Shenqi Fuzheng Injection Ameliorates Radiation-induced Brain Injury*

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Summary: Shenqi Fuzheng injection (SFI) has been confirmed to be able to alleviate brain injury in mice. This study examined the brain-protective effect of SFI on patients after cranial radiation. Lung cancer patients with brain metastasis were randomly assigned to two groups. The SFI group received cranial radiation in combination with SFI. The control group received cranial radiation alone. The changes in cognitive function were evaluated pre- and post-radiation against the Mini-Mental State Exam (MMSE), Montreal Cognitive Assessement (MoCA), Zung Self-Rating Depression Scale (SDS) and Zung Self-Rating Anxiety Scale (SAS). The changes in inflammatory factors, such as TGF-β1, TNF-α and IL-10, were also detected before, during and after radiation (15Gy/5F). The results showed that 6 months after cranial radiation, the total scores on the MMSE and MoCA scales of the patients decreased, especially memory ability. The control group experienced a more evident decline, the memory ability being the greatest. TGF-β1 and TNF-α increased shortly after radiation and decreased one month later, and the change was more conspicuous in SFI group than in control group. IL-10 increased after radiation and stayed at a high level one month later in both groups, the level being higher in the SFI group than in the control group. Our study indicated that cognitive functions, especially memory ability, were impaired after cranial radiation. SFI could alleviate radiation-induced brain injury by regulating inflammatory factors.

Key words: Shenqi Fuzheng injection; irradiation-induced brain injury; cognitive disorders; inflammation

Cranial radiation therapy (CRT) represents one of the most effective therapies for primary and secondary brain tumors[1]. Radiation-induced brain injury (RIBI) is a common sequela of whole or partial brain radiation. Neurotoxic consequences of RIBI include cognitive impairment, leukoencephalopathy, vasculopathy, and secondary neoplasms[2, 3]. Cognitive impairment, deficits in hippocampal-dependent functions, such as learning, memory, spatial information processing, occur in 50% to 90% of patients who survive for more than 6 months after CRT and exert an adverse impact on patients' quality of life[4, 5].

In the pathological cascades of RIBI, neuro-inflammation, induced by microglia, may be the main checkpoint in the mediation of many cellular interactions that contribute to dysfunction after whole brain radiation[6–9]. *In vitro* studies showed that microglia could be activated after radiation, and the activation in turn led to elevated expression of a variety of pro-inflammatory genes, including transforming growth factor (TGF)-β1, interleukin (IL)-10, IL-6, tumor necrosis factor (TNF)-α and cyclooxygenase (COX)-2[10]. Our previous studies found that the neuro-inflammation mediated by radiation-activated microglial cells played a key role in the development of RIBI[11, 12]. Several studies exhibited that cytokines were directly and indirectly implicated in the development of radiation injury[13, 14]. TGF-β1 could modulate immune inflammatory reaction, serving as a bidirectional regulator in pro- or anti-inflammation[15]. Tumor TNF-α is one of the most crucial pro-fibrosis cytokines. IL-10 can suppress inflammatory reaction and reduce the activity of macrophagocytes[14]. Therefore, in this study, we examined such biomarkers as TNF-α, TGF-β1 and IL-10 and evaluated their roles in the development of RIBI, with an attempt to find better treatments for RIBI.

Pre-clinical studies suggested that anti-inflammatory drugs might ameliorate radiation-induced cognitive impairment in patients whose brain was subjected to ionizing radiation[16, 17]. Shenqi Fuzheng injection (SFI) is a Chinese traditional herbal medicine

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and our previous study demonstrated that it alleviated radiation-induced pneumonitis and caused changes in the levels of TNF-α and TGF-β at various stages (pre-, intra- and post-radiation stages) of radiation[18]. By examining the inflammatory factors and blood-brain barrier (BBB) integrity in cranially-irradiated mice, we also found that SFI treatment relieved radiation-induced inflammatory injury[19].

The present study evaluated the effect of SFI for the treatment of RIBI, studied possibility of SFI improving cognitive function in patients with brain metastasis and explored the possible mechanism by detecting the levels of inflammatory factors after cranial radiation.

1 MATERIALS AND METHODS

1.1 Ethic Statement

The study was approved by the Education and Research Committee and the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Written informed consents were obtained from all enrolled patients in accordance with the Declaration of Helsinki.

1.2 Patient Population and Inclusion Criteria

The inclusion criteria were as follows: age ≥18 years and ≤80 years; Karnofsky Performance Status (KPS) ≥70; MRI or CT that demonstrated brain metastases from histologically-proven lung cancer; life expectancy ≥6 months; radiation dose of whole brain radiation ≥30 Gy; being well-informed about the study and having good compliance with the treatment.

