Oral JS-38, a metabolite from *Xenorhabdus* sp., has both anti-tumor activity and the ability to elevate peripheral neutrophils

LIU Min-Yu¹,², XIAO Lin², CHEN Geng-Hui³, WANG Yong-Xiang¹, XIONG Wei-Xia², LI Fei², LIU Ying², HUANG Xiao-Ling², DENG Yi-Fang², ZHANG Zhen², SUN Hai-Yan², LIU Quan-Hai²*, YIN Ming¹*

¹ School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, China; ² Department of Pharmacology, Shanghai Institute of Pharmaceutical Industry, Shanghai 200437, China; ³ Beijing Wenfeng Tianji Pharmaceuticals Ltd., Beijing 100027, China

Available online October 2014

[ABSTRACT]

**AIM:** JS-38 (mitothesolore), a synthetic version of a metabolite isolated from *Xenorhabdus* sp., was evaluated for its anti-tumor and white blood cell (WBC) elevating activities.

**METHOD:** These anti-proliferative activities were assessed *in vitro* using a panel of ten cell lines. The anti-tumor activities were tested *in vivo* using B16 allograft mouse models and xenograft models of A549 human lung carcinoma and QGY human hepatoma in nude mice. The anti-tumor interactions of JS-38 and cyclophosphamide (CTX) or 5-fluorouracil (5-Fu) were studied in a S180 sarcoma model in ICR mice. Specific stimulatory effects were determined on peripheral neutrophils in normal and CTX- and 5-Fu-induced neutropenic mice.

**RESULTS:** The IC₅₀ values ranged from 0.1 to 2.0 μmol·L⁻¹. JS-38 (1 μmol·L⁻¹) caused an increase in A549 tumor cell apoptosis. Multi-daily gavage of JS-38 (15, 30, and 60 mg·kg⁻¹·d⁻¹) inhibited *in vivo* tumor progression without a significant effect on body weight. JS-38 additively enhanced the *in vivo* anti-tumor effects of CTX or 5-Fu. JS-38 increased peripheral neutrophil counts and neutrophil rates in normal BALB/c mice almost as effectively as granulocyte colony-stimulating factor (G-CSF). In mice with neutropenia induced by CTX or 5-Fu, JS-38 rapidly restored neutrophil counts.

**CONCLUSION:** These results suggest that JS-38 has anti-tumor activity, and also has the ability to increase peripheral blood neutrophils.

**[KEY WORDS]** JS-38 (mitothesolore); *Xenorhabdus* sp.; Anti-tumor activity; Neutrophils; Apoptosis

**[CLC Number]** R965

**[Article ID]** 2095-6975(2014)10-0768-09

---

**Introduction**

Chemotherapy has become one of the major treatments of tumors. In recent years, there has been a rapid development of anti-cancer drugs, especially of the small molecule-targeting chemotherapy drugs, more than ten of which have already been approved for the market, and many more of which are in the process of being assessed in clinical trials and preclinical studies. However, the majority of the available anti-cancer drugs are cytotoxic drugs. Though these drugs are intended to target tumor cells, they usually display evident toxicity in normal cells, especially for rapidly proliferating tissues. The majority of cancer patients that are given cytotoxic drugs show varying degrees of myelosuppression. Many of the drugs can even cause life-threatening neutropenia. Even for specific small molecule-targeting chemotherapy drugs, such as the BCR-ABL tyrosine kinase inhibitor imatinib, or the multi-target inhibitors...
sunitinib and sorafenib, bone marrow suppression and peripheral blood leukopenia may occur to some degree [3]. Therefore myelosuppression and neutropenia have become a serious concern faced by every clinician prescribing tumor chemotherapy.

Neutropenia, and its subsequent infectious complications, represent the most common sources of dose-limiting toxicity of cancer chemotherapy. Two cytokines, granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF), are now in routine clinical use for the management of millions of patients undergoing chemotherapy-induced myelosuppression every year [6]. Treatment with these cytokines is an integral part of the prevention of potentially life-threatening febrile neutropenia [7]. However, the treatment requires iterative parenteral injections due to the short half-lives of most cytokines.

