A cisplatin-incorporated liposome that targets the epidermal growth factor receptor enhances radiotherapeutic efficacy without nephrotoxicity.

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ABSTRACT SUMMARY

Radiotherapy is one of the major modalities for NSCLC, but its efficacy is often compromised by cellular resistance caused by various mechanisms including the overexpression of epidermal growth factor receptor (EGFR). Although cisplatin (CDDP) has been well characterized as an effective radiosensitizer, its clinical application is limited by its severe nephrotoxic effects.

In an A549 xenograft mouse model, increased delays in tumor growth were observed in the mice treated with a combination of CDDP-incorporated liposome conjugated with EGFR antibodies (EGFR:LP-CDDP) and radiation. Notably, the EGFR:LP-CDDP treated animals showed no differences in body-weight-loss of nephrotoxicity compared with untreated control mice. In contrast, the use of CDDP caused lower body weights and poorer survival outcomes accompanied by a significant level of nephrotoxicity.

These findings suggest the feasibility of using EGFR:LP-CDDP to radiosensitize cells in a targeted manner without inducing nephrotoxic effects. The EGFR:LP-CDDP may therefore have clinical potential as part of a tailored chemoradiotherapy strategy.

INTRODUCTION

Radiotherapy (RT) is commonly used for the treatment of NSCLC but tumor control and survival outcomes remain poor for affected patients due to radiotherapy resistance. Combination therapies involving radiosensitizing drugs and RT are therefore currently recommended for NSCLC cases.

CDDP is a well known radiosensitizing agent and is administered as part of a primary intervention, particularly for advanced NSCLC treatment regimens. However, the use of CDDP is often limited as it is severely nephrotoxic. CDDP metabolites also induce nephrotoxicity through a biotransformation pathway. Hence, the development of alternatives to CDDP is of great interest.

LP is well known as a classical carrier for DDS. LP improves pharmacokinetics and biodistribution of drug. A size-controlled LP can efficiently deliver the drug to tumors through enhanced permeability and retention effect (passive targeting) and protect the drug from metabolic processes that may clear it from the body prematurely.

EGFR is frequently targeted as an anticancer therapy strategy as its overexpression. EGFR overexpression plays a major role in reducing the radiosensitivity of NSCLC cells. Recently, an active targeting approach has emerged involving the display of a tumor-specific ligand or antibody on an LP. In our current study, we conjugated an EGFR antibody to an LP containing CDDP (LP-CDDP) and evaluated its ability to enhance the efficacy of targeted radiotherapy without the adverse nephrotoxic effects of CDDP.

EXPERIMENTAL METHODS

LP-CDDP was prepared as previous described. EGFRAb were displayed on the liposome surface through 3, 3-dithiobis (sulfo succinimidyl propionate). Tris was added to reach a final concentration of 132 mg/ml for terminating reaction. The amount of EGFRAb on LP-CDDP was determined by western blot analysis. The sizes of the LP-CDDP and EGFR:LP-CDDP were measured at 25°C by dynamic light scattering (DLS) using a Zetasizer Nano-ZS device. The compounds were subjected to transmission electron microscopy (TEM).

To generate xenograft model, A549 cells (1×10⁶ cells) were injected subcutaneously into the right hind leg of Balb/c-nu nude mice. When the tumors grew to about 200 mm³, the mice were divided into the experimental groups and injected intravenously with 10 mg/kg (in the dose of CDDP) of CDDP, LP-CDDP or EGFRAb:LP-CDDP. At 2 h after administration, the tumors were irradiated with 5 Gy using 6MV photon beam linear accelerator. Tumor size and body weight of mice were measured. Tumor volume (V) was calculated by the following formula: V = 0.5 × L × S². At the final day of experiment, kidneys of mice were isolated and weighed.

To evaluate kidney function, mice were treated with CDDP, LP-CDDP or EGFRAb:LP-CDDP (10 mg/kg as the dose of CDDP). At 3 days after administration, samples of blood were taken. Blood urea nitrogen (BUN) was determined using the modified Berthelot reaction. Creatinine was measured by creatinine colorimetric detection kit. To assess nephrotoxicity, Balb/c nude mice bearing an A549-derived tumor were treated with CDDP, LP-CDDP or EGFR:LP-CDDP (10 mg/kg CDDP dose
equivalent) (n=6) and sacrificed 30 days later. The kidney, lung and livers were harvested and fixed in 4% paraformaldehyde and the tissues were embedded in paraffin and sliced at a 5 µm-thickness. The resulting sections were stained with hematoxylin and eosin and observed under a Olympus DP71.

RESULTS AND DISCUSSION

The chemoradiotherapeutic efficacy of LP and EGFR:LP containing CDDP was compared with that of free CDDP in mice bearing A549-derived tumors in the right hind leg. As shown in Figure 1A, the tumor growth in the CDDP, LP-CDDP, or EGFR:LP-CDDP treated animals was delayed compared with that of the control. In combination therapies of IR and drugs (Figure 1A, right panel), CDDP, LP-CDDP, and EGFR:LP-CDDP all enhanced the radiotherapeutic efficacy of the treatment. These results reveal a higher chemotherapeutic and chemoradiotherapeutic efficacy of EGFR:LP-CDDP among the compounds tested.

During these experiments, the body weights of the surviving animals in the groups treated with CDDP or with a combination of CDDP and IR fell to 85% of normal levels at the beginning of the therapy and then slowly recovered (Figure 1B). These results indicate that although free CDDP has anticancer effects, it is severely toxic. The other treatment groups showed equivalent survival rate and body weight profiles. On the final day of the experimental period, the kidney weights of the mice in each treatment group were compared. As shown in Figure 1C, only two treatments (CDDP and CDDP with IR) caused a significant loss in kidney weight. These results suggest that the LP formulation prevented CDDP-induced damage.

To examine and compare the effects of LP-CDDP and EGFR:LP-CDDP on renal function, the kidney injury markers BUN and creatinine were assayed in the tumor mice treated with CDDP, LP-CDDP and EGFR:LP-CDDP (figure 2). Kidney weight significantly reduced in CDDP or the combination of CDDP and IR treated mice. Normal value of creatinine was less than 1.3 mg/dl and BUN was less than 23 mg/dl. Only CDDP caused significant nephrotoxicity as expected, whilst LP-CDDP and EGFR:LP-CDDP did not show any evidence of such toxic effects.

CONCLUSION

EGFR:LP-CDDP is an effective targeted radiosensitizer in EGFR-overexpressing NSCLC cells. This maximizes the chemoradiotherapeutic efficacy of combination regimens in NSCLC cells by neutralizing both the toxicity of CDDP and the IR resistance of the cells.

REFERENCES


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