

The Anti-Proliferative Effect of 5-Fluorouracil on Tumor Is Highly Associated with the Renewal of Peripheral White Blood Cells

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Abstract

The efficacy of chemotherapy is thought to be direct killing of tumor cells, but documented studies have been shown that immunity plays a role in its effectiveness. In a pilot study to observe the bone marrow suppression and regeneration in tumor bearing mice induced by single dose injection of 5-fluorouracil (5-FU), we unexpectedly found that tumors grew fast as bone marrow mononuclear cells (BMC) and peripheral white blood cells (PWBC) were decreased quickly during myelosuppression meanwhile significantly slow as repopulating of BMC and PWBC during bone marrow regeneration after 5-FU treatment, no matter whether in low or high dose administration, but the higher the dose was, the lower of the nadir of BMC and PWBC were reached to, as well as the much more powerful duration and strength of the repopulated BMC and PWBC, suggested that the immunity might be a predominant drive in 5-FU chemotherapy. Due to the fact that BMC is the source of PWBC which is its final maturational and functional form, it could be proposed that the anti-proliferative effect of 5-FU on tumor is highly associated with the renewal of PWBC.

Keywords

5-Fluorouracil, Chemotherapy, Renewal, White Blood Cells, Bone Marrow Regeneration

*These two authors made equal contributions to this study.

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1. Introduction

5-Fluorouracil (5-FU) has been an important chemotherapeutic agent since its discovery nearly 60 years ago for the treatment of human cancer. As the nucleotide analogue of thymine, 5-FU is competitively incorporated into DNA double chains when DNA replicates during the S phase of the cell cycle and lead to cell death [1]. Thus dividing cells especially rapid cycling cells such as cancer cells, bone marrow cells, intestinal epithelial cells and hair follicle cells will be the vulnerable target of its action. This mechanism of 5-FU on cycling cells is usually thought to form the foundation of its anticancer therapy as well as its side-effects. But in the past decade, there have been reports that immunity plays a key role in cancer chemotherapy [2]-[7], and 5-FU has the ability to break immune tolerance by depletion of myeloid-derived suppressor cells (MDSC) in tumor microenvironment and spleen [4]. During a pilot experiment to observe the bone marrow regeneration of tumor-bearing mice injected with 5-FU, we unexpectedly found that the anti-proliferative effect of 5-FU on tumor, no matter whether at low or high dose, was highly associated with the renewal of peripheral white blood cells (PWBC).

2. Materials and Methods

2.1. Preparation of Tumor Bearing Mice Model

S180, a murine sarcoma cell line, was obtained from the cell bank of the Shanghai Institutes for Biological Sciences of Chinese Academy of Sciences. Cells were cultured in DMEM (Life Technologies) with 10% fetal bovine serum (Life Technologies), penicillin and streptomycin (Life Technologies). KM mice were purchased from the same institution mentioned above at 8 to 10 weeks of age, and maintained under the specific pathogen free environment following the guidelines of Animal Experimental Ethics Committee. 3×10^6 S180 cells in 100 μ L PBS (Life Technologies) were subcutaneously injected into the flank of mice. When tumor surface was around 100 mm², mice were randomly divided into three parts, two parts of these mice were administrated by a unique injection of 5-FU intraperitoneally at 12.5 mg/kg or 125 mg/kg of body weight as low-dose or high-dose groups, respectively, and the third part of mice were injected with saline and designated as control group of 5-FU at 0 mg/kg of body weight. The grouping standard for low or high dose of 5-FU was according to the references previously described elsewhere [1] [4].

2.2. Observation of Bone Marrow Regeneration and Tumor Growth

Bone marrow regeneration was monitored by counting of PWBC and bone marrow mononuclear cells (BMC) at different time points [1], while tumor growth was measured according to its increasing weight. In brief, 10 μ L of vein blood from mouse eye socket with heparin anticoagulation was pipetted and immediately mixed into 190 μ L of pre-cold 3% acetic acid (w/v). 10 μ L of the mixture was added onto hemacytometer and PWBC were counted under microscope. After peripheral blood of each mouse was collected, tumor bearing mice were sacrificed by head-neck dislocation. Following separation of femur and tumor bulk for each mouse, bone marrow cells were harvested by flushing using PBS and BMC were counted using hemacytometer after lysis of red blood cells by pre-cold ammonium chloride solution [8], meanwhile tumor bulk was weighted. Time points of detection were set at 6 hours later after injection of 5-FU or saline which designated as Day 0, and subsequent Day 3, 7, 11, 14 respectively after 5-FU or saline injection. Each time point contains 3 mice for each group.

