Cetuximab combined with cisplatin improves the prognosis of gastric cancer patients and its effect on P38 MAPK expression

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Summary

Purpose: To observe the clinical efficacy of cetuximab combined with cisplatin in gastric cancer patients, and to explore its potential mechanism so as to provide references for clinical chemotherapeutic drugs for gastric cancer.

Methods: A total of 122 gastric cancer patients undergoing chemotherapy in our hospital from August 2014 to June 2017 were enrolled and divided into cetuximab group (n=64) and cetuximab + cisplatin group (n=58) according to the chemotherapy regimen. The clinical efficacy, prognosis, adverse reactions and immune status were compared between the two groups of patients. At the same time, the expressions of serum gastric cancer markers, carcinoembryonic antigen (CEA) and vascular endothelial growth factor (VEGF) in both groups of patients were detected before and after treatment. In addition, the P38 protein expression level in cancer tissues in both groups of patients was detected via Western blotting before and after treatment.

Results: The total effective rate of clinical efficacy was 66.41 and 82.55%, respectively, in cetuximab group and the cetuximab + cisplatin group (p<0.05). The mortality and tumor metastasis rates were 16.5% vs. 3.4% and 12.3% vs. 4.5%, respectively, in the cetuximab group and the cetuximab + cisplatin group (p<0.05). The percentages of cluster of differentiation 3 (CD3), CD4, CD8, CD4/CD8 and T cell metastasis rate after treatment were significantly increased in the cetuximab + cisplatin group (p<0.05), but significantly declined in the cetuximab group (p<0.05). In addition, the levels of serum CEA and VEGF in both groups were significantly decreased (p<0.05), but they declined more significantly in the cetuximab + cisplatin group. Furthermore, it was found that cetuximab + cisplatin group had a stronger phosphorylation ability of P38 in gastric cancer tissues than the cetuximab group (p<0.05).

Conclusions: Compared with cetuximab alone, cetuximab combined with cisplatin can significantly improve the clinical efficacy, reduce the tumor metastasis rate, enhance the immune function and improve the prognosis of gastric cancer patients, whose mechanism may be related to the activation of P38 in gastric cancer tissues.

Key words: gastric cancer, cetuximab, cisplatin, efficacy, P38

Introduction

Gastric cancer is one of the major causes of cancer-related deaths in the world, especially in China [1]. Although the overall morbidity rate of gastric cancer is currently declining, the incidence rate of gastroesophageal junction tumors with extremely poor prognosis is increasing [2]. In recent years, great improvement has been made in the treatment of gastric cancer, and the surgical resection combined with chemoradiotherapy makes it possible to even cure some gastric cancer patients, but the invasion and metastasis of this malignancy seriously affect the therapeutic effect [3,4]. The probabilities of postoperative recurrence or metastasis of gastric cancer remain high in patients

receiving early radical surgery [5]. Therefore, further optimizing the therapeutic regimens for gastric cancer is of great significance in improving the long-term prognosis of patients.

Studies have demonstrated that adjuvant radiotherapy for gastric cancer after surgery can significantly reduce the local tumor recurrence rate and improve the prognosis of patients [6]. However, the long-term administration of single chemotherapeutic drugs can notably reduce the chemosensitivity of cancer cells, resulting in drug resistance of cancer cells. Compared with single-drug chemotherapy or no chemotherapy, the combination chemotherapy can improve the prognosis of patients with advanced gastric cancer and enhance the cytotoxic effect of chemotherapeutic drugs on cancer cells [7]. Cetuximab is a monoclonal IgG1 antibody [8] that can affect the epidermal growth factor receptor (EGFR) in a targeted way. Cetuximab binds to the extracellular region in the inactive structure of EGFR, competing for receptor binding through blocking the ligand binding domain. Such interaction between antibodies and receptors prevents receptor dimerization, thereby blocking the ligand-induced EGFR tyrosine kinase activation [9]. In addition, cetuximab can also induce the internalization, down-regulation and degradation of EGFR, thus inhibiting the occurrence and development of gastric cancer [10]. Studies have shown that the efficacy of cetuximab combined with 5-fluorouracil is superior to that of chemotherapeutic drugs in the treatment of metastatic gastric cancer [11]. However, the clinical efficacy of cetuximab combined with cisplatin in gastric cancer patients has not been reported yet. In the present study, the effects of cetuximab combined with cisplatin on the prognosis, clinical efficacy and immune system function of gastric cancer patients were evaluated, and the potential molecular mechanism of drug combination in improving the clinical efficacy on gastric cancer was also explored.

