**ORIGINAL ARTICLE**

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# Retention mechanism of hypoxia selective nuclear imaging/radiotherapeutic agent Cu-diacetyl-bis($N^4$-methylthiosemicarbazone) (Cu-ATSM) in tumor cells

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The retention mechanism of the novel imaging/radiotherapeutic agent, Cu-diacetyl-bis($N^4$-methylthiosemicarbazone) (Cu-ATSM) in tumor cells was clarified in comparison with that in normal tissue *in vitro*. With Cu-ATSM and reversed phase HPLC analysis, the reductive metabolism of Cu-ATSM in subcellular fractions obtained from Ehrlich ascites tumor cells was examined. As a reference, mouse brain was used. To determine the contribution of enzymes in the retention mechanisms, and specific inhibitor studies were performed. In subcellular fractions of tumor cells, Cu-ATSM was reduced mainly in the microsome/cytosol fraction rather than in the mitochondria. This finding was completely different from that found in normal brain cells. The reduction process in the microsome/cytosol was heat-sensitive and enhanced by adding exogenous NAD(P)H, an indication of enzymatic reduction of Cu-ATSM in tumor cells. Among the known bioreductive enzymes, NADH-cytochrome b5 reductase and NADPH-cytochrome P450 reductase in microsome played a major role in the reductive retention of Cu-ATSM in tumors. This enzymatic reduction was enhanced by the induction of hypoxia. Radiocopper labeled Cu-ATSM provides useful information for the detection of hypoxia as well as the microsomal bioreductive enzyme expression in tumor.

**Key words:** Cu-ATSM, hypoxia, bioreductive enzyme, microsome