Int J Clin Exp Med 2020;13(8):6224-6228
www.ijcem.com /ISSN:1940-5901/IJCEM0097688

Original Article

Celecoxib interferes with the proliferation and apoptosis of rat pituitary adenoma GH3 cells

Qinghao Wang[1], Haocong Wang[1], Tao Song[1,2]

[1]*Department of Neurosurgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250021, Shandong, China;* [2]*Department of Neurosurgery, Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University, Jinan 250021, Shandong, China*

Received May 29, 2019; Accepted October 9, 2019; Epub August 15, 2020; Published August 30, 2020

Abstract: Recently, the incidence of pituitary adenoma has been gradually increasing. Therefore, it is urgent to explore safe and effective treatments. We administered celecoxib, an early and highly selective COX-2 inhibitor, to treat rat pituitary adenoma, pair (GH3) cell proliferation, apoptosis, and hormone secretions. Here, we discuss celecoxib's mechanism that interferes with GH3 cells. We treated GH3 cells with different concentrations of celecoxib in vitro. We then analyzed the effect of the drug on GH3 cell proliferation activity using an MTT assay, and we measured the apoptosis of GH3 cells using TUNEL staining and flow cytometry. In addition, we measured the protein expressions of Bax, Bcl-2, and caspase-3 using western blot. Compared with the control group, the GH3 cell apoptosis and proliferation inhibition rates were associated with the concentration of celecoxib in the low, medium, and high dose celecoxib-groups and showed significant differences ($P<0.05$). At the same time, the Bax and caspase-3 protein expressions in the celecoxib-group were significantly increased ($P<0.05$), and the Bcl-2 protein expression in the high dose group was significantly reduced ($P<0.05$). Celecoxib can inhibit tumor cell proliferation and accelerate cell apoptosis.

Keywords: Celecoxib, pituitary adenoma, GH3 cells, proliferation, apoptosis

Introduction

Pituitary adenoma is a common intracranial tumor that occurs in the anterior and posterior pituitary lobes of the tumor. Recently, its incidence has increased dramatically, and is the most common intracranial tumor after gliomas and meningiomas [1]. An intracranial benign pituitary tumor, pituitary adenomas do not undergo aggressive growth, so their treatment is mainly surgery followed by postoperative radiotherapy and chemotherapy. Most patients respond well to treatment and there is a low recurrence rate after the treatment. However, a small number of patients with pituitary tumors suffer infiltration sieve to the sphenoid sinus and sella bone, and the surrounding tissue damages the saddle area. Such pituitary tumors are called invasive pituitary adenoma. The surgical resection rate of those tumors is low, and postoperative chemotherapy is ineffective. Therefore, it is urgent to explore more safe and effective treatments [2]. Cyclooxygenase (COX) is a key arachidonic acid metabolism factor involved in the whole metabolic process [3]. Cyclooxygenase includes two isomeric structures: COX-1 maintains the stability of the intracellular PG, and COX-2 plays a role in inducing the expression. When the COX-2 expression increases, it can participate in malignant cell proliferation and anti-apoptosis [4]. Studies have found that COX-2 is involved in the progression of pituitary adenomas, and celecoxib is a highly selective COX-2 inhibitor, mainly used to treat rheumatoid arthritis and cancerous familial bowel polyps in the clinic [5]. More recently, studies have focused on the intervention effect of celecoxib on the invasion and angiogenesis of pituitary tumors [6]. However, there is little data on the drug's effect on pituitary cell proliferation and apoptosis. In addition, the mechanism by which COX-2 and celecoxib interfere with pituitary cell proliferation and apoptosis is still unclear. Therefore, it is

urgent to determine how celecoxib works. In the present study, we completed an analysis of celecoxib intervention on GH3 cell proliferation, apoptosis, and hormone secretion to explore celecoxib's potential mechanism.

Materials and methods

Cells

Rat GH3 cells: purchased from Nanjing Grand Biotechnology Company.