Considerable efforts have been focused on identifying small molecules that are capable of elevating peripheral white blood cell (WBC) counts, though few of them have been clinically successful. The existing compounds, including lithium [8], all-trans-retinoic acid [9], arsenic trioxide (As2O3) [10], and corticosteroids [11], can elevate peripheral WBC counts under certain conditions, but they have rarely been applied in the treatment of leukopenia induced by radiotherapy and chemotherapy. Drugs that simultaneously inhibit tumor proliferation and elevate peripheral WBC counts are scarce. As an alternative approach, combinations of drugs that elevate peripheral WBC counts together with anti-tumor drugs, cytotoxic drugs, or specific targeting drugs might be used to simultaneously strengthen the anti-tumor effect and relieve leukopenia in the patient.

Xenorhabdus sp. is bacterial symbiont of soil-living endopathogenic nematodes, and has been a rich source of biologically active compounds, most notably antibiotics. Some derivatives of small molecular metabolites isolated from Xenorhabdus sp. were synthesized and their cytotoxic activity and structure-activity relationships were reported. In the present study, JS-38 (mitothiolore), a derivative of 6-amino-4H-[1,2]dithio[4,3-b]pyrrol-5-one was characterized, and demonstrated to be simultaneously effective in providing anti-tumor activity and elevating peripheral neutrophil counts. In addition, JS-38 can also be effectively combined with other chemotherapeutic agents for additive anti-tumor effects and the reversal of acquired neutropenia [12].

![Structure of JS-38](image)

**Fig. 1 Structure of JS-38**

### Materials and Methods

**Drugs and reagents**

JS-38 was obtained from Beijing Wenfeng Tianji Pharmaceuticals, Ltd., and its molecular weight of 548 and purity of 99.9% were confirmed in-house by NMR spectroscopy, mass spectrometry, and HPLC. Cyclophosphamide (CTX), 5-fluorouracil (5-Fu), cisplatin, and granulocyte colony-stimulating factor (G-CSF) were obtained, respectively, from Jiang Su Heng Rui Medicine Co., Ltd., Shanghai Xudong Haipu Pharmaceutical Co., Ltd., Qilu Pharmaceutical Co., Ltd., and Xiamen Amoytop Biotech., Ltd. All drugs were freshly prepared before use.
dissolved in sterile normal saline solution.

**Cell lines and cell culture**

All human and mouse tumor cell lines were obtained from the Cell Bank of the Chinese Academy of Sciences. A549 and NCI-H460 human lung cancer cells, SK-OV-3 human ovarian cancer cells, MCF-7 human breast cancer cells, QGY-7701 human hepatoma cells, and A375, SKMEL28, and K111 human and mouse melanoma cells were grown in DMEM supplemented with heat-inactivated fetal bovine serum and 2 mmol·L⁻¹ L-glutamine. K562 and L1210 human and mouse leukemia cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum and 2 mmol·L⁻¹ L-glutamine. All of the cell lines were grown at 37 °C in a humidified atmosphere containing 5% CO₂. Seeding densities for each cell line were empirically optimized by passing twice weekly. Quantification of cell numbers was done by hemocytometric counting, and viability determinations were made by standard trypsin blue exclusion techniques. Routine harvesting of monolayer cultures was done by standard trypsinization procedures.

**Experimental animals**

Female C57BL/6 mice, ICR mice, and BALB/c nude mice of 6 to 8 weeks were purchased from the Shanghai Laboratory Animal Center (Shanghai, China). After shipment, mice were housed in a temperature- and humidity-controlled and pathogen-free environment on a 12 h light/dark cycle for 7 days allowing acclimatization prior to experimentation. All animals were given free access to food and distilled water. All animal studies were approved by the Shanghai Institute of Pharmaceutical Industry Animal Care and Use Committee.

**Cell growth inhibition assays**

Cellular anti-proliferative assays were carried out using the MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) method, as previously described [13]. Briefly, cells (100 µL) were incubated with various concentrations of compounds in a 96-well plate for 48 h at 37 °C. Then MTT (5 mmol·L⁻¹) solution (10 µL) was added to each well. After further incubation at 37°C for 48 h, the formazan formed from MTT was extracted by adding DMSO (100 µL) for 12 h. The absorbance at 492 nm was determined using a microplate reader, and the IC₅₀ values were calculated.