2.3. Statistical Analysis

One-way analysis of variance and two-side Student's t test were used to calculate a p value between groups at different time points. A p value of less than 0.05 was considered statistical significance. All tests were performed by microsoft office excel.

3. Results

No matter whether at low (12.5 mg/kg) or high dose (125 mg/kg), mice PWBC and BMC were decreased steeply during the first three days and then subsequently increased in later 11 days in contrast to the nontreated control group (Figure 1 and Figure 2). This cycle of bone marrow suppression and regeneration induced by 5-FU single injection is a typical change pattern previously described elsewhere [1]. The higher the dose of 5-FU were

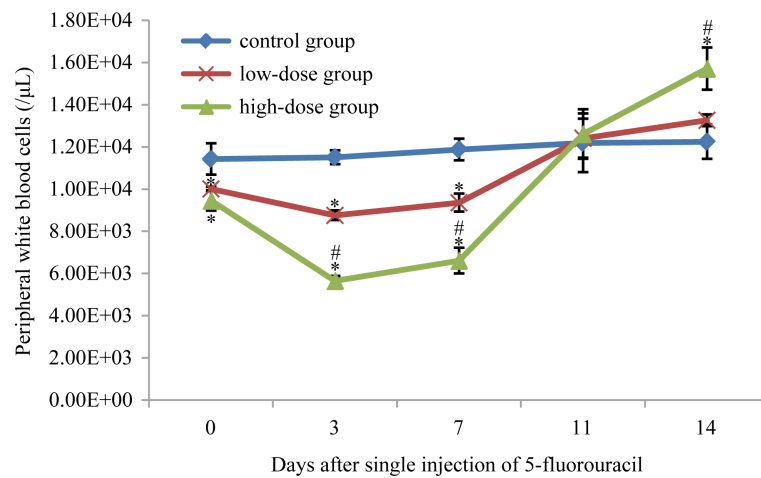


Figure 1. Changes of the number of peripheral white blood cells in tumor bearing mice after 5-fluorouracil treatment. Detection on Day 0 was carried out at 6 hours after unique injection of 5-fluorouracil intraperitoneally. Control group: injected with saline; low-dose group: with dosage at 12.5 mg/kg; high-dose group: dosage at 125 mg/kg. *: compared to control group, $p < 0.05$; #: compared to low-dose group, $p < 0.05$.

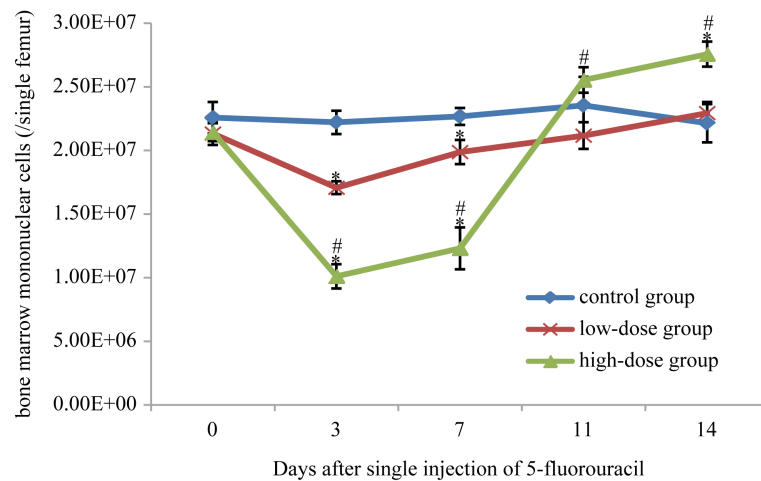


Figure 2. Changes of the number of bone marrow mononuclear cells in tumor bearing mice after 5-fluorouracil treatment. Detection on Day 0 was carried out at 6 hours after unique injection of 5-fluorouracil intraperitoneally. Control group: injected with saline; low-dose group: with dosage at 12.5 mg/kg; high-dose group: dosage at 125 mg/kg. *: compared to control group, $p < 0.05$; #: compared to low-dose group, $p < 0.05$.

administrated, the lower of the nadir of the PWBC and BMC were reached to, respectively (**Figure 1** and **Figure 2**).

