Saudi Journal of Biological Sciences 27 (2020) 606–610

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Therapeutic effect of small molecule targeting drug apatinib on gastric cancer and its role in prognosis and anti-infection mechanism

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ARTICLE INFO

Article history:
Received 28 September 2019
Revised 18 November 2019
Accepted 26 November 2019
Available online 6 December 2019

Keywords:
Apatinib
Infection
Treatment
Mechanism
Response

ABSTRACT

Objective: To investigate the effect of apatinib when treating advanced gastric cancer (GC) as well as the mechanism of preventing infection. Methods: From January 2017 to December 2018, 100 advanced GC patients had failed to receive second-line or above treatment in XX Hospital were divided into two groups according to the experimental requirements: the experimental group and the blank group. The experimental group was treated with small molecule targeted drug apatinib, while the blank group was only treated with ordinary drugs. After 4 weeks of treatment, the diagnosis and evaluation were carried out every eight weeks. In this study, the mechanism of infection prevention and prognosis was studied through the internal treatment of GC patients with apatinib. Results: until the end of the fourth week, a significant difference can be seen in the treatment effect between the experimental group as well as the blank group. In the experimental group, the proportion of partial remission + disease stability reached about 73%, while that in the blank group was only about 33%. In addition, apatinib was better than the blank group in the control of adverse reactions like hypertension, proteinuria, myelosuppression as well as diarrhea. In addition, apatinib was better than the blank group when treating AFP positive GC. In terms of the therapeutic effect of apatinib, it is much better than that of the negative group. In addition, apatinib is also better than the blank group in drug resistance for GC patients. It is found that apatinib's anti infection mechanism is to prevent the phosphorylation of vascular endothelial growth factor receptor-2 (VEGFR-2) as well as stop the downstream signal pathway, so as to inhibit the tumor angiogenesis, tumor growth and metastasis, so as to achieve treatment and reduce the probability of infection. Conclusion: the therapeutic effect of small molecule targeting drug apatinib on gastric cancer is better than that of other drugs, whether in therapeutic effect, drug resistance, adverse reactions or infection control. This study has important reference significance for the follow-up treatment of apatinib and cancer.

1. Introduction

Gastric cancer (GC) is the third most lethal cancer after lung cancer as well as liver cancer in the world. It is more common in Southeast Asia, the Middle East of Europe, the south of the United States, etc. Every year, there are 1 million new patients of GC in the world, more than 70% of them are in Southeast Asia, half of them are in China (Abdal Dayem et al., 2016). The incidence of GC is the second and the mortality is the third in China. According to the domestic research, the discovery rate of early gastric cancer is only 2–4%, most of which have reached local advanced stage or multiple metastases of the whole body, without indication of operation, so patients often miss the opportunity of surgical treatment (Hsieh et al., 2017), compared with the best support treatment, chemotherapy can make patients live a better life and live longer (Roviello et al., 2016), but the overall survival is short, the median total survival time is still less than 1 year. However, the targets of chemotherapy drugs are also commonly found in normal cells. With the increase of chemotherapy cycle, adverse reactions are more likely to occur (Xu et al., 2016).

With the development of molecular biology, a new treatment method -- tumor molecular targeted therapy is rising (Zhou et al., 2016), and molecular targeted therapy has been playing a more and more important role in the field of tumor treatment in

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2019.11.036
1319-562X/

recent years (Xu et al., 2018). Its mechanism is to block the overexpression or metastasis of cancer cells by using monoclonal antibodies or small molecule drugs, aiming at some molecular pathways in the process of tumor occurrence, development and metastasis. The related pathways of key targets are abnormally activated (Lin et al., 2017). In this way, tumor treatment can be achieved from the molecular pathway (Peng et al., 2016). In addition, the adverse reactions of targeted therapy are less than that of chemotherapy, and patients have good tolerance, which has become an important part of tumor clinical treatment (Jomrich and Schoppmann, 2016; Thomas et al., 2016). Among them, bevacizumab, an anti angiogenic drug, is one of the research hotspots. The results of avagast phase II clinical research are disappointing (Fang et al., 2017), while apatinib, a new targeted drug developed in China has been rarely reported in China (Zhao et al., 2016). The clinical data of the subjective were analyzed deeply to explore the apatinib, which include clinical efficacy analysis as well as prognostic factors (Zhang et al., 2017).

