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Cell-type-specific Augmentation of the Tumoricidal Activity of Polymeric Adriamycin Combined with Galactosamine

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The optimization of drug delivery system with approaches to a target in structure has been implicated to play a role in cancer chemotherapy, because it can reduce the adverse effects. However, this system partly reduces the direct cytotoxicity of anticancer drugs against tumor cells, in comparison to its free form. In the present study, poly (α -malic acid) adriamycin (poly ADR) coated with saccharides including galactosamine which recognizes galactose-lectin specific to hepatocytes was prepared, and its cytotoxicities against Hep G2 cells (human hepatoblastoma), AZ521 cells (human gastric cancer) and KNS cells (human lung cancer) was evaluated using an in vitro cytotoxicity assay. In both AZ521 cells and KNS cells, poly ADR as well as poly ADR coated with glucosamine, glactosamine or mannosamine provided relatively lower cytotoxicities than the free form of ADR. In contrast, Hep G2 cells were to more efficiently sensitized, compared with the free form of ADR or poly ADR combined with or without glucosamine or mannosamine ($P<0.01$, respectively). These results indicate that poly ADR coated with galactosamine used as a cell recognition element is thus applicable for targeting cancer chemotherapy in the treatment of hepatocellular carcinoma.

Key Words : polymeric drug, active targeting, saccharide poly (α -malic acid), adriamycin

Introduction

The drug delivery system contributes to an improved efficacy of cancer chemotherapy, because this system can reduce the Adverse effects (1-2). In addition, the utilization of a cell-type-specific recognition elemental device is an ideal approach for targeting cancer chemotherapy. Many attempts have been made to develop successful methods of targeting cancer chemotherapy, and recent studies have shown that saccharides function as a cell recognition motif in a variety of tumor cells (3-4). We previously reported that poly (α -malic acid) adriamycin (poly ADR) appeared to offer some advantages in cancer chemotherapy (5). However, the tissue distribution of poly ADR, when injected, was not either cell or tissue-specific in animal models. Galactose-specific asialoglycoprotein receptor is localized selectively on the surface of hepatocytes and is recognized with poly (vinylbenzyl lactonamide) (PVLA) which has galactose residue in the tip as described previously (6). In the present study, poly ADR was combined with such saccharides as glucosamine, galactosamine and mannosamine, and the cytotoxicities of these conjugates against Hep G2 cells (human hepatoblastoma), AZ 521 cells (human gastric cancer) or KNS (human lung cancer) were thus investigated using an in vitro cytotoxicity assay.

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Materials and Methods

Chemicals

The structures of poly ADR or poly ADR combined with saccharides are shown in Fig. 1. These conjugates were synthesized according to the methods reported previously (5). ADR was obtained from Kyowa Hakko Kogyo Co. Ltd (Osaka, Japan). PVLA was a generous gift from Dr. A. Akimitsu, Kanagawa Academy of Science and Technology, Kanagawa, Japan. Na ^{125}I was purchased from the Amersham Co., (Tokyo, Japan). PVLA was radiolabeled with ^{125}I by the iodogen method (7).

Uptake of ^{125}I-labeled PVLA

Tumor cells (KNS : human lung cancer, AZ521 : human gastric cancer, Hep G2 : human hepatoblastoma) were placed on a 12-well plate (1.2×10^5/ml) in 5% CO_2 at 37℃ for 24 hours. The cells were reacted with ^{125}I labeled PVLA

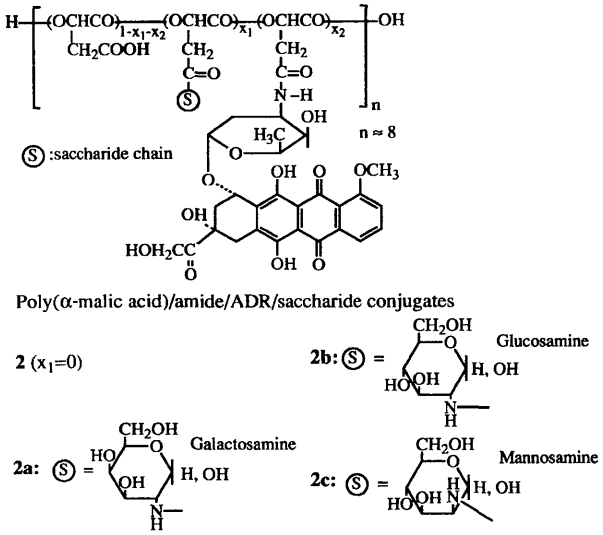

Fig. 1. Structure of poly (α -malic acid)/adriamycin/ saccharide Molecular weight is about 1,500.