But in parallel with bone marrow suppression and regeneration, tumors grafted in mice presented a rather interesting process of growth. On day 3 of myelosuppression after 5-FU treatment, the mean weights of tumors were 0.544 ± 0.137 g in low-dose group and 0.740 ± 0.182 g in high-dose group, both significantly heavier than that in control group which was at 0.302 ± 0.031 g ($p < 0.05$, respectively, **Figure 3**), indicated that the growth of tumors were significantly accelerated as PWBC and BMC were decreasing fast in groups of 5-FU treated mice in comparison to the control group, in other words, also suggested that intact immunity was fundamental for antitumor capacity of body and chemotherapeutic drug 5-FU had no direct inhibition effect in vivo on tumor proliferation.

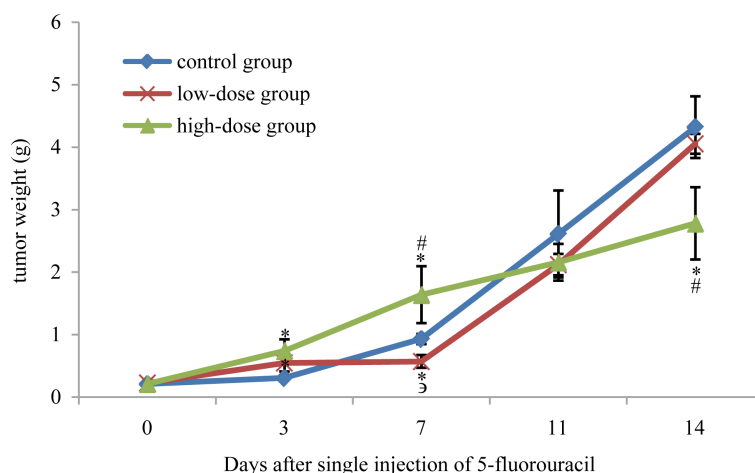


Figure 3. The growth of xenografted tumors in KM mice after 5-fluorouracil treatment. Detection on Day 0 was carried out at 6 hours after unique injection of 5-fluorouracil intraperitoneally. Control group: injected with saline; low-dose group: with dosage at 12.5 mg/kg; high-dose group: dosage at 125 mg/kg. *: compared to control group, $p < 0.05$; #: compared to low-dose group, $p < 0.05$; ∃: compared to tumor weights on day 3 of low-dose group, $p > 0.05$.

On day 7 of subsequent bone marrow regeneration, there was a differentiation of tumor growth between 5-FU treated groups, in which the mean weights of tumors were obviously lighter in low-dose group (0.569 ± 0.102 g) while much heavier in high-dose group (1.637 ± 0.455 g) than that in control group (0.927 ± 0.082 g), respectively ($p < 0.05$, **Figure 3**). This discrepancy was in consistency with the different levels of repopulating BMC ($1.99 \times 10^7 \pm 9.50 \times 10^5$ /single femur in low-dose group versus $1.23 \times 10^7 \pm 1.65 \times 10^6$ /single femur in high-dose group, $p < 0.05$) and PWBC ($9350 \pm 427/\mu\text{L}$ in low-dose group versus $6600 \pm 608/\mu\text{L}$ in high-dose group, $p < 0.05$) between 5-FU treated groups (**Figure 1** and **Figure 2**), while tumors seemed to stop growing from day 3 to day 7 in low-dose group because there was no significant increase of tumor weight between these two time points (0.544 ± 0.137 g versus 0.569 ± 0.102 g, $p > 0.05$, **Figure 3**), indicated that certain levels of renewal of BMC and PWBC induced by 5-FU treatment were evidently related to the inhibition of tumor growth in vivo.

Tumors seemed to grow much fast after day 7 in 5-FU treated and nontreated control groups (**Figure 3**), but the growth of tumors in high-dose group were relatively slow according to the decreased tumor weights (2.778 ± 0.578 g) on day 14 than that in low-dose group (4.051 ± 0.159 g) or control-group (4.318 ± 0.494 g) of the same day ($p < 0.05$, **Figure 3**). This event was accompanied by massive repopulation of BMC ($2.76 \times 10^7 \pm 9.87 \times 10^5$ /single femur) and PWBC ($15700 \pm 1000/\mu\text{L}$) in high-dose group of day 14 in comparison to those in low-dose group ($2.29 \times 10^7 \pm 8.74 \times 10^5$ /single femur and $13250 \pm 278/\mu\text{L}$, $p < 0.05$, respectively), further indicated that repopulated BMC and PWBC were associated with the growth retardation of tumors. Although the mean weights of tumors in low-dose group were lighter than that in control group on day 11 and day 14, there were no statistical significance ($p > 0.05$), suggested that the duration and strength of repopulated BMC and PWBC with antitumor capacity were probably more powerful in high-dose group than in low-dose group.