To sum up, this paper studies the effect of small molecule targeting drug apatinib on gastric cancer patients and the mechanism of preventing infection. The results showed that the therapeutic effect of apatinib, a small molecular targeting drug, on gastric cancer patients was better than that of other drugs as a whole. In terms of therapeutic effect, control of adverse reactions and drug resistance, apatinib stopped vascular endothelial growth factor receptor-2 (VEGFR-2) phosphorylation, and blocked the transmission of downstream signal pathway. So as to inhibit the formation of tumor blood vessels, so as to achieve treatment and reduce infection. Apatinib can effectively alleviate the disease and infection of GC patients, and improve the life of patients. The intervention therapy with apatinib as the core has important research value. Studying the internal mechanism of apatinib when treating GC patients, and analyzing its pro drug resistance are the study innovation. Therefore, this paper is quite meaningful.

2. Materials and methods

2.1. Research subjects

100 advanced GC patients who have failed to receive second-line or more treatment from January 2016 to December 2018 are selected. All patients are diagnosed by pathology and immunohistochemistry. Apatinib is orally administered 750–850 mg/d until the disease progressed. The clinical efficacy and adverse reactions are observed. The patients are divided into experimental group as well as blank group. The experimental group is treated with apatinib, while the blank group is treated with normal drugs only. The patient signs the informed letter. In addition, the experiment is approved by the ethics committee.

Exclusion criteria: The patients have other serious diseases such as liver and kidney dysfunction, and their life expectancy is less than 2 years. Patients cannot accept apatinib drugs. Patients cannot insist on treatment. Patient data are incomplete. Because patients change to other treatment methods during the course of treatment, it is impossible to judge the curative effect or influence the judgment of curative effect.

Inclusion criteria: Patients who have failed, progressed or relapsed second-line or more treatment in the past. Patients are equal to or older than 18 years old. According to the Eastern Cooperative Oncology Group (ECOG) score of 0–2, the survival time is expected to be more than 3 years. The clinical stage is IV. There are measurable target lesions according to the criteria for evaluating the efficacy of RECIST solid tumors. Patients with target lesions treated by radiotherapy are excluded.

2.2. Therapeutic method

Each patient in the experimental group receive the intervention of Apatinib Mesylate (Apatinib Mesylate Tablets; Jiangsu Hengrui Pharmaceutical Co., Ltd.): The patient takes 750–850 mg once a day. The patient takes the medicine 30 min after each meal. The duration of each cycle is 4 weeks, and it needs to be maintained until the disease has not been effectively improved or patients have problems with drug resistance and adverse reactions. When a patient has a grade 3 or more ADR problem associated with apatinib in the process of receiving intervention treatment, the patient can stop taking the medicine or reduce the number of doses, and then look at the next decision after observation.

2.3. Observation indicators and evaluation of therapeutic effect

The curative effect is evaluated after 4 weeks of administration and every 8 weeks after administration. The clinical efficacy of RECIST solid tumors is evaluated by response evaluation criteria, which are complete response (CR), partial response (PR), progressive disease (PD), and objective stable disease (SD), objective response (RR) = CR + PR. disease control rate (DCR) = CR + PR + SD.

Progression free survival (PFS) means the time from the beginning of apatinib administration to tumor progression, patient loss of follow-up or death. Besides, overall survival time (OS) means the time from the beginning of apatinib treatment to the time of death or loss of visit. The adverse reactions are evaluated according to the WHO standard of anti-tumor drug toxicity and side effects, and the total score is 0-Ⅳ. Among them, grade 0 indicates normal. Grade 1 indicates mild toxicity. Grade 2 indicates moderate toxicity. Grade 3 indicates severe toxicity. Grade 4 indicates life-threatening or inactive toxicity. Grade 5 indicates death from toxicity. The short-term DCR and long-term PFS as well as OS of apatinib when treating advanced GC are observed. The efficacy of AFP positive patients is compared with that of AFP negative patients.