Agents

The FBS was purchased from the Nanjing Helprin Tech Co. The RPMI 1640 medium was purchased from Guinness, owned by the Nanjing Biological Technology Co., Ltd.; The MTT was purchased from the United States Mego Company. The celecoxib was purchased from the Yangtze River Pharmaceutical Co. (Zhunzi h20120063). The TUNEL kit was purchased from the Shanghai Biological Technology Co., and the Bax and Bcl-2 antibodies were purchased from the Nanjing Liyun Biotechnology Co., Ltd. The caspase-3 and β-actin monoclonal antibodies were purchased from Nanjing Zhongshan Biotechnology Ltd. The flow apoptosis kit was purchased from the Shanghai Kaiji Biotechnology Co., Ltd.

GH3 cells

Rat GH3 cells were cultured in ab RPMI 1640 medium, at 37°C, with 5% CO_2 and a humidity of 100%. We changed the medium every two days. The GH3 cells in the logarithmic growth cycle were used last in the experiments [7].

Treatments

There were four groups: the control group (which contained the same amount of DMSO), and the low, medium, and high dose celecoxib-groups. The concentrations of the low, medium, and high doses were 20, 40, and 80 μmol/L, respectively.

GH3 cell morphology

All four groups of the GH3 cells were placed on 6-well plates, and the cell density was 1.0×10^6 cell/mL. After the drug treatment, we cultured the cells for another 72 hours, then we used an inverted phase contrast microscope to analyze the morphology of the GH3 cells.

GH3 cell proliferation

An MTT assay was used to analyze the effect of celecoxib on the GH3 cells in 96-well plates, with a seeding cell density of 1.0×10^4 cell/mL. After 24 h culture, the cells were treated with celecoxib, and then they were cultured in an RPMI 1640 medium for another 72 h. We added 20 μL MTT to each well and incubated them for 4 h, and then the supernatant was discarded and 150 μL DMSO was added. We measured the absorbance of each well at a wavelength of 570 nm using an enzyme-linked immunosorbent monitor. We then analyzed the GH3 cell proliferation inhibition rate and the inhibition rate (treated group, the average value of A/control group mean A value).

GH3 apoptosis rate

In the present study, the TUNEL method was used to analyze the effect of celecoxib on GH3 cell apoptosis. The GH3 cells on a logarithmic growth cycle were seeded in 6-well plates with a cell density of 1.0×10^4 cell/mL, and cultured for 24 h. Then the cells were treated with the different concentrations of celecoxib. According to the TUNEL reagent operating instructions, we randomly selected six high-power fields (each with ≥100 cells) and placed them under a fluorescence microscope to analyze the GH3 cell apoptosis: cell apoptosis rate = (TUNEL-positive cells/total cells) × 100%. GH3 cells on logarithmic growth cycle were seeded in 96-well plates, and cultured for 24 h. Then the cells were treated with the different concentrations of celecoxib. According to the kit's instructions, we used flow cytometry to measure the cell apoptosis rate.

The expressions of Bax Bcl-2 and caspase-3 protein in the GH3 cells

Western blot was used to determine the Bax, Bcl-2, and caspase-3 protein expressions in the GH3 cells. The GH3 cells on a logarithmic growth cycle were seeded in the 6-well plates. After 24 h culture, the cells were treated with celecoxib and then cultured in a RPMI 1640 medium for another 72 h. The cells were lysed and we extracted the total protein. 40 μg protein was used for the SDS-polyacrylamide gel electrophoresis. After that, we transferred the protein onto a PVDF membrane, and we blocked it with 5% nonfat milk. We incubated the membranes with Bax, Bcl-2, and a caspase-3

Table 1. The GH3 apoptosis rate in each group after the interventions

Groups	GH3 apoptosis rate (%)	
	TUNEL	Flow cytometry method
Control	1.71±0.59	1.36±0.45
Low-dose celecoxib group	12.54±2.14*	11.21±1.96*
Middle-dose celecoxib group	19.52±3.24*	17.21±2.74*
High-dose celecoxib group	31.25±0.59*	29.74±3.14*

Note: One-way ANOVA was performed to compare the differences among the different groups. Compared with the control group, *P<0.05 indicates a significant difference.

