An oriental herbal cocktail, ka-mi-kae-kyuk-tang, exerts anti-cancer activities by targeting angiogenesis, apoptosis and metastasis

Source:

Rigorous and systematic pre-clinical studies are necessary and essential to establish the efficacy and safety of Oriental herbs and formulas in order to transform traditional herbal practices into evidence-based medicine. Here we evaluated the anti-cancer activities of the ethanol extract of Ka-mi-kae-kyuk-tang (KMKKT), a formula of ten Oriental herbs, with a battery of in vitro and in vivo mechanism-based biomarkers involving angiogenesis, apoptosis and metastasis.

<table>
<thead>
<tr>
<th>Oriental herb/ingredients</th>
<th>Country of origin</th>
<th>Grams</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benincasa hispida (seed)</td>
<td>China</td>
<td>30</td>
<td>17.24</td>
</tr>
<tr>
<td>Bletilla striata (root and tuber)</td>
<td>China</td>
<td>15</td>
<td>8.62</td>
</tr>
<tr>
<td>Tulipa edulis (stem tuber)</td>
<td>Korea</td>
<td>15</td>
<td>8.62</td>
</tr>
<tr>
<td>Panax ginseng (root)</td>
<td>Korea</td>
<td>15</td>
<td>8.62</td>
</tr>
<tr>
<td>Phaseolus angularis (seed)</td>
<td>Korea</td>
<td>30</td>
<td>17.24</td>
</tr>
<tr>
<td>Zanthoxylum piperitum (seed)</td>
<td>Korea</td>
<td>12</td>
<td>6.9</td>
</tr>
<tr>
<td>Patrinia villosa (root)</td>
<td>China</td>
<td>15</td>
<td>8.62</td>
</tr>
<tr>
<td>Astragalus membranaceus (root)</td>
<td>Korea</td>
<td>15</td>
<td>8.62</td>
</tr>
<tr>
<td>Angelica gigas Nakai (root)</td>
<td>Korea</td>
<td>12</td>
<td>6.9</td>
</tr>
<tr>
<td>Asini gelatinum</td>
<td>Korea</td>
<td>15</td>
<td>8.62</td>
</tr>
<tr>
<td>Total amount</td>
<td></td>
<td>174</td>
<td>100</td>
</tr>
</tbody>
</table>

The results show that KMKKT suppressed the vascular endothelial responses by inhibiting basic fibroblast growth factor (bFGF)-induced ERK1/2 phosphorylation, cell migration as well as tube formation in the human umbilical vein endothelial cell model, and decreased the hypoxia-induced HIF1α and vascular epithelial growth factor (VEGF) expression in the mouse Lewis lung carcinoma (LLC) cells in vitro, and inhibited the bFGF-induced angiogenesis in chick chorioallantoic membrane model, and in the Matrigel plugs in mice. Intraperitoneal delivery of KMKKT potently inhibited the growth of the subcutaneously inoculated LLC cells in syngenic mice. In addition, KMKKT inhibited the invasion ability of the mouse colon 26-L5 cancer cells in vitro and decreased their formation of liver metastasis when intraportally inoculated in syngenic mice. Furthermore, KMKKT suppressed the growth of the human PC-3 prostate cancer xenografts in athymic nude mice and averted the cancer-related body weight loss. The in vivo cancer growth suppression was associated with a decreased microvessel density and VEGF abundance as well as an increased
PARP cleavage and the TUNEL-positive apoptosis. Together, our data support broad-spectra in vivo anti-cancer activities of KMKKT targeting angiogenesis, apoptosis and metastasis without any adverse effect on the body weight. This formula merits serious consideration for further evaluation for the chemoprevention and treatment of cancers of multiple organ sites.

Oriental medicinal herbs are rich sources of potential cancer chemopreventive and therapeutic agents, but require rigorous and systematic in vitro and in vivo pre-clinical evaluations as exemplified in the current work to transform traditional herbal practices into mainstream evidence-based medicine. To this end, we focused our work on a battery of angiogenesis functional assays for screening purposes to provide the rationale for supporting the in vivo tests of the anti-tumor efficacy and safety. Studies strongly support anti-angiogenesis properties of the KMKKT formula and reveal potential cellular and molecular pathways that are affected. These include the bFGF-stimulated endothelial ERK phosphorylation, cell motility, capillary differentiation, on one hand, and the hypoxia signaling to VEGF in the tumor cells, on the other. Further work will be necessary to identify the active herbs and chemical compounds responsible for these activities.

