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Comparison of the cancer cell−killing effect of the DOS scheme and the SOX scheme for neoadjuvant chemotherapy for gastric cancer

Ya−Ni Chen✉, Hai−Feng HU

Digestive System Department, Ansai People's Hospital in Yan'an Shaanxi Province, Yan'an City, Shaanxi Province, 717400

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ABSTRACT

Objective: To study the cancer cell-killing effect of the DOS scheme and the SOX scheme for neoadjuvant chemotherapy for gastric cancer. **Methods:** A total of 76 patients with gastric cancer who underwent preoperative neoadjuvant chemotherapy in Ansai People′s Hospital between June 2014 and October 2016 were selected and randomly divided into the SOX group who received oxaliplatin + S-1 scheme chemotherapy and the DOS group who accepted oxaliplatin + S-1 + docetaxel scheme chemotherapy. Serum tumor marker levels were measured before and after the chemotherapy, and the expression of proliferation genes, tumor suppressor genes and invasion genes in lesions were determined after chemotherapy. **Results:** Serum CA72-4, G17 and TK-1 levels of both groups of patients after chemotherapy were significantly lower than those before chemotherapy and serum CA72-4, G17 and TK-1 levels of DOS group after chemotherapy were significantly lower than those of SOX group; after chemotherapy, CyclinB, CyclinD1, CDK1, CDK4, CDK6, Vav2, MMP2, ADAM8 and ITF2 mRNA expression in surgically removed lesions of DOS group were significantly lower than those of SOX group while RASSF1A, Noxa, GKN1 and $p16^{ink4a}$ mRNA expression were significantly higher than those of SOX group. **Conclusion:** DOS neoadjuvant chemotherapy can be more effective than SOX in killing the gastric cancer cells and inhibiting the proliferation and invasion of cancer cells.

1. Introduction

Gastric cancer is the malignant digestive tract tumor with the highest incidence, it generally lacks of early typical clinical symptoms and is difficult to be diagnosed, the majority of patients have developed middle-advanced stage disease at diagnosis, and some patients have even lost the opportunity of surgical resection. Neoadjuvant chemotherapy is the new way of chemotherapy developed in recent years, and preoperative neoadjuvant chemotherapy can kill the cancer cells, shrink the tumor volume and reduce tumor blood supply to create conditions for surgical resection. Both SOX chemotherapy of oxaliplatin + S-1 and DOS chemotherapy of oxaliplatin + S-1 + docetaxel are the common neoadjuvant chemotherapy regimens for patients with gastric cancer[1,2], but it is not clear about the efficacy of the two options for neoadjuvant chemotherapy for gastric cancer. In the following study, the cancer cell-killing effect of the DOS scheme and the SOX scheme for neoadjuvant chemotherapy for gastric cancer were analyzed from the tumor marker levels in serum as well as proliferation gene, tumor suppressor gene and invasion gene expression in tumor lesions.

2. Case information and research methods

2.1 General information of gastric cancer patients

A total of 76 patients with gastric cancer who underwent preoperative neoadjuvant chemotherapy in Ansai People′s Hospital in Yan′an between June 2014 and October 2016 were selected, all patients were diagnosed with gastric cancer by gastroscopic pathological biopsy, abdomen CT or endoscopic ultrasonography indicated T3 stage, and they did not receive radiotherapy and chemotherapy as well as targeted drug therapy. Patients with

✉Corresponding author: Ya-Ni Chen, Digestive System Department, Ansai People′s Hospital in Yan′an Shaanxi Province, Yan′an City, Shaanxi Province, 717400.
Tel: 13630218363
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chemotherapy contraindications and those associated with distant tumor metastasis were excluded. Random number table was used to divide the 76 patients into DOS group and SOX group, each with 38 cases. DOS group included 22 men and 16 women that were 46-59 years old; SOX group included 23 men and 15 women that were 44-60 years old. There was no statistically significant difference in general information between the two groups (P>0.05).