**Apoptosis morphology assay**

Cells (5 × 10⁴) were cultured in 6-well plates for 24 hours and then treated with 1.0 µmol·L⁻¹ of JS-38 for 6, 12, 24, and 48 h. For the apoptosis assay, cells were harvested by trypsinization and washed twice with cold PBS. The cells were centrifuged at 1,500 r·min⁻¹ for 5 min, and then the supernatant was discarded and the pellets were resuspended in binding buffer (Hepes 10 mmol·L⁻¹/NaOH, NaCl 140 mmol·L⁻¹, CaCl₂ 2.5 mmol·L⁻¹, pH 7.4). 100 µL of the sample solution was transferred to a 5 mL culture tube and incubated with FITC-conjugated annexin V (5 µL) and propidium iodide (5 µL) for 15 min at room temp in the dark. Binding buffer (400 µL) was added to each sample tube, and the samples were analyzed by flow cytometry.

**Blood cell counting**

One hundred microliters of blood sample gathered from the mouse orbital vein was mixed with the EDTA anticoagulant solution (5 µL). After adding appropriate reagents for each blood cell type (200106FS-PAK: Dilution-L200106 FSMB.P02; Hemolysin I-L200106FSXIB.P02; Hemolysin II-L200106FSXII.B. P02; Cleansing solution-L200106FSCB.P02), erythrocytes, neutrophils, basophils, eosinophils, lymphocytes, monocytes, and platelets were counted using a HV950FS hemocytometer (Erba Diagnostics, Inc.).

**Tumor inoculation**

Allografted and xenografted cancer models were established by subcutaneous inoculation of five million B16 mouse melanoma cells into the flanks of C57BL/6 mice, or five million A549 human lung cancer cells or QGY-7701 human hepatoma cells into the flanks of BALB/c nude mice [12]. The mice were randomly allocated into control and treatment groups, and were administered oral saline (25 mL·kg⁻¹) or JS-38 at 15, 30, and 60 mg·kg⁻¹ for 7 days starting on the day after subcutaneous inoculation in allografted models or when their tumors reached about 100 mm³ in xenografted models. The tumor length and width were measured every 3 days and the volume was calculated using the formula: volume = D × d² × π/6. At the conclusion of the experiments, tumor-bearing animals were sacrificed with CO₂ asphyxiation, and tumors were isolated and weighed.

**Data analysis and statistics**

Q values were calculated with the formula: \( Q = \frac{1}{E_a \times E_b} \) [14]. The results are expressed as mean ± S.D. Statistical significance was evaluated by a one-way analysis of variance (ANOVA). The criterion for statistical significance was \( P < 0.05 \).

**Results**

**Anti-proliferative activity of JS-38 in vitro**

To assess the anti-proliferative effect of JS-38, it was compared to that of cisplatin in a panel of ten human and mouse cancer cell lines. A 48 h treatment with either JS-38 or cisplatin broadly reduced cell viability in all of the cancer cells tested as assessed by the MTT assay, though the potency was consistently greater for JS-38 than for cisplatin. The calculated IC₅₀ values of JS-38 and cisplatin ranged from 0.2 to 2.0 µmol·L⁻¹, and from 1.2 to 17.8 µmol·L⁻¹, respectively (Table 1). The anti-proliferative effects of JS-38 appeared to have no tissue specificity or species specificity.

**Effects of JS-38 on apoptosis in cancer cells**

To determine the level at which JS-38 functions to inhibit tumor growth, the effects of JS-38 on the number of apoptotic cells in vitro were assessed. A549 cancer cells were treated with 1.0 µmol·L⁻¹ JS-38 for 6, 12, 24, and 48 h and then stained with propidium iodide and analyzed by flow cytometry. While the basic apoptotic rate in A549 cancer cells was 2.6% without drug treatment, JS-38 caused...
Table 1  Anti-proliferative effects of JS-38 and cisplatin on human and mouse cancer cell lines. The cell growth was measured by the MTT assay and half inhibitory concentration (IC\(_{50}\)) values were calculated.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC(_{50}) (μmol·L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549 human lung carcinoma</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>NCI-H460 human lung carcinoma</td>
<td>6.1 ± 2.1</td>
</tr>
<tr>
<td>QGY-7701 human hepatoma</td>
<td>5.3 ± 3.1</td>
</tr>
<tr>
<td>SK-OV-3 human ovarian cancer</td>
<td>3.6 ± 2.1</td>
</tr>
<tr>
<td>MCF-7 human breast cancer</td>
<td>17.3 ± 7.9</td>
</tr>
<tr>
<td>SKMEL28 human melanoma</td>
<td>3.9 ± 1.6</td>
</tr>
<tr>
<td>A375 human melanoma</td>
<td>3.2 ± 1.3</td>
</tr>
<tr>
<td>K562 human leukemia</td>
<td>5.2 ± 2.1</td>
</tr>
<tr>
<td>K111 mouse melanoma</td>
<td>16.9 ± 5.6</td>
</tr>
<tr>
<td>L1210 mouse leukemia</td>
<td>1.7 ± 0.6</td>
</tr>
</tbody>
</table>

