Effects of Kanglaite Injection on Serum miRNA-21 in Patients with Advanced Lung Cancer

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Lung cancer is the most common type of cancer throughout the world, and the morbidity of lung cancer is continuously increasing. Patients with advanced lung cancer often cannot tolerate chemoradiotherapy. The decrease of the expression of miRNA-21 can be used as an indicator of the therapeutic effect of lung cancer. In this study, the effect of Kanglaite injection on serum miRNA-21 in patients with advanced lung cancer was investigated, providing reliable and important evidences.

Material/Methods: From March 2016 to March 2017, 120 patients with advanced lung cancer were examined; we collected detailed information and serum samples. The patients were treated by intravenous drip of Kanglaite, which was provided by Zhejiang Kanglaite Pharmaceutical Co., Ltd., China. We administered 200 ml intravenous drip once per day for a total of 21 days. Serum samples were collected from patients after treatments. In this study, 4 observation indexes were considered – KPS (Karnofsky performance score, KPS), body weight, adverse effects, and miRNA-21 level – evaluated before and after the Kanglaite treatment.

Results: Among 120 patients with advanced lung cancer, the KPS of 75 patients (63.1%) was increased after the treatment, and body weight was increased by 55.9%. In addition, serum miRNA-21 level after the Kanglaite treatment was 2.45±0.15, which was significantly lower than before treatment (3.87±0.54), (P<0.05).

Conclusions: The effect of Kanglaite injection on serum miRNA-21 in patients with advanced lung cancer was shown to significantly reduce the expression of miRNA-21, providing objective evidence for the effect of Kanglaite injection in patients with advanced lung cancer.

MeSH Keywords: **Lung Cancer • Kanglaite Injection • Serum miRNA-21 • Statistical Analysis**

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Background

Lung cancer is the most common type of cancer throughout the world, and the morbidity of lung cancer is continuously increasing. The standardized rates of male morbidity and mortality of lung cancer in East Asia are 222.1 and 155.5 deaths per 100 000 persons, respectively, while the standardized rates of female morbidity and mortality are 158.1 and 87.3 deaths per 100 000 persons, respectively [1]. Patients with advanced lung cancer often cannot tolerate chemoradiotherapy due to their poor general condition and low KPS, and only palliative treatment can be provided. In treatment of patients with advanced lung cancer, it is important to determine how to improve quality of life and prolong survival of patients with advanced lung cancer. Effective anticancer components of Kanglaite injection are extracted from a traditional Chinese medicinal plant, *Semen cocis*, which is used to prepare traditional Chinese medicine for injection. Recently, a number studies have reported that Kanglaite injection can improve quality of life of patients with advanced malignant tumors [2]. For the treatment of advanced cancer patients, the KPS score and body weight are usually used to evaluate the effect, but potential adverse effects must also be considered [2]. However, previous studies have not used objective indexes, and the corresponding validity cannot be assessed based on the objective biomolecular levels; therefore, further research is needed on the anti-tumor mechanism of Kanglaite. miRNA-21 can be used as an oncogene, and it is of great value in various solid tumors. The changes in the expression of miRNA-21 in the cell lines, tissues, and serum of lung cancer can be used as an indicator of the therapeutic effect of lung cancer treatment. In this study, the effects of Kanglaite injection on patients with advanced lung cancer were assessed by comparing serum microRNA 21 (miRNA-21) levels before and after Kanglaite injection. The achieved results establish a reliable benchmark for therapeutic effect of Kanglaite injection in patients with advanced lung cancer.

Material and Methods

General information

From March, 2016 to March, 2017, a total of 120 patients with advanced lung cancer were admitted and examined in our hospital. There were 74 males and 46 females, ages 39–75 years (average age, 58.4 years), weight 24.5–74.7 kg (average weight, 53.6 kg), and height 152–180 cm (average height, 167cm in). There3 were 68 smoking patients and 52 non-smoking patients. All patients were diagnosed as having stage IV lung cancer by surgical pathology or biopsy pathology. All patients had non-small cell lung cancer, including 70 cases of squamous cell carcinoma and 50 cases of adenocarcinomata. All of the patients were unable to tolerate chemoradiotherapy due to poor general condition, granulocytosis, abnormal liver function, and renal insufficiency. Before treatment, the KPS was ≥40; thus, other chemoradiotherapy were stopped for at least 1 month. All patients provided signed informed consent, and the study was approved by the Ethics Committee of the hospital.