2.2 Chemotherapy

SOX group of patients received oxaliplatin + S-1 chemotherapy, and the method was as follows: oxaliplatin into 130 mg/m^2, by intravenous drip, on day 1, S-1 60 mg/m^2, taken orally twice in the morning and evening, on day 1-14, and 21 d as a cycle; DOS group received oxaliplatin + S-1 + docetaxel chemotherapy, and the method was as follows: oxaliplatin into 130 mg/m^2, by intravenous drip, on day 1, docetaxel 60 mg/m^2, by intravenous drip, on day 1, S-1 60 mg/m^2, taken orally twice in the morning and evening, on day 1-14, oral administration of dexamethasone 10 mg 12 h before docetaxel application, and intramuscular injection of diphenhydramine 20 mg 0.5 h before docetaxel application. Both groups of patients received pantoprazole and tropisetron to stop vomiting during chemotherapy.

2.3 Serum tumor marker level detection

Before chemotherapy and two cycles after chemotherapy, 3ml of fasting cubital venous blood was collected from two groups of patients and centrifuged to isolate serum, and enzyme-linked immunosorbent assay kit was used to determine CA72-4, G17 and TK-1 levels.

2.4 Gene expression detection in surgically removed lesions

Lesion tissue was collected after surgical removal and added in Trizol lysis buffer to extract RNA and synthesize it into cDNA by reverse transcription, then fluorescence quantitative PCR kit was used to amplify CyclinB, CyclinD1, CDK1, CDK4, CDK6, RASSF1A, Noxa, GKN1, p16ink4a, Vav2, MMP2, ADAM8 and ITF2, and then the mRNA expression was calculated according to the amplification curve.

2.5 Statistical methods

SPSS 22.0 software was used to input serum data and gene expression data, data analysis between two groups was by t test and P<0.05 meant statistical significance in differences.

3. Results

3.1 Serum tumor marker levels before and after chemotherapy

Before chemotherapy and two cycles after chemotherapy, analysis of serum tumor markers CA72-4 (ng/mL), G17 (ng/mL) and TK-1 (pmol/mL) levels between two groups of patients was as follows: before chemotherapy, serum CA72-4, G17 and TK-1 levels were not significantly different between two groups of patients (P>0.05); after chemotherapy, serum CA72-4, G17 and TK-1 levels of both groups of patients were significantly lower than those before chemotherapy (P<0.05) and serum CA72-4, G17 and TK-1 levels of DOS group after chemotherapy were significantly lower than those of SOX group (P<0.05).

3.2 Proliferation gene expression in surgically removed lesions after chemotherapy

After chemotherapy, analysis of proliferation genes CyclinB, CyclinD1, CDK1, CDK4 and CDK6 expression in surgically removed lesions between two groups of patients was as follows: CyclinB, CyclinD1, CDK1, CDK4 and CDK6 mRNA expression in surgically removed lesions of DOS group were significantly lower than those of SOX group. Differences in CyclinB, CyclinD1, CDK1, CDK4 and CDK6 expression in surgically removed lesions were statistically significant between two groups of patients after chemotherapy (P<0.05).

Table 1.

Comparison of serum tumor marker levels before and after chemotherapy.

Groups	n	Time	CA72-4	G17	TK-1
DOS group	38	Before chemotherapy	55.42±7.92	79.41±8.39	3.20±0.52
		After chemotherapy	27.51±3.62$^{*\&}$	47.83±7.51$^{*\&}$	1.45±0.18$^{*\&}$
SOX group	38	Before chemotherapy	56.15±8.02	78.82±8.93	3.28±0.58
		After chemotherapy	40.28±5.52$^{\&}$	59.63±8.62$^{\&}$	2.16±0.28$^{\&}$

*: comparison between DOS group and SOX group after chemotherapy, P<0.05; $^{\&}$: comparison within group before and after chemotherapy, P<0.05.

Table 2.

Comparison of proliferation gene expression in surgically removed lesions after chemotherapy.