Table 2. The GH3 cell proliferation inhibition rate in each group

Group	GH3 Inhibition rates of cell proliferation (%)
Control	3.56±0.58
Low-dose celecoxib group	13.11±1.43*
Middle-dose celecoxib group	20.22±2.12*
High-dose celecoxib group	30.16±3.23*

Note: One-way ANOVA was performed to compare the differences among the different groups. Compared with the control group, *P<0.05 indicates a significant difference.

antibody (1:1000) overnight. After we washed the membranes with a TBST buffer three times, the membrane was incubated with horseradish peroxidase-labeled secondary antibody and placed at room temperature for 2 h. Then we washed it with a TBST buffer three times and then we exposed it. β-actin protein was used as a internal reference standard to analyze the value of each group's optical density (sample optical density/internal reference optical density) [8].

Statistical analysis

The data were processed using SPSS 19.0 software and are presented as the mean ± standard deviation (SD). One-way ANOVA was performed to compare the differences among the different groups. P<0.05 indicated a statistical significance.

Results

Changes in cell morphology

The number of GH3 cells in the low and medium celecoxib groups was significantly reduced, and the remaining GH3 cells became round, with reduced brightness and decreased refraction, while the GH3 cells in the control group showed increased brightness and good refraction. The volume of GH3 cells in the high-dose celecoxib group was significantly reduced and had a lower refractive index, and the majority of the GH3 cells disintegrated, had a medium color, and became cloudy.

Celecoxib interfered with the cell apoptosis

According to the results of the TUNEL assay and flow cytometry: compared with the control group, the apoptosis rates of all three celecoxib groups in the rat GH3 were significantly higher (P<0.05). And the apoptosis rates in the GH3 cells increased in a dose-dependent way according to the celecoxib concentrations (**Table 1**).

Intervention on the proliferation of the GH3 cells

According to the results of the MTT assay and the flow cytometry: compared with the control group, after being treated with celecoxib, the proliferation inhibition rate of the GH3 cells increased in a dose-dependent way according to the celecoxib concentrations, and the differences were significant (P<0.05) (**Table 2**).

The intracellular Bax, Bcl-2, and caspase-3 protein expressions in the GH3 cell

Compared with the control group, the Bax and caspase-3 proteins were significantly increased in all the celecoxib groups, and the differences were significant (P<0.05). Compared with the control group, the Bcl-2 protein expression in all the celecoxib groups was significantly reduced, and the differences were significant (P<0.05) (**Table 3** and **Figure 1**).

Discussion

Celecoxib, the main drug treatment for arthritis, selectively inhibits COX-2 expression. A previous study found that celecoxib can inhibit a variety of tumors, and it can be used with other drugs to create a synergistic anti-tumor effect

Table 3. Expression of Bax, Bcl-2 and caspase-3 proteins in GH3 cells of each group

Groups	Bax	Bcl-2	caspase-3
Control	0.56±0.21	0.69±0.19	0.10±0.03
Low-dose celecoxib group	1.21±0.31*	0.45±0.18*	0.25±0.11*
Middle-dose celecoxib group	1.45±0.32*	0.31±0.15*	0.45±0.15*
High dose celecoxib group	1.98±0.43*	0.15±0.11*	0.64±0.14*

Note: One-way ANOVA was performed for comparison of the differences among different groups. Compared with the control group, *P<0.05 indicates sign.