2.4. Extraction of DNA and RNA

Peripheral leukocyte isolation: First, 6 mL of leucocyte isolation solution is injected into 30 mL centrifugal tube (Eppendorf Co., Ltd., Germany). The next step is to evenly pour the new anticoagulant whole blood 6 mL onto the liquid that needs to be separated. At room temperature, centrifugal operation is carried out at 1000 rpm for 30 min. When the centrifugation is completed, the top layer is the diluted plasma layer, and the bottom layer is the erythrocyte layer. The liquid in the middle is slowly injected into a clean and pure centrifugal tube of 20 mL, and then 20 mL PBS solution is injected for cell cleaning. At room temperature, centrifugal operation is carried out at 250 rpm for 20 min. The remaining superfluid is discarded. PBS solution is used for cell cleaning. Finally, it is stored in a refrigerator at −90 °C (Haier, China).

Leukocyte DNA extraction: Leukocyte DNA extraction: first take out a 2.5 mL experimental EP tube, inject 30 μl protease solution into it, then add 300 μl leukocyte suspension waiting for use into it, the next step is to inject 300 μL bufferal (Qiagen, Germany) solution into EP experimental tube, completely shake the tube to achieve the purpose of complete mixing (Si Co., Ltd., USA). Then, it is allowed to stand in a water bath at 60 °C for 20 min, centrifugal operation is carried out at a speed of 15,000 rpm at room temperature for 20 min. 300 μl of anhydrous ethanol solution (Sinopharm group, China) are injected into it, and the test tube is completely shaken to achieve the purpose of complete mixing. Repeat the previous process, and put the residual in the test tube up. The clear solution is injected into the centrifuge tube of qiaamp mini (Qiagen, Germany), and the centrifuge operation is carried out at the speed of 10,000 rpm at room temperature for 2 min: take out the qiaamp min centrifuge tube, transfer the solution

in it to another clean 5 mL test tube, and then inject 600 μL bufferaw1 solution into qiaamp mini, and carry out another centrifuge operation at room temperature, transfer the solution to another clean 5 mL test tube, and then inject 600 μL bufferaw1 solution into qiaamp Mini. At room temperature, the product yield will be increased greatly after standing in water bath for 10 min. At room temperature, centrifuge at 10,000 rpm for 2 min. Then discard the centrifuge column and place the DNA extracted from the inner surface of the test tube in the refrigerator.

DNA extraction from paraffin specimens: Four paraffin sections with thickness of 10 μm are placed in a 20 mL centrifugal tube. Then, 2 mL of dimethylbenzene solution is dewaxed and the test tube is shaken completely to achieve complete mixing. At room temperature, centrifugal operation is carried out at a speed of 15,000 rpm for 5 min. The residual superficial solution in the test tube is discarded and the remaining sediment is preserved. The next step is to use absolute ethanol to fully clean the sediment and shake the test tube completely to achieve the purpose of complete mixing. At room temperature, centrifugal operation is carried out at a speed of 15,000 rpm for 5 min. The residual superficial solution in the test tube is discarded. At room temperature, the test tube is placed and dried. Then, 180 L of cell lysate and 30 L of protease K solution are injected. After shaking the tube completely to achieve the purpose of complete mixing, the membrane is used to seal it. It is necessary to place in a water bath of 60 °C until the next day, and in 100 °C for 60 min (Shanghai Baidian Co., Ltd., China). Centrifugal operation is carried out at a speed of 15,000 rpm for 5 min. When the temperature drops to room temperature, 2 L of RNA solution is injected into it. At room temperature, centrifugal operation is carried out at a speed of 15,000 rpm for 5 min. The supernatant in the test tube is transferred to another clean centrifugal test tube. Then, 300 mL buffer AL solution and 300 mL anhydrous alcohol solution is added, the test tube is shaken completely to achieve the purpose of complete mixing, and then it is placed in the DNA binding column. At room temperature, centrifugal operation is carried out at a speed of 800 rpm for 3 min. The solution from the centrifugal tube is collected and reinjected into the DNA binding column. Then, 300 L of diluted buffer GW solution is added. At room temperature, centrifugal operation is carried out at a speed of 800 rpm for 3 min. The solution from the centrifugal tube is collected and reinjected into the DNA binding column. At room temperature, centrifugal operation is carried out at a speed of 15,000 rpm for 5 min. The DNA binding column solution is transferred to a 20 mL EP test tube and dried at room temperature. At room temperature, centrifugation is performed at 1500 rpm for 5 min, and DNA binding column is discarded.