Groups	n	CyclinB	CyclinD1	CDK1	CDK4	CDK6
DOS group	38	0.31±0.05	0.28±0.04	0.38±0.07	0.56±0.08	0.22±0.04
SOX group	38	0.96±0.12	1.01±0.14	1.08±0.12	1.06±0.15	0.98±0.11
T		17.487	22.108	15.028	9.108	24.517
P		<0.05	<0.05	<0.05	<0.05	<0.05

3.3 Tumor suppressor gene expression in surgically removed lesions after chemotherapy

After chemotherapy, analysis of tumor suppressor genes RASSF1A, Noxa, GKN1 and $p16^{ink4a}$ expression in surgically removed lesions between two groups of patients was as follows: RASSF1A, Noxa, GKN1 and $p16^{ink4a}$ mRNA expression in surgically removed lesions of DOS group were significantly higher than those of SOX group. Differences in RASSF1A, Noxa, GKN1 and $p16^{ink4a}$ expression in surgically removed lesions were statistically significant between two groups of patients after chemotherapy (P<0.05).

Table 3.

Comparison of tumor suppressor gene expression in surgically removed lesions after chemotherapy.

Groups	n	RASSF1A	Noxa	GKN1	$p16^{ink4a}$
DOS group	38	2.27±0.35	2.68±0.39	1.88±0.24	2.42±0.41
SOX group	38	1.04±0.15	0.97±0.11	1.02±0.16	1.05±0.11
T		11.479	16.472	8.398	14.427
P		<0.05	<0.05	<0.05	<0.05

3.4 Invasion gene expression in surgically removed lesions after chemotherapy

After chemotherapy, analysis of invasion genes Vav2, MMP2, ADAM8 and ITF2 expression in surgically removed lesions between two groups of patients was as follows: Vav2, MMP2, ADAM8 and ITF2 mRNA expression in surgically removed lesions of DOS group were significantly lower than those of SOX group. Differences in Vav2, MMP2, ADAM8 and ITF2 expression in surgically removed lesions were statistically significant between two groups of patients after chemotherapy (P<0.05).

Table 4.

Comparison of invasion gene expression in surgically removed lesions after chemotherapy.

Groups	n	Vav2	MMP2	ADAM8	ITF2
DOS group	38	0.36±0.07	0.42±0.05	0.25±0.04	0.56±0.07
SOX group	38	0.96±0.12	1.05±0.16	1.02±0.14	0.99±0.11
T		14.948	12.775	23.219	8.347
P		<0.05	<0.05	<0.05	<0.05

4. Discussion

Docetaxel is a type of taxane chemotherapy drug synthesized from yew needle extract after structural modification, which has stronger killing effect and dissolving effect on cancer cells than paclitaxel, and can also cause intracellular microtubule aggregation and terminate cell mitosis[3]. In the study, docetaxel was added on the basis of oxaliplatin + S-1 neoadjuvant chemotherapy in order to enhance the killing effect of oxaliplatin and S-1 on gastric cancer cells by docetaxel. Serum tumor markers are commonly used to evaluate tumor malignancy, and CA72-4, G17, TK-1, etc., are proven to be able to evaluate the malignancy of gastric cancer. CA72-4 is a newly developed carbohydrate antigen, which has a good correlation with the development of gastric cancer[4]; G17 is a polypeptide hormone synthesized from the sinus cells, and the G17 secretion significantly increases in the course of gastric cancer[5]; TK-1 is a key catalytic enzyme for intracellular DNA replication and synthesis, which is massively synthesized and secreted into the blood circulation during the proliferation of cancer cells[6]. In the study, analysis of the changes in serum tumor marker levels before and after chemotherapy showed that serum CA72-4, G17 and TK-1 levels of both groups after chemotherapy were significantly lower than those before chemotherapy and serum CA72-4, G17 and TK-1 levels of DOS group after chemotherapy were significantly lower than those of SOX group. This indicates that the DOS scheme is superior to the SOX group in killing gastric cancer cells and can more effectively reduce the serum tumor marker levels.

