Intraperitoneal oxaliplatin administration inhibits the tumor immunosuppressive microenvironment in an abdominal implantation model of colon cancer

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Abstract. Recent studies have demonstrated that some chemotherapeutic drugs can enhance antitumor immunity by eliminating and inactivating immunosuppressive cells. Oxaliplatin (OXP) induces immunogenic cell death by increasing the immunogenicity of cancer cells. However, the effects of OXP on the tumor immunosuppressive microenvironment remain unclear. The aim of the present study was to evaluate the antitumor activity of OXP by intraperitoneal (i.p.) administration in an abdominal implantation model of colon cancer and tested the tumor immune microenvironment to observe whether OXP affects the local immune inhibitory cell populations. Abdominal metastasis models were established by inoculation of CT26 cells. The antitumor efficacy of OXP and the tumor immune microenvironment were evaluated. The tumors and spleens of mice were harvested for flow cytometric analysis. Cluster of differentiation (CD)-8+CD69+ T cells, regulatory T cells (Tregs), CD11b+F4/80 high macrophages and myeloid-derived suppressor cells (MDSCs) were evaluated by flow cytometric analysis. In vivo i.p. administration of OXP inhibited tumor growth in the abdominal metastasis model. Furthermore, OXP was observed to increase tumor-infiltrating activated CD8+ T cells in tumors, decrease CD11b+F4/80 high macrophages in tumors and decrease MDSCs in the spleen. These results suggested that i.p. administration of OXP alone may inhibit tumor cell growth and induce the antitumor immunostimulatory microenvironment by eliminating immunosuppressive cells.

Introduction

Chemotherapy continues to be the most widely employed therapeutic option for cancer treatment. Chemotherapeutic drugs were previously assumed to suppress body’s immune system and detrimental to the efficacy of immunotherapy because of their nonspecific cytostatic and cytotoxic effects. However, an increasing number of studies have reported that some cancer chemotherapeutic drugs stimulate anticancer immune responses under certain conditions. Doxorubicin, cyclophosphamide, gemcitabine, and paclitaxel have been proved to induce immunogenic cell death (ICD) in cancer through surface exposure of calreticulin and release of adenosine triphosphate (ATP) and high-mobility group protein box-1 (HMGB-1) (1-7).

Although ICD is the most important way to trigger a chemotherapy-induced immune response against cancer, it is not the only way (8,9). Recent studies have demonstrated that some chemotherapeutic drugs and agents can enhance antitumor immunity by eliminating and inactivating immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs), T regulatory cells (Tregs), and tumor-associated macrophages (TAMs) (10-17). Paradoxically, some chemotherapy agents, such as cyclophosphamide, melphalan and doxorubicin, have been shown to induce MDSCs or TAMs that inhibit immune responses (18-20).

Oxaliplatin (OXP) is effective against many solid tumors and is commonly used in the treatment of colorectal cancer, gastric cancer, pancreatic cancer and ovarian cancer. The major antitumor mechanism is the induction of tumor cell apoptosis by inhibition of DNA synthesis through their covalent binding to DNA in cells (21). Apart from its the cytotoxic properties, recent studies have shown that OXP has the ability to increase the immunogenicity of cancer cells and induce ICD (22). The effects of OXP on the tumor immunosuppressive microenvironment are not clearly understood, and the cellular identity of OXP-induced suppressor cells has not been well studied.

Peritoneal metastasis occurs on 40% of patients with colorectal cancer. It is one of the most common metastasis pathways and compromises the long-term survival of patients with colorectal cancer. OXP is a primarily used chemotherapeutic
Flow cytometric analysis. The tumor cells and spleen cells were harvested from the mice. Tumor cells were minced and digested in a cocktail containing collagenase i.v. (1 mg/ml) and DNase (0.1 mg/ml) in RPMI-1640 for 1 h at 37°C. Osmotic lysis was used to remove the erythrocytes from the spleen cells. Next, single-cell suspensions of tumor cells and spleen cells were stained using fluorochrome-labeled antibodies, CD4, CD8, CD69, CD11b, F4/80, and matched isotype control antibodies. The mouse regulatory T cell staining kit was used to stain intercellular FoxP3. After staining, the cells were analyzed using the FACSCalibur flow-cytometer (BD Biosciences) and the data were analyzed using the FlowJo software version 7.6 (FlowJo LLC, Ashland, OR, USA).