Methods

General data

A total of 122 gastric cancer patients undergoing chemotherapy in our hospital from August 2014 to June 2017 were enrolled. The general data of patients are shown in Table 1, and the baseline data had no statistically significant differences between the two groups of patients (p>0.05), which were comparable. The present study was approved by the Ethics Committee of our hospital, and all patients enrolled signed the informed consent.

Inclusion criteria

1) Patients pathologically diagnosed with gastric cancer (TNM stage II-III); 2) patients undergoing gastric cancer resection, including but not limited to radical surgery and extended radical surgery of gastric cancer; 3) patients without receiving any other chemoradiotherapy regimen within 3 month after surgery; 4) patients with a Karnofsky performance scale (KPS) score >60; 5) patients without tumor metastasis and recurrence before drug administration; and 6) patients without severe hepato-renal insufficiency and hematopoietic dysfunction.

Therapeutic regimens

Chemotherapy was administered to 122 gastric cancer patients 2 weeks after surgery. According to the chemotherapy regimen, the patients were divided into the cetuximab group (n=64) and cetuximab+cisplatin group (n=58). The drugs were administered for a total of 6 cycles (30 consecutive days equalled 1 cycle). Six mL peripheral blood was drawn from patients at the beginning and end of treatment and the gastric cancer tissues collected during the gastric cancer resection were stored in a refrigerator at -80°C for later use.

Efficacy of evaluation criteria

According to the evaluation criteria of the World Health Organization (WHO), the efficacy is divided into complete remission (CR): complete disappearance of tumor >1 month; partial remission (PR): shrinkage of tumor >50% >1 month; no change (NC): enlargement of tumor <25%, or shrinkage of tumor <50%; and progressive disease (PD): new lesions or enlargement of tumor >25%.

Table 1. Clinical data of patients in both groups

Group	n	Male	Female	Age (years)	Pathological type			
					Poorly differentiated adenocarcinoma	*Moderately differentiated adenocarcinoma*	*Mucous adenocarcinoma*	*Signet-ring cell carcinoma*
Cetuximab group	64	30	34	48.78±7.41	33	12	14	5
Cetuximab+ Cisplatin group	58	32	26	46.88±5.83	28	15	10	5
Total	122	62	60	-	61	27	24	10
x^2	-	0.45	0.92	0.51				
p	-	0.71	0.13	0.24				

Detection of immune cell subsets

The immune cells in the peripheral blood, including cluster of differentiation 3 (CD3), CD4, CD8, CD4/CD8 ratio and T cell rate in both groups of patients were detected *via* flow cytometry before and after treatment.

Western blotting

The gastric cancer tissues in both groups of patients were subjected to lysis buffer before and after treatment. Then the lysis buffer was centrifuged and the supernatant were taken and placed into Eppendorf (EP) tube. Total protein concentration extracted from gastric cancer tissues was detected *via* ultraviolet spectrometry, and the protein samples were subjected to an isochoric process. After the total protein was extracted, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. Then, the protein in the gel was transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA), incubated with the primary antibody at 4°C overnight, and then incubated again with goat anti-rabbit secondary antibody in the dark for 1 h. The protein bands were scanned and quantified using the Odyssey scanner (Lincoln, NE, USA), and the level of proteins to be detected was corrected using glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Detection of VEGF expression in the peripheral blood via reverse transcription-polymerase chain reaction (RT-PCR)

1. The total RNA was extracted from the peripheral blood using the TRIzol method (Invitrogen, Carlsbad, CA, USA), the concentration and purity of the extracted RNA were detected using ultraviolet spectrophotometer, and the RNA with absorbance $(A)_{260}/A_{280}$ of 1.8-2.0 was used; 2. The messenger RNA (mRNA) was synthesized into the complementary DNA (cDNA) through reverse transcriptase (RT) and stored in the refrigerator at -80°C; 3. RT-PCR system: 2.5 µL 10 × Buffer, 2 µL cDNA, 0.25 µL forward primer (20 µmol/L), 0.25 µL reverse primer (20 µmol/L), 0.5 µL dNTPs (10 mmol/L), 0.5 µL Taq enzyme (2×10^6 U/L) and 19 µL ddH$_2$O. The amplification system of RT-PCR was the same as above.

