DRUG RESISTANCE MODIFICATION BY PULSING ELECTROMAGNETIC FIELD FOR DOXORUBICIN RESISTANT MOUSE OSTEOSARCOMA CELL LINE

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Introduction
Multidrug resistance (MDR) is a major problem in osteosarcoma chemotherapy. P-glycoprotein (Pgp) which is drug efflux pump localized on cell membrane, is associated MDR in human osteosarcoma and recent report revealed that overexpression of Pgp is an indicator of poor prognosis in osteosarcoma patients[1]. Lately, a number of studies have been attempted to over come Pgp-mediated MDR in various cancers by resistance modifying agents such as verapamil, cyclosporin A, MS209, etc. However, most are not useful for patients, because of many complications. In this study, we therefore undertook to clarify drug resistance modifying effect on MDR mouse osteosarcoma by pulsing electromagneticfield stimulation (PEMFs) which is commonly used for treatment of fracture nonunion and pseudarthrosis.

Materials and methods
Pgp positive, doxorubicin (DOX) resistant murine osteosarcoma cell line (MOS/ADR1) and the parental cell line (MOS) were used for the study. The MOS/ADR1 was established by single cell culture after the exposure of the parental cell line (MOS) to six pulsed, stepwise increments of DOX. MOS/ADR1 cells were 15-fold more resistant to DOX than MOS cells[2]. PEMFs was applied to the cells at the frequency of 10Hz, signal width 25µsec and intensity of 2 and 4Gauss.

To assess reversal effect of the PEMFs on DOX resistance, cells were incubated for 72 hours in graded concentration of DOX with or without PEMFs. The dose of DOX required to produce 50% growth inhibition (IC50) was determined by MTT assay. The sensitization by PEMFs was expressed as the ratio of IC50 in the absence of PEMFs to IC50 in the presence of PEMFs.

DOX binding ability to nuclear DNA was also performed by following procedure. Cells grown on coverslips were incubated for one hour in the medium containing DOX (10µg/ml) with or without PEMFs. After washing with PBS, cells were fixed with buffered formalin for 10 minutes at room temperature. Intensity of red autofluorescence from DOX binding to DNA in each cell was measured by an epi-illumination cytofluorometer. Pgp expression in each cell was detected by indirect immunufluorescence staining method, using primary monoclonal antibody to Pgp (C219).

Results
In the absence of PEMFs, IC50 of MOS/ADR1 to DOX was 6.1µg/ml. Whereas PEMFs in the presence of PEMFs to IC50 was 3.9µg/ml at 2 Gauss intensity and 2.3µg/ml at 4 Gauss intensity( Fig. 1). DOX binding ability to nuclear DNA, which is expressed as average fluorescence intensity of DOX, increased 3 times in PEMFs treated cells, compared to that in control cells( Fig. 2 ).

Pgp expression detected by immunofluorescence in each cell did not decrease even in the presence of PEMFs (figure is not shown).

Discussion
Results of the study demonstrated that PEMFs enhanced the toxicity of DOX by increase intracellular DOX accumulation in MDR mouse osteosarcoma. It is also evident that PEMFs inhibits the function of Pgp, not overexpression and reversed DOX resistance. Because PEMFs has been used for treatment of fracture nonunion and pseudarthrosis, we believe that PEMFs could be useful for overcoming MDR in osteosarcoma patients.

References

Fig. 1 Reversal effects of PEMFs on MOS/ADR1 cells

Fig. 2 Relative fluorescence intensity of intracellular DOX
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