DNA quantitative detection: This step is mainly carried out by using fluorescence quantifier (Eppendorf, Germany). Operation is carried out according to the instructions. The first step is the solution configuration, 300 L of standard test solution. Then, the standard samples and the samples to be tested are placed in the special centrifugal tube of the fluorescence quantitative instrument. In order, standard 1 and standard 2 are placed in the hole to be tested in centrifugal tube for inspection and calibration. Then, the samples that need to be tested are placed in the measuring hole for testing, and the data are recorded accurately.

On-line sequencing: Samples identified to meet the requirements will be tested on a high-throughput sequencer. Sequencing depth of exon groups captured by each sample should be guaranteed to reach 100×. Base reading uses Illumina base-call (Ilumina Ltd., USA), and the output file format is FASTQ.

2.5. Vascular endothelial growth factor (VEGF) as well as VEGFR

The formation of fresh blood vessels is the transport of nutrients such as protein, fat and oxygen from the digestive system and lung tissue to the whole body. At the same time, the metabolic wastes such as urea, creatinine and carbon dioxide produced by the metabolism of tissues and organs in the whole body are transported from the body to the body to ensure the basic conditions for normal metabolism of the human body. Various kinds of VEGF and VEGFR regulate angiogenesis through interaction.

After the interaction of VEGF and VEGFR-2, the phosphorylation of VEGFR-2 can be carried out by itself, which can open up the next signal transmission route, thus forming a series of cascade signal transduction operations. Among them, PI3K/AKT/mTOR pathway is related to vascular permeability and cell survival, p38-MAPK is related to cell migration, Ras/MEK/Erk is related to vascular permeability and cell survival, and the activation of Ras/MEK/Erk pathway has a close relationship with endothelial cell proliferation. These reactions stimulate endothelial cells to proliferate in large numbers and form fresh capillaries, which create favorable conditions for the crazy growth and infinite proliferation of cancer cells. Among several members of the VEGF family, VEGFR-2 is considered to be the most closely related factor in the VEGF/VEGFR-2 signaling pathway. Scientists think it has more clinical significance.

2.6. Statistical method

In this study, SPSS 22.0 software is used to analyze the data. Chi-square test is used to count data, *t*-test is used for measuring data that conform to normal distribution, otherwise rank sum test is used. Kaplan-Meier method is used for the analysis of the disease-free survival time as well as the total survival time. Besides, Log-rank test is carried out. The confounding factors are corrected by the Cox risk proportional model constructed by DFS and OS, and multivariate analysis is carried out. $P < 0.05$ shows the significant difference.

3. Results

3.1. Therapeutic effect of apatinib

The therapeutic effect of apatinib on patients with advanced gastric cancer can be seen in Fig. 1. From Fig. 1, when comparing with the blank group, the treatment effect of experimental group drugs for gastric cancer patients is more prominent. Whether it is partial remission or stable condition, each aspect has a significant effect compared with the blank group. It can be seen that apatinib processes a better therapeutic as well as control effect on GC