Research methodology

Detailed information and serum samples of the 120 respondents were collected. All patients were treated by intravenous drip of Kanglaite injection, which was provided by Zhejiang Kanglaite Pharmaceutical Co., Ltd, China. The intravenous drip was administered once per day for a total of 21 days. Serum samples were collected after the treatment.

KPS was evaluated before and after the treatment.

Body weight was measured before and after the treatment.

Adverse effects during the Kanglaite treatment were recorded.

Serum miRNA-21 levels were compared before and after treatment.

Serum samples were stored at –80°C and RNA was extracted.

Before doing quantitative analysis of serum miRNA-21, an appropriate internal reference was initially chosen. Since there is no widely accepted internal reference for quantitative analysis of miRNA, the exogenous miRNA-cel-miRNA-39 was used as internal reference in this study. This miRNA has no expression in humans.

The TaqMan MicroRNA Reverse Transcription Kit and miRNA specificity neck ring structure were used for the purpose of reverse transcription.

Relevant reagents were thawed on ice.

The extracted small RNA samples served as templates for the reverse transcription reaction.

Reaction products were stored at 4°C for quantitative polymerase-chain-reaction (qPRC) analysis.

The miRNA-21 was detected by fluorogenic quantitative PCR method.

Dosages of each reaction ingredients were calculated. Each index was calculated 3 times. We used the hsa-miR-21 Taq Man® MicroRNA Assay and the sequence was GTCGTATCCAGTGCAGGG TCCGAGGTATTCGCACTGGATATTCG CACTGGATACGCTCAAC.

Table 1. Scoring criteria of the Karnofsky Performance Scale.

Physical condition	Score
Normal, asymptomatic and physical signs	100 scores
Able to perform normal activities with mild symptoms and signs	90 scores
Normal activity, with some symptoms or signs	80 scores
Can perform self-care, but cannot maintain normal life and work	70 scores
Can usually perform self-care, but sometimes needs help	60 scores
Often need to be taken care of	50scores
Cannot perform self-care needs special care and help	40 scores
Too seriously ill to perform self-care	30 scores
Seriously ill, needs to be hospitalized and receive active support treatment	20 scores
Near death	10 scores
Dead	0 scores

A relative quantitative reaction plate file was set up.

Observation indexes

KPS: The Karnofsky Performance Scale reflects a person's ability to perform certain ordinary tasks. We used KPS to measure the quality of life of cancer patients. The KPS ranking runs from 100 to 0, where 100 is perfect health and 0 is death. The specific scoring criteria are as follows (Table 1).

Body weight.

Adverse effects, defined as a harmful reaction that appears to be unrelated to the purpose of the drug under normal usage, include fever, allergy, dizziness, nausea, changes in electrocardiogram, liver function, and renal function, and phlebitis.

miRNA-21 level.

Evaluation of standards

In this study, KPS was evaluated before and after the Kanglaite treatment. Significantly effective was defined as KPS after the treatment is 20 points higher than before the treatment. Improvement was defined as KPS after the treatment is 10 scores higher than before treatment. Stable was defined as KPS remains constant after the treatment. Worsening was defined as KPS decreases by more than 10 points.

If body weight after the Kanglaite treatment increased by more than 2.5 kg compared with before the treatment, it is considered as body weight gain. If body weight after the Kanglaite treatment is decreased by over 2.5 kg compared with before the treatment, it is regarded as body weight loss. Otherwise, it is in the status of body weight stability.

Adverse effects were assessed according World Health Organization (WHO) toxicity grading scale.

A statistical analysis was carried out on miRNA-21 levels before and after the treatment.

Statistical analysis

Expressions of miRNA-21 were analyzed by $2^{-\Delta\Delta Ct}$ method. All values are expressed as means ± standard deviations (SDs) using the t test, and statistically analyzed with SPSS software, version 17.0 (SPSS Ins., IL, USA). A statistically significant is indicated by p-values (P) less than 0.05.