(10kBq/ml) for 60 minutes. The cells were washed twice gently with PBS. The radioactivity of the cells was counted using a γ -scintillation counter. The uptake of ^{125}I-labeled PVLA was expressed according to the following formula :

The uptake of ^{125}I-labeled PVLA by the cell (%) = $C/C_0 \times 100$

C : radioactivity of the cells measured

C_0 : radioactivity of ^{125}I-labeled PVLA administrated

Cytotoxic activity of the conjugates

The *in vitro* cytotoxic activity of the conjugates against tumor cell lines (KNS, AZ521 and Hep G2) was determined by an MTT assay [3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (9). Briefly, the tumor cell suspensions (1×10^5/ml) were placed on 96 well multi-plates and incubated either with the conjugates or free ADR (8 μg equivarent ADR per well) in 5% CO_2 at 37℃. Thirty minutes later, the cells were washed twice with warmed RPMI 1640 media to remove the drugs, and incubated with fresh media without free ADR or the conjugates in 5% C O_2 at 37℃. One day later, the numbers of viable cells were determined by means of MTT assay. The cytotoxic activity was calculated based on the following formula : Cytotoxic activity (%) = $T/C \times 100$

C : number of viable cells after 24h incubation without drug.

T : number of viable cells after 24h incubation with drug.

Statistical analyses

Significant differences were assessed by a one way analysis of variance (ANOVA) or Fisher's PLSD Post-hoc test.

Results

Uptake of ^{125}I-labeled PVLA

The uptake of ^{125}I-labeled PVLA by Hep G2 were significantly higher than those by either KNS cells or AZ521 (Fig. 2). It is likely that the galactose residue in the tip of PVLA recognizes the galactose-specific lectin on the cell surface of Hep G2 cells which are known to have asialoglycoprotein receptors on the cell surface.

Cytotoxic activity of conjugates

The direct cytotoxicities of poly ADR were lower than those of free form of ADR in all tumor cell lines used in this study (Fig. 3). Poly ADR combined with any of the saccharides did not enhance the cytotoxicities against either AZ521 cells or KNS cells. Similarly, combining poly ADR with glucosamine or mannosamine did not affect the cytotoxicities against Hep G2 cells, compared with poly ADR itself. However, when poly ADR was combined with galactosamine, the cytotoxicity of this conjugate against Hep G2 cells was significantly higher than poly ADR or even free form of ADR ($P<0.01$, respectively).

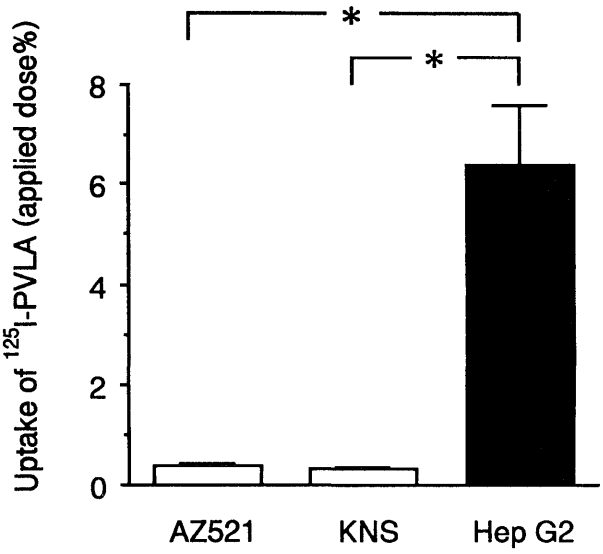

Fig. 2. Uptake of ^{125}I labeled PVLA
The data are shown as the mean ± S.D.
* $P<0.01$ by a one way analysis of variance (ANOVA) and Fisher's PLSD Post-hoc test.