Table 2  Effects of multi-daily gavage of JS-38 on body weight and B16 tumor growth in the allografted B16-C57BL/6 mouse model. Tumor weights are quantified for tumors excised from allografted C57BL/6 mice on day 10 following 7 days treatment with increasing JS-38 doses. (means ± SD, n = 10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight/g</th>
<th>Tumor weight/g</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 10</td>
<td></td>
</tr>
<tr>
<td>Saline (20 mL·kg(^{-1}))</td>
<td>20.8 ± 0.9</td>
<td>19.6 ± 1.6</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>CTX (15 mg·kg(^{-1}))</td>
<td>20.1 ± 0.5</td>
<td>18.1 ± 1.2</td>
<td>0.3 ± 0.4*</td>
</tr>
<tr>
<td>JS-38 (15 mg·kg(^{-1}))</td>
<td>19.9 ± 0.6</td>
<td>19.6 ± 1.1</td>
<td>1.5 ± 0.4*</td>
</tr>
<tr>
<td>JS-38 (30 mg·kg(^{-1}))</td>
<td>19.8 ± 1.0</td>
<td>19.2 ± 1.4</td>
<td>1.0 ± 0.3*</td>
</tr>
<tr>
<td>JS-38 (60 mg·kg(^{-1}))</td>
<td>19.8 ± 1.0</td>
<td>17.9 ± 1.8</td>
<td>0.7 ± 0.3*</td>
</tr>
</tbody>
</table>

a progressive increase in the number of apoptotic cells, with apoptotic rates of 58.7%, 68.2%, 78.2%, and 98.7% at 6, 12, 24, and 48 h post incubation.

**Anti-tumor activity of JS-38 in allograft and xenograft models in vivo**

The anti-tumor effects of JS-38 were further evaluated on B16 mouse melanoma cells allografted into C57BL/6 mice. The day after the B16 cells were implanted, four groups of tumor-bearing C57BL/6 mice (n = 10 in each group) received multiple doses each day for 7 days of gavage of either normal saline (25 mL·kg\(^{-1}·d\(^{-1}\)) or JS-38 (15, 30, and 60 mg·kg\(^{-1}·d\(^{-1}\)). Compared to the saline control, JS-38 did not significantly reduce total body weight; however, the average weight of tumors in the JS-38-treated mice decreased in a dose-dependent manner, with 48.2%, 65.2%, and 77.7% inhibition, respectively (Table 2). The reduction in tumor size following JS-38 treatment can be visualized by inspection of the excised tumors from each group of mice at the termination of the experiment (Fig. 3).

To verify these findings, the effects of JS-38 were examined in two different xenograft cancer models. For the first model, A549 human lung cancer cells were grafted into BALB/c nude mice. Four groups of A549 tumor-bearing BALB/c nude mice (n = 6 in each group) received a multi-daily gavage of saline (25 mL·kg\(^{-1}·d\(^{-1}\)) or JS-38 (15, 30, and 60 mg·kg\(^{-1}·d\(^{-1}\)) for 7 days. For the control nude mice, the A549 tumors grew exponentially and the average tumor volume increased from (116 ± 12.5) mm\(^3\) to (1,251 ± 75.1) mm\(^3\) over the 7-day observation period. Multi-daily gavage of JS-38 (15, 30, and 60 mg·kg\(^{-1}·d\(^{-1}\)) inhibited tumor growth in a dose-dependent manner, with inhibition rates after 7 days of 36.2%, 45.4%, and 51.4%, respectively (Fig. 4A). The anti-tumor effect of JS-38 was visualized by inspection of the excised tumors at the termination of the experiment (Fig. 4B).
**Fig. 3** Inhibitory effects of multi-daily gavage of JS-38 on B16 tumor growth in the allografted B16-C57BL/6 mouse model. B16 mouse melanoma cells were injected into C57BL/6 mice. The day after inoculation, mice were given multi-daily gavage of saline (20 mL·kg⁻¹·d⁻¹) or JS-38 (15, 30 or 60 mg·kg⁻¹·d⁻¹) for 7 days. Images are shown of the excised B16 tumors from all mice of each group.

