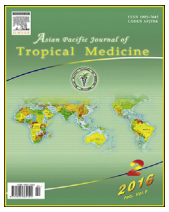
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2016.01.005

Anti-proliferation effect of zoledronic acid on human colon cancer line SW480

Fu-Shi Han[1], Mou-Bin Lin[2], Hui-Yuan Zhu[3], Ying-Qun Chen[4], Wei Shui[1], Jin-Ming Xu[1*]

[1]*Department of Medical Imaging, Affiliated Yangpu Hospital of Tongji University, Shanghai 200090, China*

[2]*Department of General Surgery, Affiliated Yangpu Hospital of Tongji University, Shanghai 200090, China*

[3]*Tongji University School of Medicine, Shanghai 200090, China*

[4]*Department of Traditional Chinese Medicine, Affiliated Yangpu Hospital of Tongji University, Shanghai 200090, China*

ARTICLE INFO

Article history:
Received 15 Nov 2015
Received in revised form 20 Dec 2015
Accepted 30 Dec 2015
Available online 11 Jan 2016

Keywords:
Zoledronic acid
Colorectal cancer
Human colon cancer line SW480
Apoptosis
Mitochondria

ABSTRACT

Objective: To investigate the anti-proliferation effect and mechanism of zoledronic acid (ZOL) on human colon cancer line SW480.

Methods: SW480 cells were treated with 0, 12.5, 25, 50, 100 and 200 μmoL/L of ZOL for 48 h, and CCK-8 assay was employed to obtain the survival rate of SW480 cells. SW480 cells were treated with 25 μmoL/L of ZOL for 0, 12, 24, 48 and 72 h, and then the survival rate was obtained. SW480 cells of the ZOL group were treated with 25 μmoL/L of ZOL for 48 h, while cells of the CsA + ZOL group were pretreated with 10 μmoL/L of CsA for 0.5 h and then treated with 25 μmoL/L of ZOL for 48 h. Then the survival rates of SW480 cells of the control group, ZOL group and CsA + ZOL group were determined. Flow cytometry was employed to detect the apoptosis rate and the mitochondrial transmembrane potential ($\triangle\Psi m$) of the three groups and Western blot was used to detect the expressions of cyt C in the cytosol of the three groups.

Results: ZOL inhibited the proliferation of SW480 cells, and the inhibition rate positively correlated with the concentration of ZOL and the action time ($P < 0.01$). The cell survival rate and the $\triangle\Psi m$ of the ZOL group were greatly lower than those of the control group, while the apoptosis rate and the expression of cyt C in the cytosol were obviously higher than those of the control group. All the differences showed distinctly statistical significances ($P < 0.01$). The cell survival rate and the $\triangle\Psi m$ of the CsA + ZOL group were all lower than those of the control group, but substantially higher than those of the ZOL group; while the apoptosis rate and the expression of cyt C in the cytosol were higher than those of the control group, but distinctly lower than those of the ZOL group. All the differences were statistically significant ($P < 0.01$).

Conclusions: ZOL can induce the apoptosis in human colon cancer line SW480 and then inhibit the proliferation of SW480 cells directly by opening the mitochondrial permeability transition pore abnormally, decreasing $\triangle\Psi m$, and releasing the cyt C into the cytosol. And the effect enhances with the increases of the concentration of ZOL and the action time.

1. Introduction

In recent years, the transformation to delicate diet structure and intensive life style cause rapidly increase of colorectal cancer morbidity by nearly 5% every year, which far exceeds 2% of the international average standard [1]. The latest investigation report on cancer epidemiology showed that the morbidity and mortality rates of colorectal cancer in 2010 in our country reached 16.14% and 7.55%. Among them, most patients were males. The morbidity and mortality of male patients were 18.75% and 9.10%, respectively [2]. Colorectal cancer has obviously become one of the most dangerous killers for human health which severely threatens people's life and health. The grim situation has set off an alarm bell for medical workers to attach importance to the treatment research of the disease. As the newest nitrogen-containing

*Corresponding author: Jin-Ming Xu, Chief Physician, Department of Medical Imaging, Affiliated Yangpu Hospital of Tongji University, Tengyue Road No. 450, Shanghai 200090, China.
Tel: +21 65690520
E-mail: hanfushi007@126.com

Peer review under responsibility of Hainan Medical College.
Foundation project: This study is supported by National Natural Science Foundation (No. 81272480).

bisphosphonate, the bone suppression absorption activity of zoledronic acid (ZOL) increases by 2000 times as compared to that of the flexor chloride sodium phosphate and reaches the 200 times of that of pamidronate [3], which is widely employed to prevent and treat solid tumor bone metastases and delay the development of the cancer-caused bone damage. In the further clinical experiences, it is proved that ZOL can not only prevent bone diseases, but also have effects on the cancer activity which can inhibit the proliferation and reduce the activity of cancer cells. However, researches on effects of ZOL were mainly on diseases, such as breast cancer, prostate cancer, squamous-cell carcinoma and so on [4–6]. There are few researches on the effects of ZOL on colorectal cancer. Therefore, the study aims to propose a new development direction for the treatment of colorectal cancer by investigating the anti-proliferation effect and mechanism of ZOL on human colon cancer line SW480, and now it is reported as follows.