4. Discussion

Our unexpected findings in a pilot experiment for observation of bone marrow suppression and regeneration in tumor bearing mice injected with chemotherapeutic drug 5-FU showed that the inhibition of tumor growth in vivo was surprisingly coincident with repopulation of BMC and PWBC, furthermore there was at least no direct function of anti-proliferation by 5-FU itself on tumor growth with decreasing of BMC and PWBC during myelosuppression. With considering that BMC is the source of PWBC which is its final maturational and functional form, it could be proposed that the anti-proliferative effect of 5-FU on tumor is highly associated with the renewal of PWBC.

It is not an up to date fact that immunity plays a role in chemotherapy. Documented studies have been shown

that chemotherapy in noncytotoxic concentrations is capable to break immune tolerance and enhance anti-tumor immunity. Immune tolerance is referred as the immunosuppressive capabilities of tumor-induced inhibitory cells which are mainly comprised of regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC) and tumor-associated macrophages (TAM) [9]. Cyclophosphamide or gemcitabine with low dose treatment can selectively deplete Treg in patients with cancer or in tumor bearing mice [10]-[12]; Paclitaxel with noncytotoxic dose can inhibit MDSC and restore the anti-tumor activity of CD8⁺ T cells [13]; and 5-FU shows a pronounced effect on MDSC depletion [4]. Overall, approaches to deplete endogenous suppressive cell populations can improve the efficiency of anti-tumor immunity. All of these cells are originally derived from bone marrow progenitor cells and also components of PWBC in circulation, thus these studies support the raw data obtained from our preliminary experiment.

The prominent finding of our study was that the efficacy of chemotherapeutic drug 5-FU on tumor proliferation instead of inhibition was observed as BMC and PWBC were declined rapidly during myelodepression, while growth suppression of tumor with repopulating of BMC and PWBC was displayed during bone marrow regeneration after 5-FU treatment no matter at low or high dose. This strongly suggests a concept that at least intact immunity is indispensable to the capability of 5-FU on tumor cell killing *in vivo* which is finally associated with the renewal of PWBC after myelodepression. Based on this new concept, low dose chemotherapy that will not lead to PWBC decrease is probably much more ideal treatment for cancer. Though no direct inhibitive effect of chemo-drugs on tumors *in vivo* was not previously reported, there have been numerous studies recently on the efficacy of low dose chemotherapy in animal models and patients with cancer [3]-[5] [10]-[16]. Besides, studies show that prolonged survival in patients with cancer is related to T-cell replenishment after chemotherapy [6], and that MDSC or Treg levels will be recovered about 7 - 10 days after chemotherapy administration [14], thus metronomic chemotherapy can persistently deplete immunosuppressive cells and activate anti-tumor immunity in patients with cancer [10] [16] [17]. These studies further support our findings.

There are reports that antitumor-immune response can be greatly amplified by low dose chemotherapy in combination with immunotherapy such as adoptive T cells, vaccines, antibodies and cytokines [7] [14] [18]-[20], but the best dose to be determined according to the levels of immunosuppressive cells is a problem in its application. In contrast with these cells mentioned above, as a conventional and routine blood examination, PWBC are much easier to be obtained and counted fast under inexpensive, unwounded and automatic condition. Thus, our findings predict a very simple and feasible index used for evaluating what optimal dose should be administered for any of chemo-drugs, though our data are preliminary and need further confirmation. Therefore, due to this reason, extensive investigation and discussion on the findings in this study are meaningful and urgent for the sake of great improvement of cancer therapy.

5. Conclusion

Our findings in an observation for bone marrow depression and regeneration of tumor bearing mice induced by single injection of chemotherapeutic drug 5-FU indicated that the anti-proliferative effectiveness of 5-FU on tumor is highly associated with the renewal of PWBC.

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Abbreviations

5-FU: 5-fluorouracil

BMC: bone marrow mononuclear cells

PWBC: peripheral white blood cells

MDSC: myeloid-derived suppressor cells

DMEM: dulbecco's modified eagle medium

PBS: phosphate Buffered Saline

Treg: regulatory T cells

TAM: tumor-associated macrophages