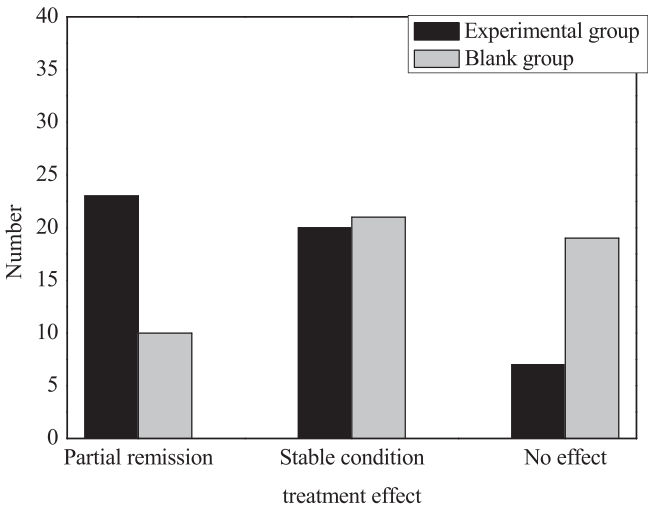

Fig. 1. Study on the therapeutic effect of apatinib on advanced GC patients.

patients than ordinary drugs. Therefore, for advanced GC patients, apatinib is a very effective treatment and control drug. Although it cannot completely cure the disease, it can temporarily control the disease and bring less pain to patients.

3.2. A study of apatinib on AFP positive and negative GC

The study of apatinib's control rate for AFP positive as well as negative GC can be seen in Fig. 2. From the figure, we can see that apatinib's treatment effect for AFP positive as well as negative GC patients is significantly different. Apatinib's treatment effect for AFP positive patients is far greater than that for AFP negative patients. The disease control rate of AFP positive gastric cancer patients is 64.64%, so it can be seen that the therapeutic effect of apatinib is better for the positive patients. However, no significant difference can be seen between the blank group and AFP positive and negative patients, which basically keeps the same state, which also shows that apatinib has certain selectivity for the treatment of gastric cancer patients.

3.3. Study on adverse reactions after taking medicine

The adverse reactions of gastric cancer patients treated with apatinib are shown in Fig. 3. From the figure, we can see that the adverse reactions of gastric cancer patients after operation have some good improvement trends under the intervention of two groups of drugs. No matter for hypertension, proteinuria, myelosuppression, hand foot syndrome and diarrhea, the apatinib works better than that other grugs in blank group. Among them, the incidence of hypertension is 42.63%, 4 cases of grade 3–4, accounting for 7.33% The incidence of proteinuria is 25.67%, and 2 cases (3–4 grade) accounts for 3.33% The incidence of leukopenia is 45.67%. The incidence of HFS is 24.00%. It can be seen that apatinib has a more significant effect on the postoperative adverse reactions of advanced GC patients than other drugs. The discomfort on the patient's body was relieved.

3.4. Study on body pain in advanced GC patients after administration of apatinib

A study of body pain in GC patients after the administration of apatinib is shown in Fig. 4. As can be seen from the figure, the effect of apatinib on pain relief of patients in both groups is better, regardless of the level of classification. It can be seen that apatinib has a good control effect on the body pain of patients with GC compared with other common drugs. In either category, there are more people in the trial group than in the control group, which will greatly alleviate the pain of patients at the body level, and it is very important for patients.

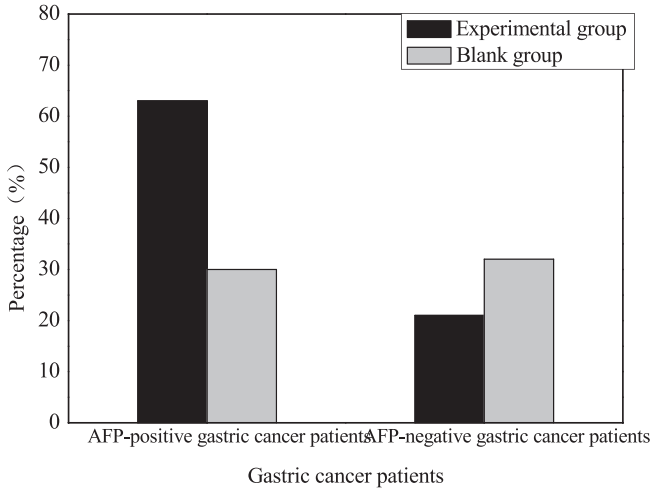

Fig. 2. Study on the control rate of apatinib to AFP positive and negative gastric cancer.

