論 文 要 旨

Synergistic effect of arsenic trioide, vismodegib and temozolomide on glioblastoma.

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【序論及び目的/Introduction and Purpose】

Primary brain tumours of glial origin (gliomas) are the most common neoplasms of the central nervous system in the adult population. Each year, about 5–6 cases out of 100,000 people are diagnosed with primary malignant brain tumours, of which about 80% are malignant gliomas, with more than half of them being glioblastomas. It has a slight male predominance, and affect mostly patients between 45-70 years of age. Despite the current new multimodal treatment strategies including surgical resection, irradiation, and chemotherapy; GBM is an aggressive neoplasms associated with high mortality due to infiltrative growth and recurrence with a uniformly fatal course. In clinical practice, combination therapy is often used to enhance the cytotoxicity and reduce the adverse effects of chemotherapeutic drugs. In the present study, we demonstrated that the combination of VIS, ATO and TMZ suppressed the growth of GBM.

【材料方法および結果/Materials Methods and results】

Three Glioblastoma cell lines; Glioblastoma of unknown origin (GUO) [U-87MG (ATCC® HTB-14™) (RRID:CVCL_0022)], U138MG human malignant GBM and U251MG cell lines were used to examine the efficacy of ATO, VIS or TMZ. WST-1 results showed that, there was a dose-dependent inhibition in cell proliferation when all the cell lines were treated with different concentrations of the drugs.

We then examined the effect of treating these cell lines with Hh inhibitors VIS or ATO in combination with TMZ in a dose-dependent manner. Five different concentrations of either a single drug or a fixed drug ratio of the combined drugs; ATO and TMZ and VIS and TMZ were used. Cell viability was assessed by WST-1 assay. The CalcuSyn median effect model was used to calculate the CI values and to analyse whether the drug combinations were synergistic, antagonistic, or additive. There was a marked inhibition in the proliferation of the GBM cell lines, when combination treatment was used unlike when a single agent was used. We then examined the ability of 300 μM TMZ when combined with 1 μM ATO/30 μM VIS to cause DNA damage and apoptosis in GBM cells following treatment for 48 h. Western blot analyses using γH2AX and cleaved caspase-3 revealed that there was higher expression of γH2AX and cleaved caspase-3 when the drugs were combined, unlike when they were used as single agents. Mouse xenograft models also showed that the combination of ATO and TMZ, VIS and TMZ, significantly inhibited GBM proliferation in vivo compared with the vehicle or single drug administration.

【結論及び考察/Discussion】

Temozolomide (TMZ) is an alkylating agent used in the treatment of GBM. Arsenic trioxide (As_2O_3, ATO), it's a Hh pathway inhibitor, used for the treatment of acute promyelocytic leukaemia (APL). Vismodegib (VIS) is a novel small molecule antagonist of the Hh pathway that binds to smoothened (SMO) and leads to the inhibition of aberrant activation of the Hh pathway.

Despite the impressive tumour regression achieved by targeting the Hh pathway with ATO and VIS in this study, resistance has also been reported in previous studies, thus conferring the need for a combination therapy. Combination therapy is frequently used in clinical practice to improve therapeutic effect and reduce the toxicity of anticancer drugs. Combined treatment with either ATO and TMZ or VIS and TMZ was better at inhibiting GBM growth *in vitro* and *in vivo* than single-drug therapy. We believe this is the first study to show the synergistic effect of ATO/VIS with TMZ on GBM as determined by the CI–isobologram method of Chou-Talalay.

In conclusion, these findings indicate that combination of Hh pathway inhibitors and TMZ may be an attractive therapeutic approach for treating GBM.