**Fig. 4** Inhibitory effects of multi-daily gavage of JS-38 on tumor growth in xenografted BALB/c nude mouse models. A549 (A) or QGY-7701 (C) human lung carcinoma cells were injected into BALB/c nude mice. After solid tumors grew to about 100 mm³, mice were given multi-daily gavage of saline (20 mL·kg⁻¹·d⁻¹) or JS-38 (15, 30 or 60 mg·kg⁻¹·d⁻¹) for 7 days. B, D. Images are shown of the isolated tumors that were excised from the mice from each group (means ± SD, n = 6).

Synergistic anti-tumor effects of JS-38 with cyclophosphamide and 5-fluorouracil

It was postulated that JS-38 might be of greater effectiveness and utility when combined with other chemotherapy drugs. To assess this possibility, the anti-tumor interactions of JS-38 and the nonspecific cell cycle inhibitor cyclophosphamide (CTX) [15] or the S specific inhibitor 5-fluorouracil (5-Fu) [16] were further studied in a S180 sarcoma model in ICR mice. Six groups of tumor-bearing ICR mice (n = 10 each) received saline (25 mL·kg⁻¹·d⁻¹), JS-38 (7.5 mg·kg⁻¹·d⁻¹, gavage), CTX (15 mg·kg⁻¹·d⁻¹, intravenously), JS-38 + CTX, 5-Fu (12.5 mg·kg⁻¹·d⁻¹, intravenously), or JS-38 + 5-Fu for 7 days. At the end of the experiment, tumors were isolated and weighed. Individual treatment with JS-38, CTX and 5-Fu inhibited tumor growth by 19.4%, 45.1%, and 55.0% on average, while the combination of JS-38 and CTX inhibited tumor growth by 62.3% and the combination of JS-38 and 5-Fu inhibited tumor growth by 65.6%. Interaction Q values indicate that JS-38 has a synergistic anti-tumor effect with both CTX and 5-Fu (Table 3). These results verify the efficacy of JS-38 in an in vivo mouse tumor model and also demonstrate that it functions additively with two cytotoxic chemotherapy agents.

Specific stimulatory effects of JS-38 on peripheral neutrophils in normal and CTX- or 5-Fu-induced neutropenic mice

Most cytotoxic chemotherapeutic agents depress bone marrow and reduce peripheral leukocytes [17]. To determine whether JS-38 causes a similar reduction in leukocyte number, peripheral leukocytes were measured following JS-38 treatment of normal BALB/c mice. Five groups of mice (n = 10 per group) received multi-daily administrations of saline (10 mL·kg⁻¹), JS-38 (15, 30, or 60 mg·kg⁻¹·d⁻¹, gavage) or G-CSF.
Table 3  Additive anti-tumor effects of multi-daily administrations of JS-38 (7.5 mg/kg/day, gavage) and cyclophosphamide (CTX, 7.5 mg/kg·d⁻¹, intravenously) or 5-fluorouracil (5-Fu, 12.5 mg/kg·d⁻¹, intravenously) in the allografted S180-ICR mouse model (means ± SD, n = 10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 10</th>
<th>Tumor weight/g</th>
<th>Inhibition/%</th>
<th>Q value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>20.5 ± 2.1</td>
<td>22.7 ± 2.6</td>
<td>3.6 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JS-38</td>
<td>21.6 ± 1.6</td>
<td>20.8 ± 2.2</td>
<td>2.9 ± 0.4</td>
<td>19.4</td>
<td></td>
</tr>
<tr>
<td>CTX</td>
<td>20.0 ± 1.6</td>
<td>19.4 ± 1.9</td>
<td>2.0 ± 0.4</td>
<td>45.1</td>
<td></td>
</tr>
<tr>
<td>JS-38 + CTX</td>
<td>20.6 ± 1.7</td>
<td>18.6 ± 1.9</td>
<td>1.4 ± 0.4</td>
<td>62.3</td>
<td>1.12*</td>
</tr>
<tr>
<td>5-Fu</td>
<td>20.9 ± 1.5</td>
<td>22.6 ± 1.8</td>
<td>1.6 ± 0.4</td>
<td>55.0</td>
<td></td>
</tr>
<tr>
<td>JS-38 + 5-Fu</td>
<td>22.0 ± 1.6</td>
<td>22.7 ± 2.7</td>
<td>1.2 ± 0.4</td>
<td>65.6</td>
<td>1.03*</td>
</tr>
</tbody>
</table>