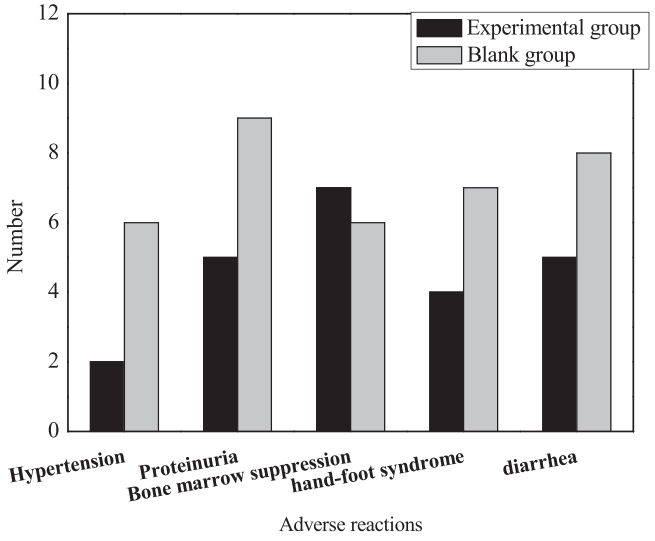

Fig. 3. Adverse reactions of gastric cancer patients after taking apatinib.

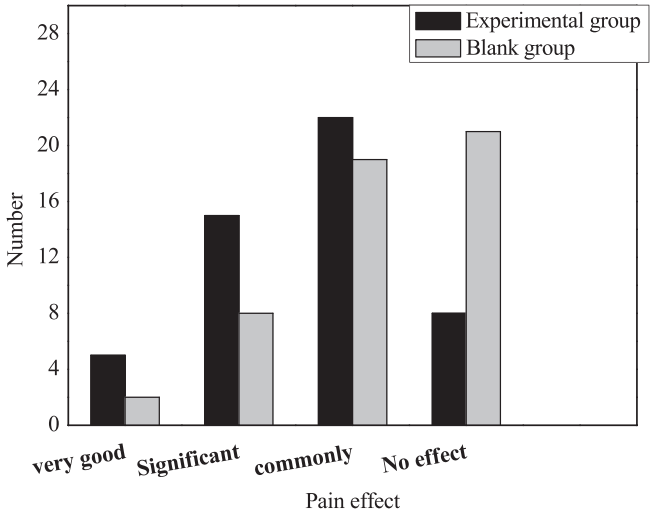

Fig. 4. Pain study of gastric cancer patients after taking apatinib.

3.5. Study on diarrhea in GC patients

A study of diarrhea in GC patients treated with apatinib can be shown in Fig. 5. The control of diarrhea between the experimental group and the blank group is obviously different. The effect of apatinib on relieving diarrhea is better in the experimental group. Therefore, apatinib has a good control effect on diarrhea of gastric cancer patients compared with other common drugs, which will greatly relieve the pain of patients at the physical level, and has a very important significance for the follow-up treatment of patients.

3.6. Resistance of apatinib to gastric cancer

The resistance of apatinib to GC is shown in Fig. 6. The drug resistance of the experimental group is mainly concentrated in

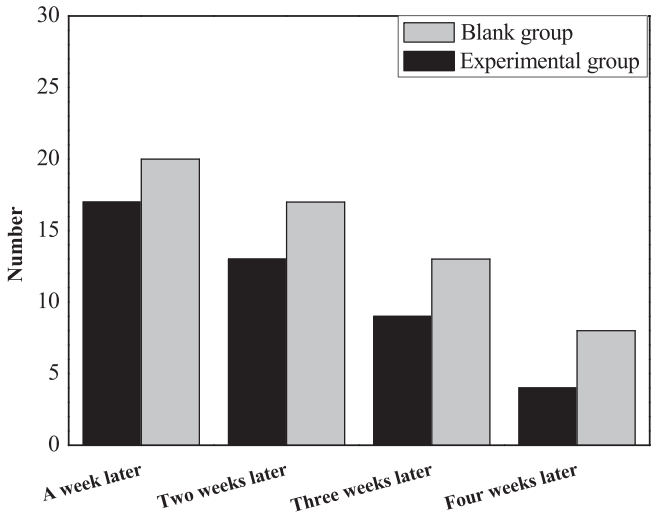

Fig. 5. Study on diarrhea of GC patients after taking apatinib.

