Tetramethylprazine, a potential adjuvent to inhibit cancer metastasis through CXCR4/SDF-1 pathway

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Chuanxiong (Ligusticum wallichi Franchat) was first described in a traditional Chinese medicine book, Shennong Bencaojing, written in 200 BC. It has been used in clinical treatment for more than 2000 years (1). The bioactive component, 2, 3, 5, 6-tetramethylpyrazine (TMP) was extracted from Chuanxiong in 1973 (2). Currently, there are 36 pharmaceutical factories that produce injections or tablets in China (3,4). These medicines were firstly used in treatment of patients with neural disease with mild side effects, such as, ischemic, cerebral infarction, Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis (MS) (5–8). However, accumulating evidence has confirmed that combining TMP with other treatments could significantly attenuate multidrug resistance of chemotherapy and inhibit proliferation and metastasis of cancer cells (9). This mini-review is focused on the application and mechanism of TMP to proliferation and metastasis of cancer cells.

TMP application in patients

Chuanxiong combining with other Chinese herbs was wildly used in treatment of malignant tumor, such as, gliomas (10), lung cancer (11), ovarian cancer (12), gastric carcinoma (13), breast cancer (14), liver cancer (15), bladder cancer (16), acute lymphocytic leukemia (17) etc. Since the bioactive component, TMP, was identified in 1977, there have been an overwhelming number of applications of tetramethylpyrazine hydrochloride (TMPH) as a highly efficient clinical treatment for tumor patients. For example, Han et al had treated 36 patients with different types of tumors through chemotherapy with tetramethylpyrazine injection, which had higher efficacy than using chemotherapy only. The outcome demonstrated that the patients in the treatment group obtained a more significant therapeutic effect than the control (18). Additionally, Xue et al reported that the radiotherapy with adjuvant TMP had a great effect on inhibition metastases of brain tumor (19).

The mechanism of TMP on anti-tumor activity

Although Ligusticum and TMP are wildly used in the treatment of patients, the precise molecular mechanisms behind TMP's anti-tumor activity are not well defined. Ancient Chinese medical books classically described that Ligusticum plays an important role in invigorating blood circulation, promoting the flow of Qi, dispelling wind, and alleviating pain (20,21). Modern medical technology further demonstrated that TMP could promote blood circulation and remove blood stasis by assaying for whole-blood viscosity, blood pressure, and platelet aggregation rate (22-24).

For its antitumor mechanism, there are several points. Clinical evidence has confirmed that combining TMPH or Chuanxiong with other treatments can significantly attenuate multidrug resistance (MDR) of chemotherapy and increase the sensitivity of cancer cells to radiation in cancers such as nasopharyngeal, lung, breast, renal and ovarian cancer (25-27). One of the underlying mechanism of TMP reverse MDR might be its down-regulating effect of P-170, a key protein related to MDR (25), indicating that TMP can effectively increase the objective response ratio in patients with short-term effect, worthy to be popularized. Therefore, attenuating multidrug resistance is suggested as the main contributor to TMPH's antitumor capabilities. In addition, according to reducing blood viscosity, managing coagulation and improving the microcirculation around ischaemic tissue (28,29), the other scientists hypothesize that TMP may increase the radiation and chemotherapy sensitivity of patients by improving the microcirculation around the tumor tissue, thereby reducing the side effects on the organs at risk (25,30). Thus, it seems logical to suggest that a correlation exists between the tumor-inhibition and neural-protection bioactivities of TMP (31). Furthermore, Cao et al found that TMP exerts antitumor effects by inducing apoptosis and autophagy in hepatocellular carcinoma (32).

Many studies focused on identifying the molecular targets of TMP in antitumor, and reported that VEGF plays a key role in TMP-medicated inhibition of tumor. Angiogenesis is known closely related to tumor metastasis, and VEGF plays an important role of it. The suppression of VEGF proteins leads to the inhibition of angiogenesis and metastasis, which might be one of the mechanism underlying the anti-tumor effect of TMP (33-35). However, more evidence demonstrated that other pathways are involved in TMP's function. Jia et al suggested that TMP suppressed angiogenesis and tumor growth of lung cancer via blocking the BMP/Smad/Id-1 signaling pathway (36). Yan et al revealed that TMP induces cell differentiation toward the neuronal phenotype through activation of the PI3K/Akt/Sp1/TopoII pathway (37). Yeom et al further demonstrated that TMP improve skin cancer and inflammation by decreasing melanogenic factors (TRP1, MITF, and MAPK) and factors (TNFα, IL-1β, IL-8, and GM-CSF) (38).

Our previous studies also investigated the molecular targets of TMP in antitumor. We discovered that CXCR4/SDF-1 pathway is a novel mechanism underlying TMP-mediated tumor inhibition (39-46). CXCR4 is a G-protein-coupled receptor with 7 transmembrane-spanning domains that is expressed in various endothelial and tumor cells, which plays an important role in all three fundamental aspects of cancer: proliferation, migration, invasion and metastases. We demonstrated that TMP significantly downregulates the expression of CXCR4 in cells of C6 glioma cells (39), retinoblastoma cells (43,45), vascular endothelial cells (43), and HELA cells, thereby suppressing tumor cell migration, proliferation and angiogenesis (46). TMPH causes malignant glioma regression *in vivo*. Tumour growth was significantly inhibited in rats treated with TMPH compared with tumour growth in control. Microcirculation in the implants was sparser in the TMPH-treated rats than that in the control rats, as measured by FITC-dextran stainin. Compared with CXCR4 antagonist, AMD3100, TMP is more effective on tumor inhibition.

In addition, we demonstrated that TMP protects cerebral neurocytes and inhibits glioma by regulating chemokine receptor CXCR4 expression both *in vitro* and *in vivo* (39,40). Down-regulation of CXCR4 expression in cerebral neurocytes by TMP can inhibit somatic Ca^{2+} increase. Accordingly, low level somatic Ca^{2+} could decrease glutamate releasing from glia cells. Thus, TMP induces neural protection. On the other hand, down-regulation CXCR4 expression in glioma cells by TMP can effectively inhibit the cell viability and migration of cultured C6 glioma cells. Similarly, the neurotoxicity caused by glutamate released from glioma cells is attenuated by TMP treatment, which reduces the damage to neural cells around glioma. This study firstly elucidated the mechanism of TMP-mediated suppression of C6 gliomas and neural protection, which could explain the molecular mechanism of its clinical application (19,49,50).

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However, TMP is a multiple-function traditional Chinese medicine. Apart from CXCR4/SDF-1 axis, caspase-3 and PARP pathway, BMP/Smad/Id-1 signaling, NF-κB-dependent mechanism and etc have also been shown involved in anti-tumor effect of TMP (51-53). Moreover, the activation of CXCR4 affects gene transcription (54). For example, CXCR4/YY1 inhibition impairs VEGF network and angiogenesis during malignancy (55). YY1 forms an active complex with HIF-1α at VEGF gene promoters and increases VEGF transcription and expression. Thus, the mechanism of the TMP-mediated antitumor could be very complicated, more investigation is required.

Conclusion

In conclusion, the application and molecular mechanism of TMP indicate that TMP would be a useful adjuvant medicine in inhibition of cancer cell migration, proliferation, and neural protection. However, currently TMP is only used in some oriental countries, such as China and Korea. Hopefully more studies could be performed to extend its application in clinical therapy in other countries.

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