* Additive interactions as determined by Q values

(22.5 µg·kg⁻¹·d⁻¹, subcutaneously) for 7 days. The peripheral blood cells were monitored on days 2, 5, 7, and 12 during and after the 7-day treatment. As shown in Fig. 5A, multi-daily gavage of JS-38 progressively stimulated the peripheral leukocyte count in a dose-dependent manner. The effect of JS-38 gavage at the highest dose (60 mg·kg⁻¹·d⁻¹) was comparable to that of subcutaneous G-CSF (22.5 µg·kg⁻¹·d⁻¹). The stimulatory effects of both JS-38 and G-CSF were reversed after the termination of administration, as the increase in blood leukocytes dropped to the pretreatment levels on Day 12. Like G-CSF, the stimulation of JS-38 on peripheral leukocytes is specific, given that there were no significant effects on erythrocytes, basophils, eosinophils, lymphocytes, monocytes, or platelets (data not shown). These results suggest that in addition to promoting tumor reduction, JS-38 has the feature of increasing the leukocyte count, both when administered alone and when administered in combination with other chemotherapeutic agents.

**Discussion and Conclusions**

These results show that JS-38 has a strong efficacy for inhibiting tumor cell growth in vitro and reducing tumor progression in vivo following engraftment of the mouse melanoma cell line B16 in a C57BL/6 model and of the human lung adenocarcinoma and hepatoma cell lines A549 and QGY-7701 in nude mice. Additive anti-tumor effects of JS-38 with CTX [18] or 5-Fu [19] were also indicated by the reduction in the size of S180 sarcomas in an ICR mouse model. Animal weight did not drop markedly and there was no obvious abnormality in behavior. The results indicate that JS-38 exhibits its marked anti-tumor effects without tissue specificity or species specificity, suggesting the possibility that it could be of potential for the treatment of clinical human cancers. Although the mechanism underlying the anti-tumor effect is not known, the results suggest that induction of tumor cell apoptosis may be a primary cause for inhibition of tumor growth by JS-38.

In general, side effects of anti-tumor drugs are common and serious. Target-specific drugs tend to have fewer and less severe side effects than traditional cytotoxic compounds; however, side effects of target-specific drugs may still pose a concern. The most common side effect of cytotoxic anti-cancer drugs, which also explains the necessity for dose limitation, is the inhibition of bone marrow hematopoietic cells [20]. This inhibition is most detrimental to white blood cells because they have a short life cycle and proliferate quickly [21]. Because infections during decreases in white blood cells may lead to fatal outcome, the peripheral white blood cell count and classification (especially neutrophilic granulocyte counts) are a major consideration in the monitoring of anti-tumor drugs [22]. However, in contrast to traditional cytotoxic drugs, it was found that JS-38 provided a dose-dependent increase in peripheral blood leukocyte counts and neutrophilic granulocytes. Thus, JS-38 has anti-tumor
Table 4  The effect of JS-38 and G-CSF on the number of leukocytes. Mice received multi-daily administration of saline (20 mL·kg⁻¹·d⁻¹), JS-38 (15, 30 or 60 mg·kg⁻¹, gavage), or granulocyte colony-stimulating factor (G-CSF, 22.5 µg·kg⁻¹·d⁻¹, subcutaneously) for 7 days (means ± SD, n = 10).

<table>
<thead>
<tr>
<th>Counts/mL</th>
<th>Saline (20 mL·kg⁻¹)</th>
<th>JS-38 (mg·kg⁻¹)</th>
<th>G-CSF (22.5 µg·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells (× 10⁶)</td>
<td>6.9 ± 0.8</td>
<td>14.4 ± 2.7</td>
<td>17.9 ± 3.9</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.0 ± 0.3</td>
<td>7.4 ± 1.7</td>
<td>13.5 ± 3.0</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>4.4 ± 0.6</td>
<td>6.5 ± 1.1</td>
<td>4.0 ± 1.9</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.04 ± 0.03</td>
<td>0.04 ± 0.02</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Red blood cells (× 10⁹)</td>
<td>7.3 ± 0.7</td>
<td>7.6 ± 0.7</td>
<td>8.2 ± 0.8</td>
</tr>
<tr>
<td>Platelets (× 10⁶)</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>