Statistical analysis. GraphPad Prism version 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA) was used to perform statistical analysis and create figures. Results are presented as the mean ± standard error. The unpaired Student's t-test was used for paired comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

Inhibitory effect of i.p. OXP administration against abdominal metastasis of colon cancer. To investigate the inhibitory effect of i.p. OXP administration on tumor growth in case of abdominal metastasis in mice, we established the abdominal metastasis model as mentioned above. The mice were randomized into PBS and OXP (5 mg/kg/injection) groups and, each with 10 mice. The tumor nodules of each mouse were cut, counted, and weighed after euthanasia. As shown in Fig. 1 (n=6), i.p. OXP administration significantly reduced the weight (0.04±0.012) g and number (26.00±9.539) of tumor nodules compared with PBS administration (2.350±0.142) g and (267.7±5.897) (P<0.0001).

i.p. OXP administration significantly increased activated T cells in the tumors of the abdominal implantation model. Tumor cells were harvested from the OXP and PBS groups and single-cell suspensions were made. Further, activated CD8+ T cells in each group were analyzed by flow cytometry. CD69 is a marker of T-cell activation. We determined whether i.p. OXP administration increases the number of activated CD8+ T cells (CD8+CD69+ population) in the tumors. As shown in Fig. 2 (n=3), i.p. OXP administration significantly increased the number of CD8+CD69+ positive T cells in tumor tissue compared with PBS administration (P<0.05). The percent of CD8+CD69+T cells in the two groups were 6.135±0.125 and 4.275±0.135%, respectively.

i.p. OXP administration had no effect on Tregs (CD4+Foxp3+T cells) of spleen in the abdominal implantation model. The fork-head/winged helix transcription factor, FoxP3, is a relatively specific marker for Treg cells (26). The Treg cells in the tumor are very few and flow cytometry is difficult to detect. We detected the tumor for Treg cells and we failed. We evaluated whether i.p. OXP administration had effect on Treg cell populations (CD4+FoxP3+ population) of spleens in the abdominal metastasis mice. Spleens from the two groups of mice were harvested, and the percentage of CD4+FoxP3+
The percentages of Tregs in spleens was 22.37±3.132% in the group with i.p. administration and 23.37±2.748% in the control group with PBS administration, which was not a significant difference (P>0.05) (Fig. 3) (n=3).

i.p. OXP administration reduced TAMs (CD11b+F4/80 high) of the tumor in the abdominal implantation mode. We examined the effects of i.p. administration on the TAMs (CD11b+F4/80 high) of the tumor in the abdominal implantation mode. We examined the effects of i.p. administration on the TAMs (CD11b+F4/80 high) (27). Single-cell suspensions of tumor tissue from the OXP group and PBS group were harvested and analyzed for CD11b+F4/80 high macrophages cells by flow cytometry. As shown in Fig. 4 (n=3), OXP administration significantly decreased the number of CD11b+F4/80 high cells in tumor tissue compared with PBS administration (P<0.01). The percentages population was analyzed. The percentages of Tregs in spleens was 22.37±3.132% in the group with i.p. administration and 23.37±2.748% in the control group with PBS administration, which was not a significant difference (P>0.05) (Fig. 3) (n=3).

i.p. OXP administration reduced TAMs (CD11b+F4/80 high) of the tumor in the abdominal implantation mode. We examined the effects of i.p. administration on the TAMs (CD11b+F4/80 high) (27). Single-cell suspensions of tumor tissue from the OXP group and PBS group were harvested and analyzed for CD11b+F4/80 high macrophages cells by flow cytometry. As shown in Fig. 4 (n=3), OXP administration significantly decreased the number of CD11b+F4/80 high cells in tumor tissue compared with PBS administration (P<0.01). The percentages
of CD11b+F4/80\textsuperscript{high} cells in the OXP and control groups were 0.797±0.383 and 7.743±0.69%, respectively.

i.p. OXP administration reduced MDSCs (CD11b+Gr-1+ cells) of the spleen in the abdominal implantation model. Spleens from the two groups of mice were collected, and the percentage of MDSCs was detected using the CD11b and Gr-1 antibodies by flow cytometry. The percentage of MDSCs in spleens was 6.12±1.344% in the group with i.p. OXP administration and 20.73±3.897% in the control group with PBS administration. Thus, i.p. OXP administration significantly decreased the number of MDSCs in spleens compared with the control in the abdominal metastasis model (P<0.05) (Fig. 5) (n=3).