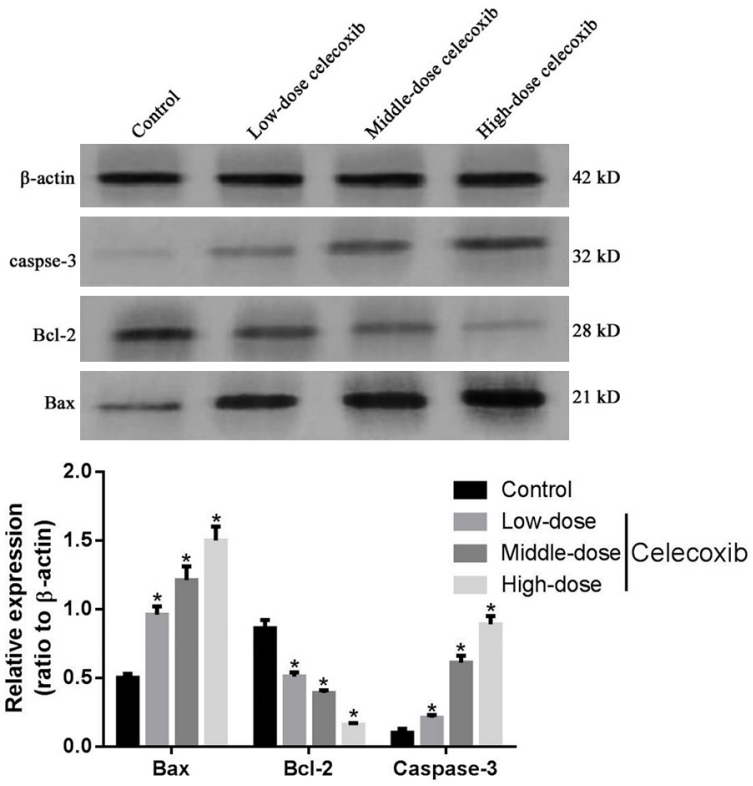

Figure 1. The expressions of the Bax, Bcl-2, and caspase-3 proteins in GH3 cells. One-way ANOVA was performed to compare the differences among the different groups. Compared with the control group, *P<0.05 indicates a significant difference.

[9-12]. Combined with other drugs, it enhances the sensitivity of cancer chemotherapy drugs. Currently, celecoxib's anti-tumor activity is being tested in clinical trials to determine whether it can significantly reduce the incidence of colon cancer [13-16]. Celecoxib was found to inhibit proliferation in PC PANC-I cells *in vitro* and play a catalytic role in the process of apoptosis. Meanwhile, celecoxib can inhibit the occurrence of carcinogen-induced rat gastric cancer and promote the apoptosis of gastric cancer cells [17-20].

In the present study, after rat pituitary adenoma GH3 cells were treated with celecoxib, compared to the control group, the cells showed significant morphological changes, had increased apoptosis, and the rates of cell proliferation inhibition and apoptosis were significantly increased in a dose-dependent way (P<0.05), suggesting that celecoxib significantly inhibits cell proliferation and promotes cell apoptosis. Compared with the control group, the Bax and caspase-3 proteins in the celecoxib group were significantly increased (P<0.05). Compared with the control group, the Bcl-2 protein expression was significantly reduced (P<0.05). Therefore, celecoxib's mechanisms that accelerate rat pituitary adenoma GH3 cell apoptosis may involve the upregulation of Bax and caspase-3 and the downregulation of Bcl-2.

In summary, the present study found that celecoxib inhibits tumor cell proliferation and accelerates cell apoptosis, findings which will provide basic experimental evidence for the clinical use of COX-2 inhibitors to treat pituitary adenoma.

Acknowledgements

This work was supported by a grant from the Shandong Provincial Key Research and Development Project (no. 2015GSF118085).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Tao Song, Department of Neurosurgery, Shandong Provincial Hospital Affiliated to Shandong First Medical University, No. 324, Jingwei 7th Road, Jinan 250021, Shandong, China. Tel: +86-0531-87938911; Fax: +86-0531-87938911; E-mail: chendi32186@yeah.net

References

[1] Zada G, Woodmansee WW, Ramkissoon S, Amadio J, Nose V and Laws ER Jr. Atypical pituitary adenomas: incidence, clinical characteristics, and implications. J Neurosurg 2011; 114: 336-344.

[2] Saito T, Tamura D and Asano R. Usefulness of selective COX-2 inhibitors as therapeutic agents against canine mammary tumors. Oncol Rep 2014; 31: 1637-1644.

[3] Bocca C, Bozzo F and Miglietta A. COX2 inhibitor NS398 reduces HT-29 cell invasiveness by modulating signaling pathways mediated by EGFR and HIF1-alpha. Anticancer Res 2014; 34: 1793-1800.

[4] Onguru O, Scheithauer BW, Kovacs K, Vidal S, Jin L, Zhang S, Ruebel KH and Lloyd RV. Analysis of Cox-2 and thromboxane synthase expression in pituitary adenomas and carcinomas. Endocr Pathol 2004; 15: 17-27.

[5] Vidal S, Kovacs K, Bell D, Horvath E, Scheithauer BW and Lloyd RV. Cyclooxygenase-2 expression in human pituitary tumors. Cancer 2003; 97: 2814-2821.

