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DEPLETION OF O^6-METHYLGUANINE-DNA METHYLTRANSFERASE ACTIVITY AND POTENTIATION OF 1-(4-AMINO-2-METHYL-5-PYRIMIDINYL) METHYL-3(2-CHLOROETHYL)-3-NITROSOUREA ANTITUMOR EFFECT BY STREPTOZOTOCIN

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O^6-methylguanine-DNA Methyltransferase (MGMT) can specifically repair the DNA demage induced by chloroethylnitrosoureas (CENU) such as 1-(4-amino-2-methyl-pyrimidinyl) methyl-3-(2-chloroethyl)-3-nitrosourea (ACNU), constituting the molecular basis of tumor cell resistance to CENU. The present study demonstrated that sensitization of resistant tumor cells to ACNU could be achieved by streptozotocin (STZ) treatment which could deplete MGMT activity *in vitro* and *in vivo*. It suggested that depletion of the molecular basis of tumor cell resistance to chemotherapeutic agents might be a practicable way to improve the effectiveness of tumor chemotherapy.

Key words: Methyltransferase, Streptozotocin, ACNU, Tumor cell line, Drug resistance.

Drug resistance remains one of the most serious problems in cancer chemotherapy. Recently, to reverse or deplete the molecular basis of tumor cell resistance to chemotherapeutic agents has become a new direction in cancer research along with the elucidation of the mechanisms involved.[1] O^6-methylguanine-DNA methyl-transferase (MGMT) can specifically repair the DNA damage induced by chloroethylnitrosoureas (CENU) such as 1-(4-amino-2-methyl-5-pyrimidinyl) methyl-3-(2-chloroethyl)-3-nitrosourea (ACNU) and 1, 3-bis-(2-chloroethyl)-1-nitrosourea (BCNU), thus constituting the molecular basis of tumor resistance to CENU. Mer^- tumor cells deficient in MGMT activity, are highly sensitive to CENU; while Mer^+ tumor cells proficient in MGMT activity, are highly resistance to CENU.[2-4] The following study aims at depleting MGMT activity by streptozotocin (STZ) pretreatment in order to increase ACNU killing effect on Mer^+ human tumor cells.

MATERIALS AND METHODS

Cell Culture

Mer^+ HeLa S3 and Mer^- HeLa MR cell lines were kindly provided by Mituo Ikenaga, Radiation Biology Center, Kyoto University, Japan. Human hepatoma SMMC-7721 and human cervical

carcinoma Cc801 cell lines were obtained from the Second Military Medical College, Shanghai and Cancer Institute, Chinese Academy of Medical Sciences, Beijing respectively. The characteristics of the four cell lines were listed in Table 1. All cells were cultured in DMEM supplemented with 10% new-born calf serum (Gibco) at 37℃, 5% CO_2.

Table 1. *Characteristics of four human tumor cell lines*

Cell line	MGMT activity (pmol/mg)	ACNU D_{10} (μg/ml)	Mer phenotype
HeLa S3	1.10	107	+
SMMC-7721	0.72	77	+
Cc801	0.39	39	+
HeLa MR	0.10	10	-

Drugs

ACNU was a gift of Dr. S. Minato, Institute of Sciences and Technology, Tokyo, Japan. STZ was obtained from Nacalai Tesque Inc., Kyoto, Japan. For *in vitro* use, drugs were dissolved in distilled water; For animal experiment, drugs were dissolved in 0.85 Nacl, pH 7.4.

Assayof MGMT Activity

Cells exposed to various concentrations of STZ at 37℃ for 1 hour were collected and assayed immediately according to the method described previously.[3] MGMT activity was expressed as pmol of methyl groups removed from O^6-methylguanine-DNA per mg of protein in the cellular extracts.

Cell Survival Assay

Appropriate numbers of cells were seeded into 6 cm diameter dishes and inubated for 20 hours to allow complete adhesion to the dishes. The cells were then exposed to one of the following drug protocols: (1) no drugs; (2) STZ only or ACNU only for 1 hour at 37℃; (3) STZ for 1 hour followed by ACNU for an additional hour at 37℃. After 10 days of incubation in fresh medium, colonies were counted. The D_{10} values, which correspond to the ACNU concentrations required to give 10% of cell survival were estimated from the survival curves and used as indexes of ACNU sensitivity.

Animal Treatment

BALB/c nude mice were purchased from the Labaratory Animal Center, Institute of Microbiology, Beijing. When the volume of tumorgrafts was in the range of 100 — 200 mm^3 within 7 — 10 days after the injection of 6×10^6 HeLa S3 cell into the flank of the nude mice, animals were randomized into four groups consisting of treatment with vehicle alone, STZ alone, ACNU alone, or STZ and ACNU in combination. Tumor volume (mm^3) was calculated from the equation: length $\times$ width^2 $\times$ 0.5.