Oxaliplatin: an effective treatment in colorectal cancer

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Abstract

Colorectal cancer is one of the most common malignancies around the world. Oxaliplatin is a new platinum compound with promising activity in colorectal cancer treatment. Based on the analysis of preclinical data. Because Oxaliplatin can lead to ICD (Immunogenic cell death), the study aims to figure out whether increasing concentrations and treatment durations with Oxaliplatin can kill colorectal cancer cells to test whether Oxaliplatin has a significant effect on colorectal cancer treatment. Using the human colorectal cancer cell line HCT116, the cancer cells will be treated with Oxaliplatin and PBS (negative control) at concentrations 1 mM, 10 mM, or 100 mM in a dose-dependent and duration-dependent manner (1,3,6,12,24 hours). Measure killing in vitro with MTT assay and measure tumor size in vivo. Also, measure HMGB-1 released by western blot and CRT by FACS. The positive control is cisplatin, and the negative is PBS/DMSO for in vitro and saline solution for in vivo. The study's findings will supply insight into the latent capacity of Oxaliplatin as a treatment for colorectal cancer in the future.

Keywords: Oxaliplatin (trans-/-diamino cyclohexane oxalatoplatinum; L-OHP), HMGB-1, colorectal cancer, HCT116, ICD

1. Introduction

Colorectal cancer (CRC) is known as one of the most commonly malignant tumors in the world because of its high relapse and mortality rates [1]. Though surgery is considered the only curative treatment for colorectal cancer, it is undeniable that chemotherapy is also widely used for the majority of patients [2,3]. Before the mid-1990s, the only effective drug has the limited activity in CRC was 5-fluorouracil (5-FU). However its response rates (RRs) of nearly 10–15% and mOS(median overall survival) of 10 months Citation is not ideal [4]. Except 5-FU, irinotecan and oxaliplatin are also applied to CRC treatment these years and have led to significant progress in CRC treatmen. Oxaliplatin (trans-/-diamino cyclohexane oxalatoplatinum; L-OHP) is a new platinum derivative for the treatment of advanced colorectal cancer [5]. However, after short-term use of oxaliplatin, drug resistance often occurs, leading to treatment failure and poor prognosis. Drug resistance limit is an obstacle for oxaliplatin in the treatment of colorectal cancer [6,7].

Recently, it has been found that some platinum derivatives such as Oxaliplatin and so on have the ability to induce cancer cell apoptosis and ICD (Immunogenic cell death) [8,9]. Unlike normal apoptosis, the ICD of cancer cells can elicit effective anti-tumor immune responses by activating dendritic cells (DCs) and subsequently activating specific T cell responses [10-12]. Tesniere et al. [13] reported that Oxaliplatin can delay the growth rate of colorectal cancer cells by guiding ICD. In this study, they studied the therapeutic effect of oxaliplatin on mouse cancer and evaluated the effectiveness of oxaliplatin and the effectiveness of the blocking checkpoint (apd-l). Compared to cisplatin, oxytocin stimulates the strongest ICD in Louis lung cancer cells and mouse models by activating CC and promoting T cell infiltration. These results show that oxaliplatin has a high potential for the treatment of colorectal cancer[14-16].

Hypothesis: Predict that increasing concentrations and treatment durations with oxaliplatin will kill HCT116 cells in vitro and shrink HCT116 xenografts in mice and increase ICD markers both invivo and in vitro.

2. Materials

2.1. *Cell line*

The human colorectal cancer cell line HCT-116 will be purchased commercially.

2.2 *Drug treatments*

The positive control is cisplatin treatment, and the negative control is dimethyl sulfoxide/phosphate-buffered saline (DMSO/PBS) all will be obtained commercially. Oxaliplatin will be treated nvarious concentrations and durations.

3. Methods

3.1 *Preparing cancer cell line*

The cells were cultured in Culture medium containing fetal bovine serum.

3.2 *Cell Viability Assay*

The all samples' cytotoxicity was assessed by MTT. The cancer cells were fixed in a 96 week microscope plate. Cells were then treated with different concentrations of oxaliplatin (positive control) and PBS (negative control). Then remove the drug and add fresh medium to each well. After 72 °, MTT can be added and cell viability measured (10% to 90% of cell viability).

3.3 *Cell Apoptosis*

After transfection, the cells were treated by 1, 10 or 100 mM (1, 2 and 3 days) of oxaliplatin or PBS. The method for measuring apoptosis is to collect cells and then stain them with annexin V-isothiocyanofluorescein (FITC) and propidium iodide (PI) in the dark for 15 minutes. Subsequently, the stained cells were analyzed by flow cytometry and apoptosis was determined by the percentage of AnnexV-positive cells.

