake obviously reduced when melanoma cells were incubated in the normal medium with glucose compared with in the medium without glucose. These results suggested that the internalization of CKA/HP-β-CD complex into melanoma cells is enhanced with GLUT1-mediated endocytosis. Our findings indicate Kojic acid is useful for a ligand residue in order to melanoma-targeting drug delivery.

References

1. Y. Liu, C. Weihe, W. Zhang, et al., A small-molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth *in vitro* and *in vivo*., Can. Ther., 11., 1672-82 (2012).