The exclusion criteria were as follows: KPS <70; being poorly informed about radiation; half-way termination of radiation with total dose <30 Gy; follow-up time <6 months or being lost to follow up; suffering from severe cerebrovascular diseases or psychiatric symptoms; having received cranial radiation.

1.3 SFI

SFI (Batch No. Z19990065) was prepared from the medicinal herbs provided by Livzon Pharmaceutics Ltd. (China). The medicinal herbs included *Radix codonopsis* (root of *Codonopsis pilosula*; Chinese name: Dangshen) and *Radix astragali* (root of astragalus; Chinese name: Huangqi), which are approved by the State Food and Drug Administration of the People's Republic of China in 1999.

1.4 Radiation

The patients were randomly assigned, at a ratio of 1:1, into two groups: an SFI group, in which patients received radiation in combination with intravenous injection of SFI (250 mL every day for 4 weeks) and a control group in which patients were subjected to radiation alone. The dose distribution of the whole brain radiation was evaluated by the CMS XIO 4.2 three-dimensional (3-D) treatment planning system (Elekta, Sweden). The patients with equal or more than 4 lesions received whole brain 3-D conformal radiotherapy with 36 Gy in 10 daily fractions while the patients with less than 4 lesions legible was given a boost dosage to 51 Gy for local lesions after whole brain radiotherapy of 30 Gy in total 17 fractions.

1.5 Assessment Scales

Before and every 3 months after radiation, all patients were assessed against various scales by a certified doctor. The Montreal Cognitive Assessment (MoCA) scale[20, 21] and Mini-Mental State Exam (MMSE) scale[22] were applied for the evaluation of the cognitive impairments. The Zung Self-Rating Anxiety Scale (ZSAS)[23] and Zung Self-Rating Depression Scale (ZSDS)[24] were used to evaluate the changes of patients' emotion status such as the level of anxiety and depression.

1.6 Detection of Cytokines

Ten mL blood samples were collected respectively in the two groups before, during and one month after radiation (15 Gy/5 F) for the determination of the levels of serum inflammatory cytokines. Serum TGF-β1, TNF-α and IL-10 levels were detected by enzyme-linked immunosorbent assay (ELISA) kits (Boster, China) following the kit instructions.

1.7 Study Design

In this study, we assessed the effect of cranial radiation on cognitive function and detected changes of inflammatory factors in long-term brain tumor survivors. All patients underwent systematic physical examination, tests of complete blood cells, and routine biochemical tests. Each subject was required to complete the questionnaires, including the MMSE, the MoCA, the ZSAS and the ZSDS. Venous blood samples were taken in the two groups to detect plasma TNF-α, TGF-β1 and IL-10 before, during and after radiation (15 Gy/5 F) .

1.8 Statistical Analysis

The objective of this study was to assess the effect of SFI on overall cognitive performance of patients after cranial radiation. All the quantitative data were expressed as mean±standard error of the mean (SEM). Significance in differences between groups was assessed by the one-way ANOVA, followed by Student's *t* tests using SPSS 18.0 software package (SPSS, USA). A P value of <0.05 was considered to be statistically significant.

2 RESULTS

2.1 Pretreatment Characteristics of Participants

From January 2014 to December 2016, 100 NSCLC patients with brain metastasis from the Cancer Center, Union Hospital, Wuhan, China, with valid data were included into the study. The clinical characteristics of the patients in the two groups showed no significant difference (table 1). The median age was 56 years

Table 1 Demographic and clinical variables in two groups

Characteristics	Control group (*n*=52)	SFI group (*n*=48)
Age (years)		
Median	57.96	53.91
Range	37 to 77	28 to 74
Sex		
Male	42 (81%)	35 (73%)
Female	10 (20%)	13 (27%)
Smoking history		
Yes	44 (85%)	40 (83%)
No	8 (15%)	8 (17%)
ECOG performance status		
0–1	50 (96%)	45 (94%)
2	2 (4%)	3 (6%)
Pathology		
Adenocarcinoma	40 (77%)	40 (83%)
Squamous cell carcinoma	3 (6%)	2 (4%)
Small cell lung cancer	9 (17%)	6 (13%)
Number of brain metastasis		
≤3	22 (42%)	21 (44%)
>3	30 (58%)	27 (56%)
Dose of brain radiation (Gy)		
36	30 (58%)	27 (56%)
51	22 (42%)	21 (44%)
Education		
>Middle school	45 (86%)	43 (90%)
Primary school	5 (10%)	4 (8%)
Illiteracy	2 (4%)	1 (2%)