Statistics

SPSS 22.0 software (IBM, Armonk, NY, USA) was used for the analysis of all data. Measurement data were expressed as mean ± standard deviation, and t-test was used for the comparison of data between two groups. Percentages (%) were used to express the percentage data, and x^2 test was used for data analysis. No other statistical tests were used in this study. P<0.05 suggested that the difference was statistically significant.

Results

Comparison of clinical efficacy between the two groups

After 6 cycles of chemotherapy, the total clinical effective rate was 66.41% and 82.55%, respectively, in the cetuximab group and the cetuximab + cisplatin group, and the difference was statistically significant (p<0.05; Table 2).

Comparison of prognosis between the two groups of patients

The prognosis of patients in the cetuximab group and the cetuximab + cisplatin group was analyzed, including 1-2-year and 3-4-year mortality rates, 1-2-year and 3-4-year metastasis rates and related complications, such as diarrhea, nausea and vomiting, alopecia and hand-foot-mouth syndrome. The results revealed that both mortality and metastasis rate in the cetuximab + cisplatin group were lower than those in the cetuximab group. In addition, the incidence of diarrhea, nausea, vomit-

Table 2. Comparison of clinical efficacy between the two groups of patients

Group	n	CR	PR	SD	PD	Total effective rate (%)	x^2	p
Cetuximab group	64	30	18	12	4	66.41	0.08	0.002
Cetuximab + Cisplatin group	58	30	10	10	8	82.55		
Total	122	60	28	22	12	74.58		

For abbreviations see text

Table 3. Comparison of prognosis between the two groups (%)

Group	n	Mortality rate			Metastasis rate			Related complications			
		1-2 years	3-4 years	Total	1-2 years	3-4 years	Total	Diarrhea	Nausea and vomiting	Alopecia	Hand-foot-mouth syndrome
Cetuximab group	64	4.2	12.3	16.5	3.2	9.1	12.3	24	34	37	12
Cetuximab + Cisplatin group	58	1.3	2.1	3.4	1.4	3.1	4.5	18	26	28	22

ing and alopecia in the cetuximab + cisplatin group were lower than those in the cetuximab group, but the number of patients with hand-foot-mouth syndrome was significantly higher than that in the cetuximab group (Table 3).
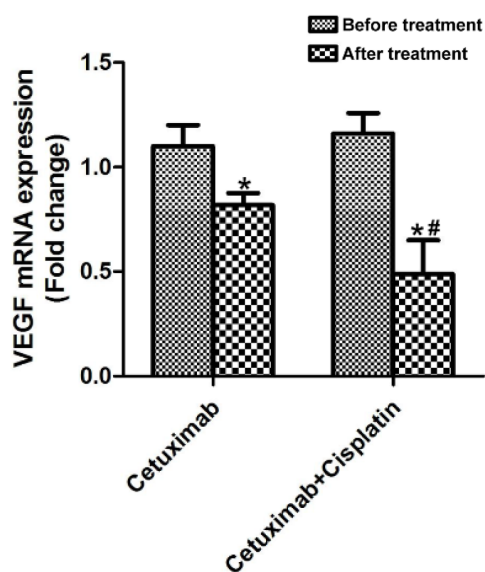
Comparison of immune function before and after treatment between the two groups of patients

The immune status of both groups of patients was evaluated after 6 cycles of therapy. The results showed that the percentages of CD3, CD4 and CD8, CD4/CD8 ratio in the peripheral blood had significant changes in the cetuximab group and the cetuximab + cisplatin group before and after treatment (p<0.05). All the above indexes were obviously increased in the cetuximab + cisplatin group (p<0.05), while they were markedly inhibited in the cetuximab group (p<0.05; Table 4), indicating that the drug combination can significantly improve the immune function of patients.

Table 4. Changes in immune indexes in the two groups of patients before and after treatment (%)

Group	CD3	CD4	CD8	CD4/CD8
Cetuximab group				
Before treatment	55.1	31.0	23.5	1.3
After treatment	50.1*	28.6*	22.4*	1.1*
Cetuximab + Cisplatin group				
Before treatment	55.8	32.4	24.8	1.8
After treatment	63.2*	45.6*	26.4*	2.1*

*p<0.05, compared with before treatment.