Serum tumor markers in gastric cancer patients are from the constantly proliferating cancer cells, and CDC25B, CyclinB, CyclinD1, CDK1, CDK4 and CDK6 are the important molecules that influence the cell cycle to adjust the gastric cancer cell proliferation. CDK1 can form complexes with CyclinB and accelerate the cell cycle from G2 to M phase[7]; CDK4 and CDK6 can form complexes with CyclinD1 and accelerate the cell cycle from G1 to S phase[8,9]. Under the action of a variety of CDK and Cyclin molecules, the process of cell cycle from G1 to phase S and from G2 to M phase is significantly accelerated, thus promoting cell growth and proliferation. In the study, analysis of above proliferation gene expression in surgically removed lesions after chemotherapy showed that CyclinB, CyclinD1, CDK1, CDK4 and CDK6 mRNA expression in surgically removed lesions of DOS group after chemotherapy were significantly lower than those of SOX group. It means that DOS chemotherapy can be more effective than SOX chemotherapy in reducing proliferation gene expression, blocking cell cycle and inhibiting cell proliferation.

In the process of the gastric cancer cell proliferation, cell proliferation is not only related to the acceleration of the cell cycle, but also related to the expression deletion of multiple tumor suppressor genes. RASSF1A, Noxa, GKN1 and p16ink4a are the cancer-suppressor genes closely associated with gastric cancer. RASSF1A is the spliceosome of Ras-association domain family 1, which is able to increase the cell cycle checkpoint function of Rb gene, lead to the cell cycle arrest, thus lead to apoptosis and cell proliferation inhibition[10]; Noxa is the pro-apoptotic member of the Bcl-2 family, which can increase the mitochondrial membrane permeability to cytochrome C and induce apoptosis[11]; GKN1 is a product during gastric mucosal cell growth, which has protective effect on gastric mucosa cells, and can also promote the repair of normal gastric mucosa and inhibit the growth of cancer cells[12]; p16ink4a is a negative regulation molecule of cell cycle, which inhibits the activation of CDK4 and CDK6 to impede the cell cycle progression and inhibit cell proliferation[13]. In order to further clarify

the killing effects of DOS and SOX on gastric cancer cells, above tumor suppressor gene expression in surgically removed lesions after chemotherapy were analyzed in the study, and the results showed that RASSF1A, Noxa, GKN1 and p16ink4a mRNA expression in surgically removed lesions of DOS group after chemothreapy were significantly higher than those of SOX group. This means that the DOS chemotherapy can more effectively increase the expression of tumor suppressor genes than SOX chemotherapy, and then inhibit the cancer cell proliferation and kill the cancer cells by inhibiting the function of tumor suppressor genes.

In the course of gastric cancer, cancer cells will infiltrate the surrounding tissues on the basis of continuous proliferation, which have the characteristics of invasive growth. Vav2, MMP2, ADAM8 and ITF2 are the molecules closely related to the invasion of gastric cancer cells. Vav2 is a member of Vav family, which has a promoting effect on the activation of invasion molecule MMP2, and has inhibitory effect on the activation of the invasion suppressor TIMP1 and TIMP2[14]; ADAM8 has disintegrin structure domain and metalloproteinase structure domain, can exert intercellular adhesion and proteolysis effects, and can not only degrade a variety of ingredients in extracellular matrix, but can also promote cancer cells to break away from the primary lesion and adhere to adjacent tissue[15]; ITF2 is a member of the bHLH protein family, it is regulated by the Wnt pathway and is able to initiate gene expression after translocation into the nucleus, and it promotes the migration, adhesion and invasion of cancer cells. In order to further clarify the DOS and SOX effects on gastric cancer cell invasion, above invasion gene expression in surgically removed lesions after chemotherapy were analyzed in the study, and the results showed that Vav2, MMP2, ADAM8 and ITF2 mRNA expression in surgically removed lesions of DOS group after chemotherapy were significantly lower than those of SOX group. This indicates that the DOS chemotherapy can more effectively reduce the invasion gene expression than SOX chemotherapy, and then inhibit the invasion and metastasis of cancer cells.

In conclusion, it is believe that both DOS scheme and SOX scheme can kill the gastric cancer cells and inhibit the cancer cell proliferation and invasion, and these effects of the DOS scheme are more significant than those of SOX scheme.

References

[1] Pfeiffer P, Qvortrup C, Krogh M, Schoennemann K, Vestermark LW, Jensen HA, et al. S-1 in combination with docetaxel and oxaliplatin in patients with advanced gastro-esophageal adenocarcinoma: two parallel phase 1/2a studies. *Acta Oncol* 2017; **56**(1): 46-51.