3.4 *Western blot*

Colon cancer cells were treated with oxaliplatin and PBS (negative control) at dose- and duration-dependent concentrations of 1, 10 or 100 mM (1, 2 and 3 days) using the human colorectal cancer cell line HCT116. After treatment, protein extraction was performed with RIPA lysate and protease inhibitors such as PMSF. Protein quantification was performed with BSA Coomassie Brilliant Blue. SDS-PAGE gel was prepared and protein samples were dissolved. Subsequently, fractionated proteins are electrophoretically transferred from the gel to the PVDF membrane and the membrane is blocked with neutral protein (BSA or milk casein). Incubation of the membrane with primary antibody specific for target protein and incubation of the membrane with HRP-labeled secondary antibody specific for primary antibodies. Finally, incubate the blot with chemiluminescent HRP substrate and expose it to film.

3.5 Statistical Analysis

The statistical significance of all numerical data obtained from Cell Viability Assay, Cell Apoptosis, Western Blot and Cell Research is analyzed with the student's T-test, setting a significance level to $p<0.05$(see Table 1).

Table 1. Combination of possible results (CR)

Combination of possible results (CR)	oxilaplatin increases HMGB1 in vivo by confocal microscopy	oxilaplatin increases HMGB1 in vitro by western blot	oxilaplatin increases killing by MTT	decreasing tumor size in vivo	Supporting hypothesis?
CR1	+	+	+	+	Yes
CR2	+	+	+	-	P
CR3	+	+	-	+	P
CR4	+	+	-	-	P
CR5	+	-	+	+	P
CR6	+	-	+	-	P
CR7	+	-	-	+	P
CR8	+	-	-	-	P
CR9	-	+	+	+	P
CR10	-	+	+	-	P
CR11	-	+	-	+	P
CR12	-	+	-	-	P
CR13	-	-	+	+	P
CR14	-	-	+	-	P
CR15	-	-	-	+	P
CR16	-	-	-	-	NO

Note: "+" represents the result of the experiment conducted supports the hypothesis, "-" represents the result contradicts the hypothesis.

4. Results

Little is known about Oxaliplatin's treatment effect on colorectal cancer. Therefore, to test the preclinical therapeutic effects of Oxaliplatin, this study applies Oxaliplatin treatment to HCT116 cell lines.

PR1 fully supports the hypothesis as all in vivo models support the effectiveness of Oxaliplatin in limiting the migration and invasion of colorectal cancer cells.

Oxaliplatin may increases HMGB1 in vivo by confocal microscopy and in vitro by western blot. Moreover, it increases killing by MTT. However, it not show obvious influence on decreasing tumor size in vivo.(PR2)

Alternatively, it decreases tumors' size but not increases HMGB1 in vivo by confocal microscopy and in vitro by western blot and killing by MTT.(PR15)

Oxaliplatin only increases HMGB1 in vivo by confocal microscopy and in vitro by western blot. But it can't increases killing by MTT. Oxaliplatin decreasing tumor size in vivo may either be favored or not (PR3 and PR4, respectively) . In contrast, Oxaliplatin decreases HMGB1 in vivo by confocal microscopy and in vitro by western blot but increases killing by MTT. Oxaliplatin increasing tumor size in vivo may either be favored or not .(PR13 and PR14, respectively)

Another possible set of outcomes is when Oxaliplatin increases HMGB1 in vivo by confocal microscopy and killing by MTT but decreases HMGB1 in vitro by western blot. Oxaliplatin increasing tumor size in vivo may either be favored or not.(PR5 and PR6, respectively) In contrast, when it decreases killing by MTT, it increasing tumor size in vivo may either be favored or not.(PR7 and PR8, respectively)

When Oxaliplatin decreases HMGB1 in vivo by confocal microscopy and increases HMGB1 in vitro by western blot,it increases killing by MTT and decrease tumor size in vivo may either be favored or not. (PR9 and PR10, respectively) In contrast, when Oxaliplatin increases killing by MTT, increasing killing by MTT and decrease tumor size in vivo may either be favored or not. (PR11 and PR12, respectively)

PR16 fully rejects the hypothesis that Oxaliplatin has antimetastatic potential against colorectal cancer cells as Oxaliplatin did not demonstrate significant effectiveness in inhibiting the migration and invasion in vivo, suggesting Oxaliplatin has no effect on colorectal.

5. Conclusion

In conclusion, this paper examines the potential of Oxaliplatin to repress the metastasis of colorectal cancer cells in vivo and in vitro studying the effect of Oxaliplatin on the migration and invasion of colorectal cells. The results of the study would indicate whether Oxaliplatin can potentially be used as a treatment for colorectal cancer to reduce the tumor size in vivo and the amount of HCT116. These findings can prove the potential of Oxaliplatin as a treatment for colorectal cancer in the future.

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