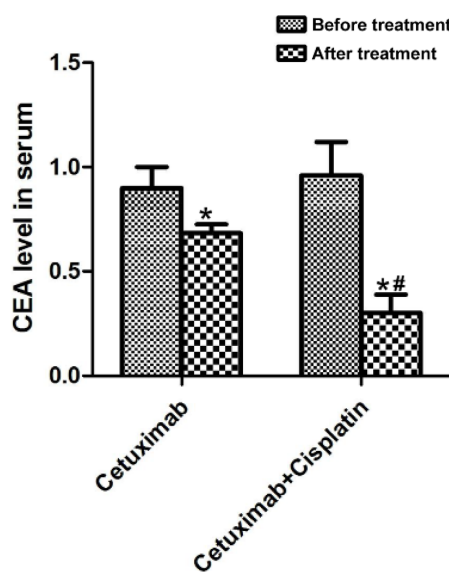*Expression of serum VEGF in both groups of patients before and after treatment*

As shown in Figure 1, the serum VEGF expression in the cetuximab group after treatment decreased 0.88 times than before treatment (p<0.05), and the serum VEGF expression in the cetuximab + cisplatin group after treatment decreased 0.43 times than before treatment (p<0.05). Statistically significant differences were found in the changes in serum VEGF expression in both groups of patients before and after treatment, but the changes were more pronounced in the cetuximab + cisplatin group than that in the cetuximab group (p<0.05).

*Expression of serum CEA in both groups of patients before and after treatment*

The expression level of serum gastric cancer CEA marker in both groups of patients was evaluated before and after treatment. As shown in Figure 2, the serum CEA level in both groups of patients was obviously decreased after treatment (p<0.05), and it declined more significantly in the cetuximab + cisplatin group than in the cetuximab group.

Expression of P38 protein in gastric cancer tissues in the two groups of patients before and after treatment

To further explore the molecular mechanism of increased efficacy after drug combination, the expression level of P38 protein in gastric cancer tissues of the two groups of patients was detected before and after treatment. As shown in Figure 3,

Figure 1. Expression of serum VEGF before and after treatment in the cetuximab group and cetuximab + cisplatin group. *p<0.05 vs. before treatment within the group and #p<0.05 vs. after treatment between the two groups, showing statistically significant differences.

Figure 2. Expression of serum CEA in patients before and after treatment in the cetuximab group and cetuximab + cisplatin group. *p<0.05 vs. before treatment within the group and #p<0.05 vs. after treatment between the two groups, showing statistically significant differences.
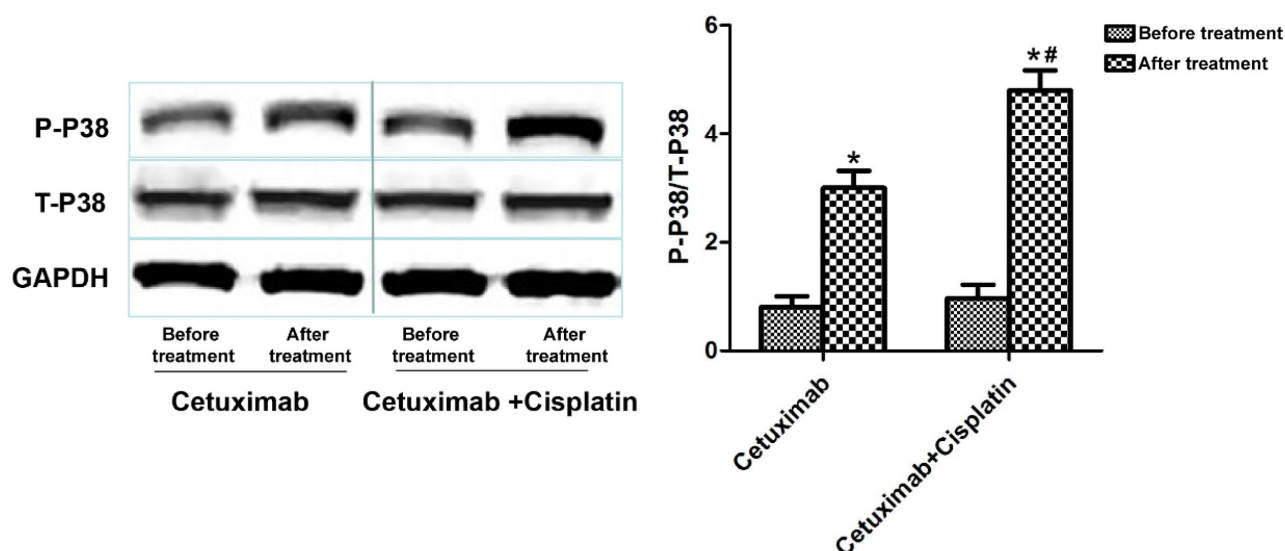
Figure 3. Expression of P38 protein in gastric cancer tissues of patients before and after treatment in the cetuximab group and the cetuximab + cisplatin group. *p<0.05 vs. before treatment within the group and #p<0.05 vs. after treatment between the two groups, displaying statistically significant differences.

the therapeutic regimens in both groups could activate the P38 protein expression in gastric cancer tissues (p<0.05), but the phosphorylation ability of P38 was stronger in the cetuximab + cisplatin group than in the cetuximab group (p<0.05).