2. Materials and methods

2.1. Cell lines, experimental materials and methods

Six concentration levels of ZOL were established and every level was treated with three holes parallel sample for subculture until the concentration of the logarithmic phase of SW480 (Shanghai Cells of Chinese Academy of Sciences) became 2×10^4/mL after digestion and dilution. They were placed on 96-well plates with 0.1 mL/well for anchorage growth under a suitable environment. After 24 h, the medium was changed into a fresh medium which had ZOL (Novartis Co. Ltd, Switzerland) and the concentrations of ZOL of each hole were 0, 12.5, 25, 50, 100 and 200 μmoL/L. After 48 h of the infiltration with ZOL, 0.01 mL of CCK-8 reagent (Shanghai LI Rui Biotechnology Co., Ltd.) was added along the hole wall into each hole. The reagent and the medium were mixed by taping the culture plate. After 4 h of full reaction, the light absorption values at 450 nm of each group were detected. The experience was conducted three times to investigate the effects of ZOL on anti-proliferation of SW480 in different concentrations.

Five time levels were established and meanwhile parallel sample with three holes in each level was treated. SW480 cells in the logarithmic phase with the concentration of 2×10^4 mL were cultured on 96-well plates with 0.1 mL/well. After adherent culture, the medium was changed into a fresh medium and then they were continuously cultured for 72 h. Then in the corresponding time points, 25 μmoL/L of ZOL was added into them. After ZOL was infiltrated for 0, 12, 24, 48 and 72 h, CCK-8 reagent was added. After 4 h of full reaction, the light absorption values at 450 nm of each group were detected. The experience was conducted three times to investigate the effects of ZOL on anti-proliferation of SW480 in different time periods.

Three control groups were formed and meanwhile parallel sample with three holes was treated. SW480 cell suspension with the concentration of 4×10^5/mL was inoculated on 6-well plates for anchorage growth under a suitable environment. After 24 h, the medium of the CsA + ZOL group was changed into a fresh medium which contained 10 μmol/L of cyclosporine A for pretreatment. The media of the control and the ZOL groups were changed into equal number of fresh media. After CsA had infiltrated 0.5 h, 25 μmoL/L of ZOL was respectively added into the ZOL and CsA + ZOL groups. The three groups were continuously cultured for 48 h, and then they were proceeded as follows: ① 0.01 mL of CCK-8 reagent was added along the hole wall into each hole. After 4 h of full reaction, the cell activity was tested in the light of the CCK-8 kit instruction to study the differences of the survival rate of SW480 cells; ② cells treated with digestion, washing, dilution and other operations were collected and their apoptosis rates were determined by FACS-Calibu flow cytometry (BD Company, USA); ③ suspensions of each group which had made form cell's digestion and washing were collected and fully mixed with 1 mmoL/L of JC-1 (Shanghai Yisheng Biotechnology Co., Ltd.). Then after shading cultivation for 20 min, cells were collected by centrifugation. After washing and diluting many times, the fluorescence intensity was detected by flow cytometry to investigate the difference of the mitochondrial transmembrane potential ($\triangle\Psi$m); ④ cells after homogenate of each group were centrifuged at 12000 g for 15 min at 4 °C. Then, the supernatant was collected and its expression quantity of cyt C was tested by Western Blot to investigate the differences of the content of cyt C in the cytosol in each group.

2.2. Statistical methods

SPSS 19.0 was used for statistical analysis. Cell survival rate, apoptosis rate, $\triangle\Psi$m, and the protein content of cyt C were all represented with mean ± SD. As for the data of the cell survival rates which were treated with ZOL in different concentration levels and time levels, ANOVA was used to analyze multi-group comparison and *SNK-q* was used to test comparison between two groups. As for the data of the control groups, comparison between two groups was tested by *t*-test. $P < 0.05$ showed statistical significance.

3. Results

3.1. Dose-dependent effects of zoledronic acid on SW480 cell survival rate

The cell survival rate which had detected in the concentration of 0 μmoL/L of ZOL was regarded as the reference standard. The cell survival rates of SW480 cells which were treated with 12.5, 25, 50, 100 and 200 μmol/L of ZOL for 48 h dropped to (64.23 ± 3.23)%, (51.88 ± 3.06)%, (44.81 ± 2.93)%, (39.28 ± 2.67)% and (30.24 ± 3.34)% respectively. With the increase in the infiltrating concentration of ZOL, the survival rates of SW480 in its different concentration levels decreased step by step, and the comparison differences had distinctly statistical significances ($P < 0.01$). Compared with the untreated group, cell survival rates of SW480 cells in the different concentration levels of ZOL were significantly lower. Also, the comparison differences had distinctly statistical significances ($P < 0.01$) (Table 1).

3.2. Time-dependent effects of zoledronic acid on SW480 cell survival rate

The cell survival rate of ZOL which had detected after infiltrating for 0 h was regarded as the reference standard. The cell survival rates of SW480 cells which were respectively treated with 200 μmol/L of ZOL for 12, 24, 48 and 72 h dropped to (90.77 ± 2.82)%, (73.38 ± 2.49)%, (51.88 ± 3.06)% and (26.25 ± 1.79)%. With the extension of the infiltration time in