Results

KPS

Before Kanglaite treatment, KPS mainly ranged between 40 and 60, with 48.1 on average. After conducting the treatment, the average patients' KPS increased by 14.4 to 62.5, and mainly varied between 50 and 70. There were 45 cases of improvement, 30 cases of significant improvement, 40 cases of stability, and 5 cases of worsening. In general, the KPS of the 75 patients (63.1%) was increased after the treatment. The obtained results are shown in Table 2. There was a statistically significant difference (P=0.03).

Body weight

The average body weight before the Kanglaite treatment was 52.9 kg, while the average body weight after the treatment

Table 2. Patients' KPS before and after doing the Kanglaite treatment.

Groups	40–49	50–59	60–69	70–79	80–89
Before	52	47	12	4	5
After	20	65	19	7	9
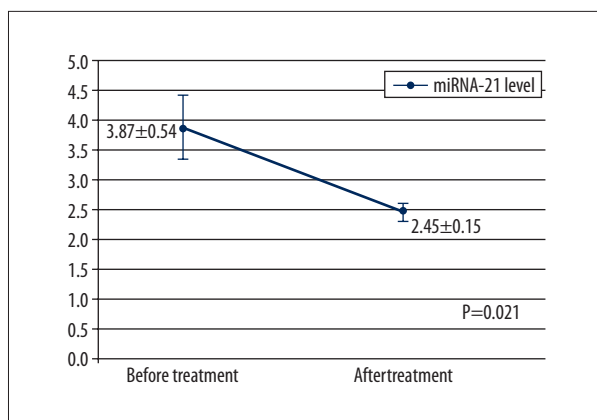
Figure 1. miRNA-21 level before and after Kanglaite treatment.

was 58.4 kg. There were 67 cases (55.9%) of body weight gain, 45 cases (37.1%) of body weight stability, and 8 cases (6.7%) of body weight loss. The difference was statistically significant (P=0.02).

The above data analysis shows that the Kanglaite injection improve appetite and general condition of patients, leading to increased body weight.

Untoward effects

Adverse effects were assessed according to the WHO toxicity grading scale. No patients showed changes in electrocardiography (ECG) or liver and kidney function, and there was no anaphylaxis. One patient had slight phlebitis and recovered well. There was no statistically significant difference (P=0.8).

Serum miRNA-21 levels

Serum miRNA-21 levels after the Kanglaite treatment were 2.45±0.15, which was significantly lower than before treatment (3.87±0.54) (Figure 1, t=2.31, P=0.021, n=120).

Discussions

According to clinical studies, the Kanglaite injection is a new, reliable, and effective broad-spectrum anticancer drug that can enhance immunity and it shows a significant therapeutic effect; however, it can cause minor adverse effects [3]. It is noteworthy that in spite of the great advantages of the Kanglaite, its anti-tumor mechanism still requires deep and systematic studies. According to the instructions of Kanglaite injection, its period of treatment is 21 days.

Research shows that Kanglaite injection can improve the symptoms of advanced lung cancer cachexia [4]. Kanglaite injection significantly improves the condition of patients with

malignant tumors and it improves appetite and KPS [5]. KPS is the Karnofsky functional status score standard. It is an important index to evaluate the quality of life of patients with advanced lung cancer. This study confirmed that Kanglaite injection can significantly improve KPS.

microRNA/miRNA is an endogenous, non-encoding, single-stranded, micromolecular RNA [6]. Recently, high-throughput chip detection methods and high-sensitivity real-time reverse transcription PCR (real-time RT-PCR) systems have been widely used. The achieved results demonstrated that miRNA level is closely related with incidence, development, and prognosis of many tumors. Some studies revealed that miRNA plays a significant regulatory role as well. The role of maladjusted miRNA was used as a marker related with cancer diagnosis, prognosis, and treatment, and has been receiving increased research attention.