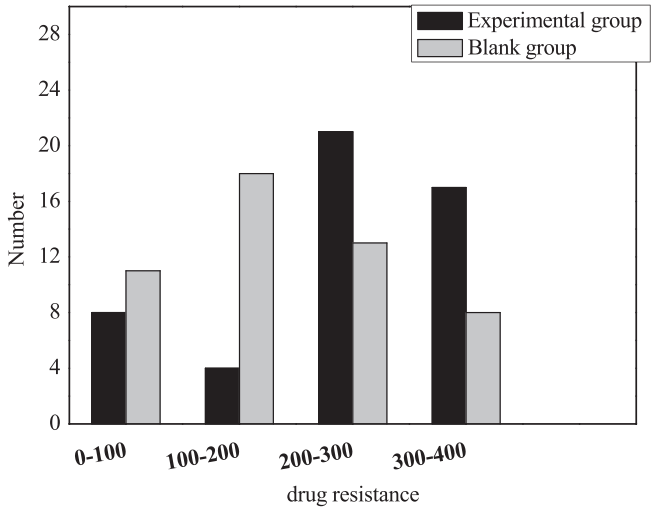

Fig. 6. Drug resistance of apatinib to gastric cancer patients.

200–300 and 300–400 days, accounting for about 80% of the population. The drug resistance of blank group is mainly concentrated in 0–100 days and 100–200 days, accounting for about 70%. Thus, for patients with gastric cancer, the new drug resistance of apatinib is far better than that of ordinary drugs, which plays a very important role in the continuous treatment of patients and maintains the body's stable acceptance of drugs.

4. Discussion

The clinical knowledge of apatinib is less. In this study, the effect of apatinib when treating advanced GC and the mechanism of preventing infection is mainly studied. The results show that the treatment effect of the experimental group is significantly different from that of the blank group. In terms of disease control, the proportion of partial remission + disease stability in the experimental group is about 73%, while that in the blank group is only about 33%. In addition, it is better than the blank group in the control of adverse reactions like hypertension, proteinuria, myelosuppression as well as diarrhea. In addition, apatinib in drug resistance for gastric cancer patients also is better than the blank group of general drugs. Apatinib can inhibit the phosphorylation of VEGFR-2, block the transmission of downstream signal pathway, inhibit the formation of tumor blood vessels, inhibit the growth and metastasis of tumor, so as to achieve the treatment and reduce the risk of infection.

Therefore, the application of apatinib in GC patients is studied by different groups and treatment. Apatinib can effectively alleviate the disease and infection of patients with advanced gastric cancer and improve their lives. The intervention therapy with apatinib as the core has important research value. There are also some limitations in this study. For example, the size of samples is not large enough, which makes the results a little less persuasive, and there are also differences in the condition and physical quality of each patient, which will cause interference to the experimental results. In the later study, it is necessary to increase the size of the samples, and patients with similar conditions and physical fitness can be selected, to decrease some other factors' interference. This study has important reference value for later researchers. At present, apatinib is mostly used as a single drug in GC advanced patients at or above the third line, and it is also combined with some chemotherapy drugs. However, it still needs a lot of high-quality randomized, controlled prospective clinical trials to study whether the combination of chemotherapy will have better clinical efficacy, which chemotherapy drug is better in efficacy and tolerance, and whether apatinib can be used in the second-line or earlier treatment in advance may bring greater clinical benefits. Further search for biomarkers that can predict and recognize the benefit of apatinib needs more clinical observation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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