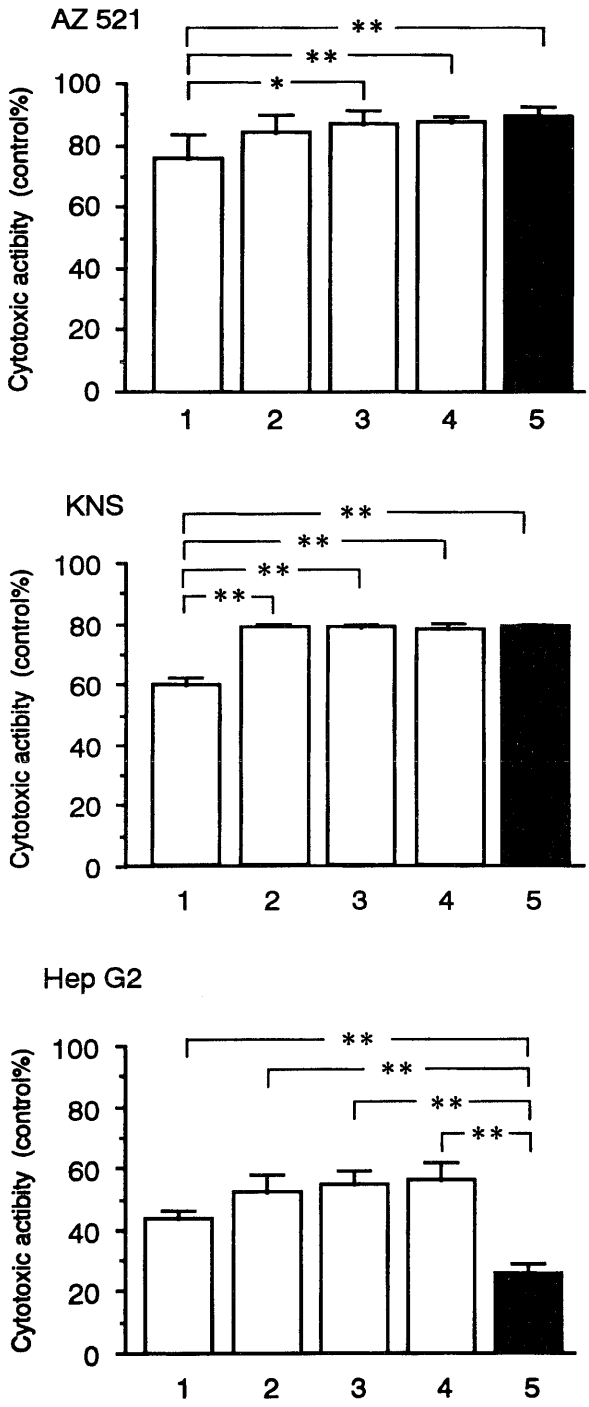

Fig. 3. Cytotoxic activity of conjugates
1 : free ADR, 2 : poly (α -malic acid)/ADR, 3 : poly (α -malic acid)/glucosamine, 4 : poly (α -malic acid)/mannosamine, 5 : poly (α -malic acid)/galactosamine
The data are shown as the mean ± S.D.
* $P<0.05$, ** $P<0.01$ by a one way analysis of variance (ANOVA) and Fisher's PLSD Post-hoc test.

Discussion

The macromolecular anticancer drugs are not able to strengthen the direct cytotoxicities against tumor cells by itself, but they can reduce the adverse effects in comparison with the low molecular anticancer drug (8). ADR can be modified by poly (α -malic acid), thus resulting in a macromolecular form poly ADR. In the present study, poly ADR was combined with saccharides (glucosamine, galactosamine, mannosamine) as a cell recognition elemental device for targeting chemotherapy. Radiolabeled PVLA which has a galactose residue in the tip was selectively incorporated into Hep G2 cells, but not in AZ521 cells or KNS cells. Since previous studies showed that Hep G2 cells had galactose-lectin specific to hepatocytes on their cell surface, PVLA thus seems to be incorporated into Hep G2 cells through this cell surface receptor (9). By in vitro cytotoxicity assay, poly ADR combined with any of the saccharides did not enhance the cytotoxic activities against either AZ521 cells or KNS cells, but rather represented relatively lower cytotoxicities, compared with the free from of ADR. Similarly, poly ADR combined with glucosamine or mannosamine did not strengthen the tumoricidal activity against Hep G2 cells. In contrast, poly ADR combined with galactosamine resulted in a more pronounced growth inhibition of Hep G2 cells than the free form of ADR or other forms of poly ADR. These results thus suggest that galactosamine acts as a cell recognition motif and also enhances the incorporation of poly ADR into Hep G2 cells, thus leading to an efficient tumoricidal effect on Hep G2 cells. For targeting cancer chemotherapy, the drug delivery system is a key factor involved in the selective accumulation of anticancer drugs in tumor cells together with minimizing systemic adverse reaction. Recent advances in diagnostic techniques now allow us to detect hepatocellular carcinoma at an early stage. Nevertheless, however, there are few surgical candidates due to either widespread intrahepatic involvement or a lack of hepatic reserve due to the coexistence of advanced cirrhosis. Our above described approach is thus considered to be potentially effective as a cancer chemotherapy modality for the treatment of hepatocellular carcinoma.

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