In 2005, Brio [7] screened miRNA spectrum from lung cancer tissues and cell strains for the first time by using a microarray chip. He reported that 29 strains of miRNA had expression dysregulation among the abnormally expressed miRNA spectrum. Among them, 12 strains were downregulated. Specifically, variations of miRNA-125b, miRNA-145, miRNA-21, and miRNA-155 were the clearest. A number of relevant studies also demonstrated that dysregulation of microRNA expression is significantly correlated with pathological features and types of molecules related to lung cancer, including estrogen receptors (ER), progesterone receptors (PR), lump volume, invasion to vessel, and Ki-67. In addition, normal cell tissues can be clearly distinguished from relevant cancer tissues based on miRNA expression in 15 strains. Mattie et al. [8] detected the miRNA expression spectrum in 20 lung cancer biopsy specimens by the same method, demonstrating that lung cancer of different molecular subtypes with human epidermal growth factor receptor 2 (HER-2) and hormone receptors can be distinguished by miRNA expression spectrum. It is well known that lung cancers of different molecular subtypes have very different prognoses and treatment and can be used in combination therapies. In recent years, Blenkiron et al.[9] reported that the miRNA expression spectrum can be used to distinguish 5 molecular subtypes of lung cancer. It is clear that the molecular subtypes of different types of lung cancer are closely associated with prognosis and responses to different therapies. Hence, it can be demonstrated that miRNA expression spectrum can be used in making clinical decisions. Tavazoie et al. [10] screened MDA-MB-231-derived lung cancer cell lines, which contain strong distant metastasis capacity. Some scholars have observed that distant metastasis of lung cancer is closely associated with abnormal expression of miRNA. Two miRNAs play a vital role in inhibiting distant metastasis of tumor. If these 2 miRNAs have no expression or low expressions in patients with lung cancer, there is a high risk

of metastasis, and the corresponding survival rate is far lower than in patients with other types of lung cancer. In summary, miRNA plays a significant role in prognosis judgment and therapy selection for patients with lung cancer.

It has been reported that miRNA-21 expression is upregulated in in all high-grade tumors, such as colorectal cancer, lung cancer, and gastric cancer [11]. This demonstrates the universal role of miRNA-21 in tumorigenesis. Many studies have reported that miRNA-21 can be a cancer gene [12] and plays a crucial role in many solid tumors. In addition, miRNA-21 expression is upregulated in cell lines, tissues, and serum with of patients with lung cancer. Moreover, it is closely correlated with lymph node status as well as prognosis in patients with early-stage lung cancer and can be used as an observation index of therapeutic effect [13].

Recently, some scholars reported that miRNA can be detected in serum and has stable expression. Compared with the complicated and very invasive miRNA detection methods in lung cancer tissue, peripheral blood serum can be easily collected and assessed. In serum, miRNA is remarkably stable and can survive for a long time, and also can resist degradation of RNA enzyme [14]. Kanglaite is a diphasic, broad-spectrum, anti-tumor drug, which depresses many kinds of tumor cells. Some previous studies demonstrated that Kanglaite can make tumor cells stagnate at G2-M stage and induce cell apoptosis [15]. In China, some studies reported that Kanglaite injection can inhibit expressions of downstream cyclin D1 and cyclin E, and prevent tumor cells developing into stage S. This is the relevant mechanism of proliferation inhibition [16]. Additionally, a recent study reported that Kanglaite injection combined with gefitinib has an outstanding apoptosis promotion effect on

A549 cell lines of lung adenocarcinoma, and Kanglaite injection can increase sensitivity of A549 cells of human lung adenocarcinoma to gefitinib [17]. In clinical practice, the combination of Kanglaite and neoadjuvant chemotherapy can relieve marrow inhibition of patients with locally advanced breast cancer and increase their immunity and chemotherapy tolerance [18]. Moreover, it can increase the chemotherapy efficacy in these patients. Relevant mechanisms might be downregulated expressions of Ki-67 and survivin [19]. miRNA-21 promotes the proliferation, invasion, and migration of hepatoma cells by downregulation of PTEN expression [20].

Conclusions

Currently, there is no study concerning the effects of Kanglaite injection on serum miRNA-21 level in China. The present study disclosed the effects of Kanglaite injection on serum miRNA-21 in patients with advanced lung cancer, showing it can significantly reduce the expressions of miRNA-21. Our findings provide a reliable benchmark for further investigation of therapeutic effects of Kanglaite injection in patients with advanced lung cancer.

Further research is needed to determine which target genes are involved in the role of miRNA-21 in lung cancer, aiming to lay foundations for further interpretation of pathogenesis of lung cancer, and may aid exploration of new potential molecular therapeutic targets.

Conflicts of interest.

None.

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