As far as the in vivo anti-tumor/metastasis efficacy and safety are concerned, we demonstrated a dose-dependent suppression by KMKKT of the mouse LLC-tumor growth as well as the establishment of liver metastasis by the mouse colon cancer cells. Furthermore, we showed that KMKKT was effective against human PC-3 PCa xenograft growth. In the mouse LLC model and the PC-3 human cancer xenograft model our data showed changes of the in vivo biomarkers that were consistent with the involvement of an inhibition of angiogenesis (decreased vWF staining and VEGF expression), an induction of caspase-mediated apoptosis (cleaved PARP, TUNEL-positive staining), and an inhibition of cancer proliferation (decreased PCNA staining) to mediate the anti-cancer growth activity. The PC-3 human xenograft studies are important for two reasons. The first is the demonstration of the in vivo efficacy of KMKKT against human cancer cell growth. Especially considering that PC-3 cells represent advanced androgen-independent prostate cancer, it is reasonable to suggest that KMKKT could be more efficacious against early lesions during carcinogenesis in a chemoprevention context. The second is the immunodeficient nature of the host mice. Because Chinese and Oriental herbal medicine often emphasizes on the enhanced immuno-surveillance and immuno-protection to account for anti-cancer activities, the results of the xenograft studies therefore suggest that KMKKT can exert anti-tumor effects under immuno-compromised conditions, which can be quite common in cancer patients during or after intense chemo- and radiation therapies. Taken together, our data strongly suggest that the KMKKT cocktail can be a promising chemopreventive and therapeutic modality by targeting multiple biological and pathological processes critical for cancer growth and metastasis. It should be recognized that in the current work aiming more for the proof of concept than practicality, we used i.p. injection of the KMKKT extract to bypass gastrointestinal processing to establish the anti-cancer efficacies. It will be very important for us to evaluate its efficacies and bioavailability with oral delivery in future work for the
practical application of KMKKT in cancer chemoprevention and treatment.
Concerning the potential therapeutic use of KMKKT, we want to point out that the manners in which we have evaluated the anti-cancer activities in the LLC model and the PC-3 xenograft model by delivering KMKKT several days after tumor inoculation, and the anti-metastatic activity by administering the formula after 9 days of colon tumor cell inoculation support such a treatment hypothesis for established tumors. This should be tested in future studies.

In addition to the demonstration of the anti-tumor and anti-metastasis efficacies, a salient feature of the animal studies is the lack of any observable adverse effect of KMKKT on the body weight of the treated mice. As shown in both the immuno-competent C57B mice and the immunodeficient Balb/C nude mice treatment with the highest tested dose of 100 mg of KMKKT per kg body weight through i.p. injection did not decrease the body weight when compared to the control mice. Especially in the nude mice studies, KMKKT treatment reversed the body weight loss in the PC-3 tumor bearing mice. This effect could be important for invigorating the cancer patients during and after chemo- and radiation treatments and for averting the cachexia caused by advanced-stage cancers.

The current work identified multiple biological processes including angiogenesis, apoptosis and metastasis as potential targets of the anti-cancer activities of the KMKKT formula. Guided by the reductionistic paradigm of Western medicine, we are carrying out additional studies to identify the active herb(s) and particularly their active chemical compounds for the various activities observed here. As a testimony of the feasibility of the activity-guided approach, we have recently discovered a potent anti-androgen signaling activity of KMKKT and identified the pyranocoumarin compound decursin from the Angelica gigas root in KMKKT as a novel anti-androgen compound (5). Conceptually, it will be interesting and important to determine whether the anti-tumor efficacies of KMKKT can be reconstituted by a few key herbs or the active chemicals identified from each.

In summary, the KMKKT cocktail at an i.p. dosage of 100 mg/kg is safe to the mice and is efficacious against angiogenesis, solid tumor growth and metastasis in four in vivo models. These desirable activities merit a serious consideration for the evaluation of KMKKT in primary carcinogenesis models for cancer chemoprevention and in additional pre-clinical models for therapeutic applications to lay a solid foundation for translational work in humans.

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