Discussion

Gastric cancer is the fourth most common cancer in the world and the second major cause of cancer-related deaths [12]. The mechanism of growth, invasion and migration of gastric cancer is complex, involving a variety of proteins and signaling pathways [13]. In recent years, a variety of targeted drugs have gradually come into the market, offering good clinical efficacy in cancer treatment. Cetuximab is the first targeted monoclonal antibody marketed in China and initially used in the treatment of colorectal cancer and head-neck malignant tumors [14]. Studies have shown that the mutation status of Kirsten rat sarcoma viral oncogene homolog (KRAS) can serve as a negative predictive marker for the treatment of colorectal cancer with cetuximab [15]. Studies have also demonstrated that cetuximab can significantly inhibit the growth of xenografts of two kinds of gastric cancer cells, SGC-7901 (wild-type KRAS) and YCC-2 (G→A mutant-type KRAS). After treatment with cetuximab, the apoptosis level of gastric cancer SGC-7901 cells was increased significantly, while the apoptosis level of gastric cancer YCC-2 cells showed no significant changes. In addition, the expression levels of the EGFR-RAS-MEK signaling pathway-related proteins in gastric cancer SGC-7901 cells were obviously up-regulated after

treatment with cetuximab. The above results suggest that the anti-gastric cancer effect of cetuximab depends on the KRAS status in gastric cancer tissues [16].

EGFR is one of the most important related factors in the proliferation, survival, apoptosis, migration and tumorigenesis of various tumor cells [17]. The high-expression EGFR is one of the sensitive markers for the poor prognosis of gastric cancer patients [18]. Studies have shown that cetuximab achieved a good therapeutic effect in many phase II clinical studies on metastatic gastric cancer, but the overall survival time was not prolonged. The abnormal activation of EGFR and its downstream signaling pathway is considered as one of the most important mechanisms of drug resistance to cetuximab [19]. According to clinical data, there is a significant positive correlation between EGFR and RANKL expression levels in gastric cancer patients. At the same time, *in vitro* studies have manifested that RANKL activates the EGFR signaling pathway in gastric cancer cells, leading to drug resistance to cetuximab [20]. The RANKL/RANK pathway is closely related to a variety of metastatic malignant tumors and hormone-induced breast cancer [21,22]. During osteoclast differentiation, the RANKL/RANK signaling pathway can regulate the EGFR expression under a negative feedback mechanism [23]. These research results indicate that the main mechanism of reduced sensitivity of gastric cancer cells to cetuximab is the abnormal activation of the RANKL/RANK signaling pathway.

Moreover, inhibiting P38 can significantly reduce the cytotoxic effect of cetuximab in colorectal cancer cells in *in vivo* and *in vitro* models of colon

cancer. At the molecular level, cetuximab activates the transcription factor FOXO3a and promotes its nuclear translocation through the P38-mediated phosphorylation, leading to up-regulation of its target genes P27 and BIM, ultimately inducing apoptosis and inhibiting proliferation of cancer cells. In the present study, it was found for the first time that cetuximab combined with cisplatin could significantly increase the efficacy of chemotherapeutic drugs, improve the prognosis of patients and reduce the incidence of complications. In addition, it was also found that cetuximab combined with cisplatin could reduce the expression level of serum VEGF in gastric cancer patients, and activate the phosphorylation of P38 protein. It is speculated that the increased efficacy of chemotherapeutic drugs due to drug combination may be related to the activation of P38 in cancer cells by cisplatin.

However, there are still some limitations in the present experiment: 1) The cell experiments were not designed for verification, and 2) whether the increased efficacy of chemotherapeutic drugs due to drug combination depends on the activation of P38 was not explored.

Conclusions

In conclusion, this study confirms for the first time that cetuximab combined with cisplatin can improve the anti-gastric cancer effect of cetuximab, whose mechanism may be related to the activation of P38 by cisplatin.

Conflict of interests

The authors declare no conflict of interests.

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