[2] Liu Y, Feng Y, Gao Y, Hou R. Clinical benefits of combined chemotherapy with S-1, oxaliplatin, and docetaxel in advanced gastric cancer patients with palliative surgery. *Onco Targets Ther* 2016; **7**(9): 1269-1273.

[3] Chen MH, Lin J, Hsiao CF, Shan YS, Chen YC, Chen LT, et al. A phase II study of sequential capecitabine plus oxaliplatin followed by docetaxel plus capecitabine in patients with unresectable gastric adenocarcinoma: the tcog 3211 clinical trial. *Medicine (Baltimore)* 2016; **95**(3): e2565.

[4] Yu J, Zhang S, Zhao B. Differences and correlation of serum CEA, CA19-9 and CA72-4 in gastric cancer. *Mol Clin Oncol* 2016; **4**(3): 441-449.

[5] Nejadi-Kelarijani F, Roshandel G, Semnani S, Ahmadi A, Faghani B, Besharat S, et al. Diagnostic values of serum levels of pepsinogens and gastrin-17 for screening gastritis and gastric cancer in a high risk area in northern Iran. *Asian Pac J Cancer Prev* 2014; **15**(17): 7433-7436.

[6] Chen Z, Guan H, Yuan H, Cao X, Liu Y, Zhou JI, et al. Serum thymidine kinase 1 is a reliable maker for the assessment of the risk of developing malignancy: A case report. *Oncol Lett* 2015; **10**(3): 1669-1673.

[7] Gao SY, Li J, Qu XY, Zhu N, Ji YB. Downregulation of Cdk1 and cyclinB1 expression contributes to oridonin-induced cell cycle arrest at G2/M phase and growth inhibition in SGC-7901 gastric cancer cells. *Asian Pac J Cancer Prev* 2014; **15**(15): 6437-6441.

[8] Yawata K, Osada S, Tanahashi T, Matsui S, Sasaki Y, Tanaka Y, et al. The significant role of cyclin d1 in the synergistic growth-inhibitory effect of combined therapy of vandetanib with 5-fluorouracil for gastric cancer. *Anticancer Res* 2016; **36**(10): 5215-5226.

[9] Huang S, Ye H, Guo W, Dong X, Wu N, Zhang X, et al. CDK4/6 inhibitor suppresses gastric cancer with CDKN2A mutation. *Int J Clin Exp Med* 2015; **8**(7): 11692-11700.

[10] Balgkouranidou I, Matthaios D, Karayiannakis A, Bolanaki H, Michailidis P, Xenidis N, et al. Prognostic role of APC and RASSF1A promoter methylation status in cell free circulating DNA of operable gastric cancer patients. *Mutat Res* 2015; **778**: 46-51.

[11] Rath S, Das L, Kokate SB, Pratheek BM, Chattopadhyay S, Goswami C, et al. Regulation of Noxa-mediated apoptosis in Helicobacter pylori-infected gastric epithelial cells. *FASEB J* 2015; **29**(3): 796-806.

[12] Altieri F, Di Stadio CS, Federico A, Miselli G, De Palma M, Rippa E, et al. Epigenetic alterations of gastrokine 1 gene expression in gastric cancer. *Oncotarget* 2017; **8**(10): 16899-16911.

[13] Guo L, Huang C, Ji QJ. Aberrant promoter hypermethylation of p16, survivin, and retinoblastoma in gastric cancer. *Bratisl Lek Listy* 2017; **118**(3): 164-168.

[14] Tan BB, Li Y, Fan LQ, Zhao Q, Liu QW, Liu Y, et al. Upregulated Vav2 in gastric cancer tissues promotes tumor invasion and metastasis. *Tumour Biol* 2017; **39**(5): 1010428317698392.

[15] Huang J, Bai Y, Huo L, Xiao J, Fan X, Yang Z, et al. Upregulation of a disintegrin and metalloprotease 8 is associated with progression and prognosis of patients with gastric cancer. *Transl Res* 2015; **166**(6): 602-613.