Discussion

In vivo i.p. administration of OXP inhibited the growth of tumors in the abdominal metastasis mouse model. Specifically, OXP treatment significantly increased the number of tumor-infiltrating activated CD8+ T cells, decreased the number of CD11b+F4/80\textsuperscript{high} macrophage in tumors, and decreased the number of MDSCs in spleens. These results suggest that i.p. OXP administration might weaken the tumor immune inhibitory microenvironment.

In the tumor microenvironment, tumor cells can promote immunoinhibitory pathways. This is the main reason by virtue of which tumor cells can escape from the host immune system and is impediment of antitumor immunotherapy (28-31). Tregs, MDSCs, immature dendritic cells (DC), and alternatively activated macrophages (M2) are the main immunosuppressive cells (28,32,33). It is one of the most important aims of cancer research to alter the immunosuppressive tumor microenvironment by eliminating and/or inactivating these immunosuppressive cells.

The cytotoxicity of chemotherapeutic agents is nonspecific and can decrease lymphocytes for a short time. Short-term lymphocytopenia may be beneficial for cancer patients (34-36). Lympho-depletion results in a decrease in the number of immunosuppressive cells, such as Tregs and MDSCs. Recent studies have demonstrated that, apart from their direct cytotoxic effects, several cytotoxic chemotherapeutic drugs may block immunoinhibitory signal networks and enhance antitumor immunity (31).

MDSCs, a heterogeneous population of undifferentiated myeloid cells, accumulate in the tumor microenvironment (37). MDSCs are the main immunosuppressive cells in tumors, including those of colorectal cancer (38,39). It has been demonstrated that some anticancer agents can enhance anticancer
immunity by eliminating or inhibiting MDSCs. Gemcitabine combined with 5-fluourouracil (5FU) has been reported to induce MDSCs apoptosis and deplete MDSCs (15). It has also been shown that Taxanes (docetaxel, paclitaxel) can impair the function of MDSCs and promote MDSCs differentiation into dendritic cells (16,40). Doxorubicin was also observed to decrease MDSCs in the spleen and tumor tissue and weaken the inhibitory function of residual MDSCs (11,41). These chemotherapeutic agents may inhibit MDSCs by different mechanisms. 5FU may selectively kill MDSCs because the expression of thymidylate synthase in MDSCs is lower (15). Docetaxel can block Stat3 phosphorylation and then induce development of M1 macrophages (16). Doxorubicin may eliminate MDSCs because the proliferation status of MDSCs is higher than that of T cells or NK cells (11). In our study, we demonstrated that i.p. OXP administration decreased MDSCs in the spleen in the abdominal metastasis model. This effect has been previously observed in patients with advanced colorectal cancer (19). FOLFOX (folinic acid, 5FU, and OXP) and FOLFIRI (folinic acid, 5FU, and irinotecan) are the commonly used treatment regimens for advanced colorectal cancer. Kanterman et al (19) revealed that FOLFOX and FOLFIRI have opposite effects on MDSCs and immune status in patients with advanced colorectal cancer. For example, FOLFOX reduced the accumulation of MDSCs in blood, whereas FOLFIRI enhanced the suppressive environment (19).