Fig. 5  Specific stimulatory effects of JS-38 on the number of blood neutrophils in normal BALB/c mice and neutropenic BALB/c mice following treatment with cyclophosphamide (CTX) or 5-fluorouracil (5-Fu). A. Mice received multi-daily administration of saline (20 mL·kg⁻¹·d⁻¹), JS-38 (15, 30 or 60 mg·kg⁻¹, gavage) or granulocyte colony-stimulating factor (G-CSF, 22.5 µg·kg⁻¹·d⁻¹, subcutaneously) for 7 days. The effect of JS-38 and G-CSF on the number of leukocytes (A) and the lack of effect on the numbers of erythrocytes, basophils, eosinophils, lymphocytes, monocytes, or platelets are shown. B, C. For the neutropenic model, mice received multi-daily intraperitoneal injection of CTX (100 mg·kg⁻¹·d⁻¹) for 3 days (B), or a single dose of 5-Fu (150 mg·kg⁻¹) (C) to induce neutropenia. On the fourth day, and each subsequent day for 8 days, multi-daily administration of saline (20 mL·kg⁻¹·d⁻¹), JS-38 (15, 30, 60 mg·kg⁻¹·d⁻¹, gavage), or G-CSF (22.5 µg·kg⁻¹·d⁻¹, subcutaneously) was given. (means ± SD, n = 10)
development of orally-available, small molecule compounds to specifically stimulate blood leukocytes could theoretically provide a viable alternative to G-CSF and GM-CSF, but prior efforts have generally had limited success [24]. For example, lithium (as lithium carbonate), a most widely prescribed mood stabilizer for the therapy of bipolar disorders [25], was found to be an effective inducer of granulopoiesis [26], and may reduce erythropoiesis [27]. In addition, lithium has a narrow therapeutic window, and intoxication may occur at normal or low doses [28]. Therefore, the specificity and reversibility may make JS-38 an ideal anti-neutropenic agent for potential further development.

Combined chemotherapy is an emerging clinical trend and has been applied for the purpose of reducing side effects, decreasing drug resistance, and increasing curative efficacy [29]. In this paper, a combined therapy regimen for JS-38 with either CTX or 5-Fu was described demonstrating the additive anti-tumor effects of these drugs in combination. With the therapeutic doses used in this study, animal weight did not drop markedly, and there was no obvious abnormality in animal behavior. These factors indicate that combined chemotherapy with JS-38 may permit the use of reduced therapeutic doses of chemotherapeutic agents. Furthermore, to determine the impact of JS-38 on the recovery of granulocyte levels following treatment with cytotoxic chemotherapy agents, neutropenia was induced by CTX or 5-Fu. The results demonstrate that JS-38 can alleviate the lowest point of decrease in white blood cells, shorten the neutrophil recovery time and even increase the number of neutrophils. The effects were similar to those of G-CSF, demonstrating dose-dependence and a tolerance for high doses. Therefore, the combined application of JS-38 with other cytotoxic agents might help to prevent the risk of infection during treatment with cytotoxic drugs.

In summary, JS-38 has strong anti-cancer activity both in vitro and in vivo and activity in increasing peripheral neutrophil counts. Combination therapy of JS-38 with cytotoxic drugs produces additive anti-tumor effects, alleviates the lowest point of decrease in neutrophils and shortens the neutrophil recovery time. These results suggest that JS-38 has anti-tumor actions, and has the ability to increase peripheral blood neutrophils. Because JS-38 can induce apoptosis in tumor cells together with its ability to increase neutrophil numbers, studies on the mechanism and various biological activities of this compound may lead to the creation of more specific chemotherapeutic agents or anti-neutropenic agents with low toxicity.

Acknowledgements

We thank Gong Nian at Shanghai Jiao Tong University School of Pharmacy for her skilled table and figure drafting.

References


Cite this article as: LIU Min-Yu, XIAO Lin, CHEN Geng-Hui, WANG Yong-Xiang, XIONG Wei-Xia, LI Fei, LIU Ying, HUANG Xiao-Ling, DENG Yi-Fang, ZHANG Zhen, SUN Hai-Yan, LIU Quan-Hai, YIN Ming. Oral JS-38, a metabolite from Xenorhabdus sp., has both anti-tumor activity and the ability to elevate peripheral neutrophils [J]. Chinese Journal of Natural Medicines, 2014, 12 (10): 768-776.