[6] Ding XL, Zhu C, Qiang H, Zhou XR and Zhou GX. Enhancing antitumor effects in pancreatic cancer cells by combined use of COX-2 and 5-LOX inhibitors. Biomed Pharmacother 2011; 65: 486-490.

[7] Liu M, Li CM, Chen ZF, Ji R, Guo QH, Li Q, Zhang HL and Zhou YN. Celecoxib regulates apoptosis and autophagy via the PI3K/Akt signaling pathway in SGC-7901 gastric cancer cells. Int J Mol Med 2014; 33: 1451-1458.

[8] Seo KW, Coh YR, Rebhun RB, Ahn JO, Han SM, Lee HW and Youn HY. Antitumor effects of celecoxib in COX-2 expressing and non-expressing canine melanoma cell lines. Res Vet Sci 2014; 96: 482-486.

[9] Uddin MJ, Crews BC, Ghebreselasie K, Huda I, Kingsley PJ, Ansari MS, Tantawy MN, Reese J and Marnett LJ. Fluorinated COX-2 inhibitors as agents in PET imaging of inflammation and cancer. Cancer Preven Res 2011; 4: 1536-1545.

[10] Telliez A, Furman C, Pommery N and Henichart JP. Mechanisms leading to COX-2 expression and COX-2 induced tumorigenesis: topical therapeutic strategies targeting COX-2 expression and activity. Anticancer Agents Med Chem 2006; 6: 187-208.

[11] Liu CM, Hong CY, Shun CT, Hsiao TY, Wang CC, Wang JS, Hsiao M and Lin SK. Inducible cyclooxygenase and interleukin 6 gene expressions in nasal polyp fibroblasts - possible implication in the pathogenesis of nasal polyposis. Arch Otolaryngol Head Neck Surg 2002; 128: 945-951.

[12] Shimizu S, Fujii G, Takahashi M, Nakanishi R, Komiya M, Shimura M, Noma N, Onuma W, Terasaki M, Yano T and Mutoh M. Sesamol suppresses cyclooxygenase-2 transcriptional activity in colon cancer cells and modifies intestinal polyp development in Apc (Min/+) mice. J Clin Biochem Nutr 2014; 54: 95-101.

[13] Vaish V and Sanyal SN. Role of sulindac and celecoxib in chemoprevention of colorectal cancer via intrinsic pathway of apoptosis: exploring NHE-1, intracellular calcium homeostasis and calpain 9. Biomed Pharmacother 2012; 66: 116-130.

[14] Sun PX, Liu YH, Ying HQ and Li SY. Action of db-cAMP on the bystander effect and chemosensitivity through connexin 43 and Bcl-2-mediated pathways in medulloblastoma cells. Oncol Rep 2012; 28: 969-976.

[15] Howe LR and Dannenberg AJ. A role for cyclooxygenase-2 inhibitors in the prevention and treatment of cancer. Semin Oncol 2002; 29: 111-119.

[16] Vidal S, Kovacs K, Bell D, Horvath E, Scheithauer BW and Lloyd RV. Cyclooxygenase-2 expression in human pituitary tumors. Cancer 2003; 97: 2814-2821.

[17] Leahy KM, Ornberg RL, Wang Y, Zweifel BS, Koki AT and Masferrer JL. Cyclooxygenase-2 inhibition by celecoxib reduces proliferation and induces apoptosis in angiogenic endothelial cells in vivo. Cancer Res 2002; 62: 625-631.

[18] Bloomer CW, Kenyon L, Hammond E, Hyslop T, Andrews DW, Curran WJ and Dicker AP. Cyclooxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR) expression in human pituitary macroadenomas. Am J Clin Oncol Cancer Clin Trials 2003; 26: S75-S80.

[19] Han X, Li H, Su L, Zhu W, Xu W, Li K, Zhao Q, Yang H and Liu H. Effect of celecoxib plus standard chemotherapy on serum levels of vascular endothelial growth factor and cyclooxygenase-2 in patients with gastric cancer. Biomed Rep 2014; 2: 183-187.

[20] Ghate NB, Hazra B, Sarkar R and Mandal N. Heartwood extract of Acacia catechu induces apoptosis in human breast carcinoma by altering bax/bcl-2 ratio. Pharmacogn Mag 2014; 10: 27-33.