TAMs are activated by cancer cells. Studies have shown that the proportion of TAMs is as high as 60% in the tumor stroma (42). Most of TAMs in tumor microenvironments are of the M2 phenotype. Macrophages can be phenotypically polarized by the microenvironment. There are two main polarized groups: classically activated (M1) and alternatively activated (M2) macrophages (10). M1 macrophages are generally considered pro-inflammatory and secrete interleukin-12, oxygen species, nitric oxide and tumor necrosis factor. In contrast, M2 macrophages are considered to have anti-inflammatory effect and induce production of interleukin-10 and transforming growth factor (TGF-β). M1 and M2 macrophages can transform into each other (43) IL-10 and TGF-β, which are produced by various of tumor cells in the tumor microenvironment, induce M2 polarization. TAMs can promote tumor cell growth and metastasis, angiogenesis, adaptive immunity and stroma formation by producing various growth factors (44-46). Previous studies have shown that TAMs are correlated with poor prognosis in a variety of cancers (47,48). Thus, TAMs are now considered as a promising target for anticancer therapy (49-51). However, little research has been conducted on the influence of traditional chemotherapy drugs on TAMs. Buhtoiarov et al (25) found by employing a combined chemotherapy regimen (vinristine, cyclophosphamide, and doxorubicin) alone or with immunotherapy, caused the phenotype of TAMs in tumors to change from M2 to M1. In contrast, Dijkgraaf et al (20) found that cisplatin or carboplatin in vitro induce M2 macrophages in tumor cell lines. The peritoneal cavity normally harbors naïve macrophages that play essential roles in regulating tissue repair and inflammatory responses. Cancer cells can polarize peritoneal macrophages toward an M2 phenotype. M2 macrophages were found to be increased in malignant ascites in xenograft models (52-54). Depletion of macrophages was shown to block ovarian tumor progression and inhibit tumor-associated ascites in vivo (55). In our study, i.p. administration of OXP in vivo inhibited peritoneal tumor growth and ascites accumulation in the abdominal metastasis model and markedly decreased TAMs in tumors. It suggests that i.p. OXP might decreased TAMs in the tumor microenvironment and induce antitumor effects.

In addition, Tregs, another type of immunosuppressive cell, can control anticancer immune response (56). Cyclophosphamide, paclitaxel, and temozolomide at low-doses have been reported to selectively reduce the number of Tregs and improve the effect of immunotherapy (14,57,58). However, in our study, i.p. OXP did not show any effect on the proportion of Tregs compared with control.

Utilizing chemotherapeutic agents to interfere with immunosuppressive cells is an appealing strategy. Our study showed that i.p. OXP administration may transform the immunosuppressive microenvironment into an immunostimulatory microenvironment. These findings would be useful to design more effective chemoinmunotherapeutic strategies. In fact, OXP combined with immunotherapy has demonstrated synergistic antitumor effects in previous research. For example, Gonzalez-Aparicio et al (59) found that OXP combined with liver-specific expression of interleukin-12 reduces the immunosuppressive microenvironment and inhibited tumor metastasis in colorectal cancer mice models. In our previous study, we also found that OXP plus interleukin-7 inhibited tumor growth accompanying significant infiltration of activated T cells in the tumor microenvironment (60).

In our previous study, we found that i.v. administration of OXP alone exerted no antitumor effect and had no effect on the number of TAMs (CD11b+F4/80 high cells) in the lung metastasis model of colon cancer. In this study, we found that i.p. administration of OXP alone inhibits the growth of tumor and decreased the number of TAMs and MDSCs in abdominal metastasis model of colon cancer. Immune reactions induced by OXP differ with the different routes of administration. We speculated that i.p. OXP administration resulted in very high concentrations of OXP in the tumor environment in the peritoneal metastasis model; hence, different immune reactions were induced as compared with i.v. OXP administration. Our results suggest the presence of an unrecognized underlying mechanism when OXP is administrated via intraepithelial route; however, further studies are required to obtain the details of the mechanisms of immuno-stimulatory properties of OXP.

In the current, we showed that i.p. OXP administration may induce an immune response against tumors by weakening the immunosuppressive microenvironment in abdominal metastasis of colon cancer in mice. These findings may aid in the designing of more effective chemoinmunonatherapeutic strategies.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

X-CC and H-FG designed the present study, and analyzed and interpreted the data. H-FG performed flow cytometry and was a major contributor in writing the manuscript. LZ and JH performed the animal experiments and flow cytometry. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All experiments were approved by the Animal Ethics Committee of Sichuan University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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