ECOG: Eastern Cooperative Oncology Group

(ranging from 37 to 77 in control group and from 28 to 74 in SFI group). About two thirds of the patients were men and had history of smoking. Most patients had a performance-status score of 0 to 1. Most of the patients were adenocarcinoma and small cell carcinoma. Half of the patients had more than 3 lesions in the brain (58% in control group; 56% in SFI group) and received a total dose of 36 Gy of whole brain radiation. Other patients who were suitable for boost dosage received a total dose of 51 Gy for local lesions after whole cranial radiation of 30 Gy.

2.2 Changes in Cognitive Function after Radiation and Effect of SFI

Before and every 3 months after radiation, all the subjects were assessed for cognitive function against the MoCA and MMSE scales. The patients were also subjected to emotional assessment against the ZSAS and ZSDS scales before and every 3 months after radiation. The scales evaluate the influence of whole cranial radiation on cognitive ability and emotion.

The changes in the MMSE scores and each cognitive variable of the patients before and every 3 months after cranial radiation were shown in fig. 1. The total MMSE scores declined, principally in scores of memory ability and verbal ability. The decrease in the control group was more significant than in the SFI group in practically all measures, suggesting that SFI had a protective effect on RIBI.

The changes in the MoCA scores of all measures were shown in fig. 2. Memory ability dropped significantly, reached the lowest point at the 6th month and then leveled off. The executive capability and the orientation force declined slightly. Naming, calculative, verbal and orientation abilities showed no significant changes during the period. The total MoCA scores also decreased and arrived at the lowest point at the 6th month. SFI group showed a less decline after radiation, which suggested a protective effect of SFI. The tests against these scales suggested that the cranial

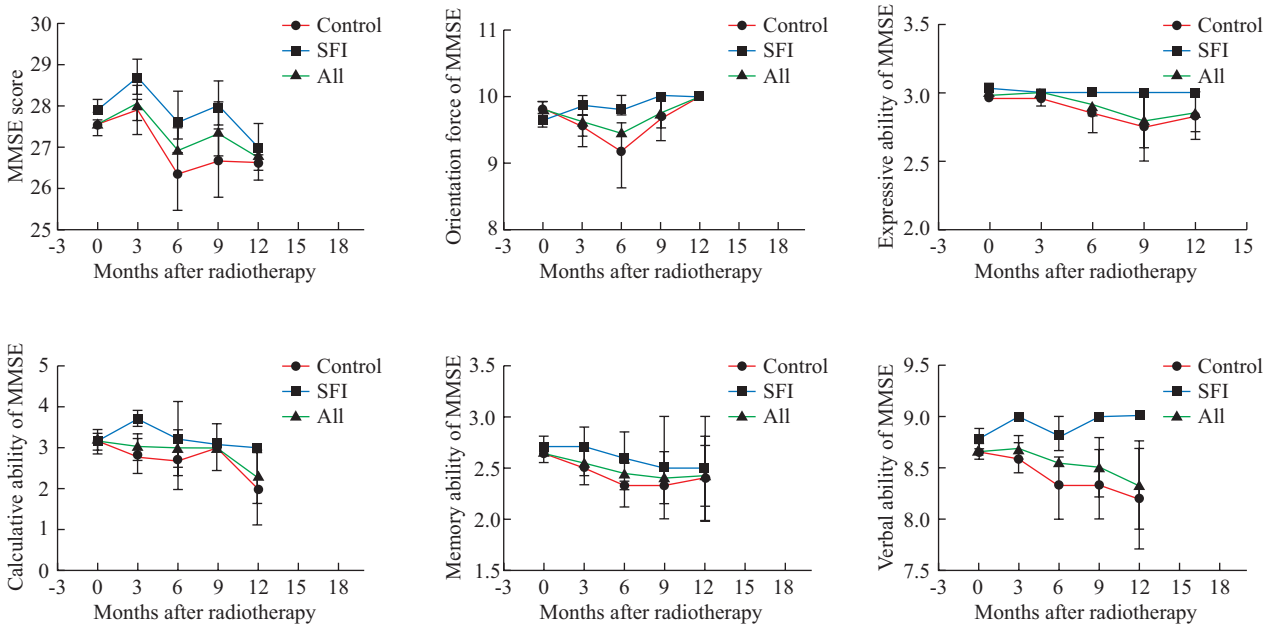

Fig. 1 Changes in mean score and each item of the MMSE scale

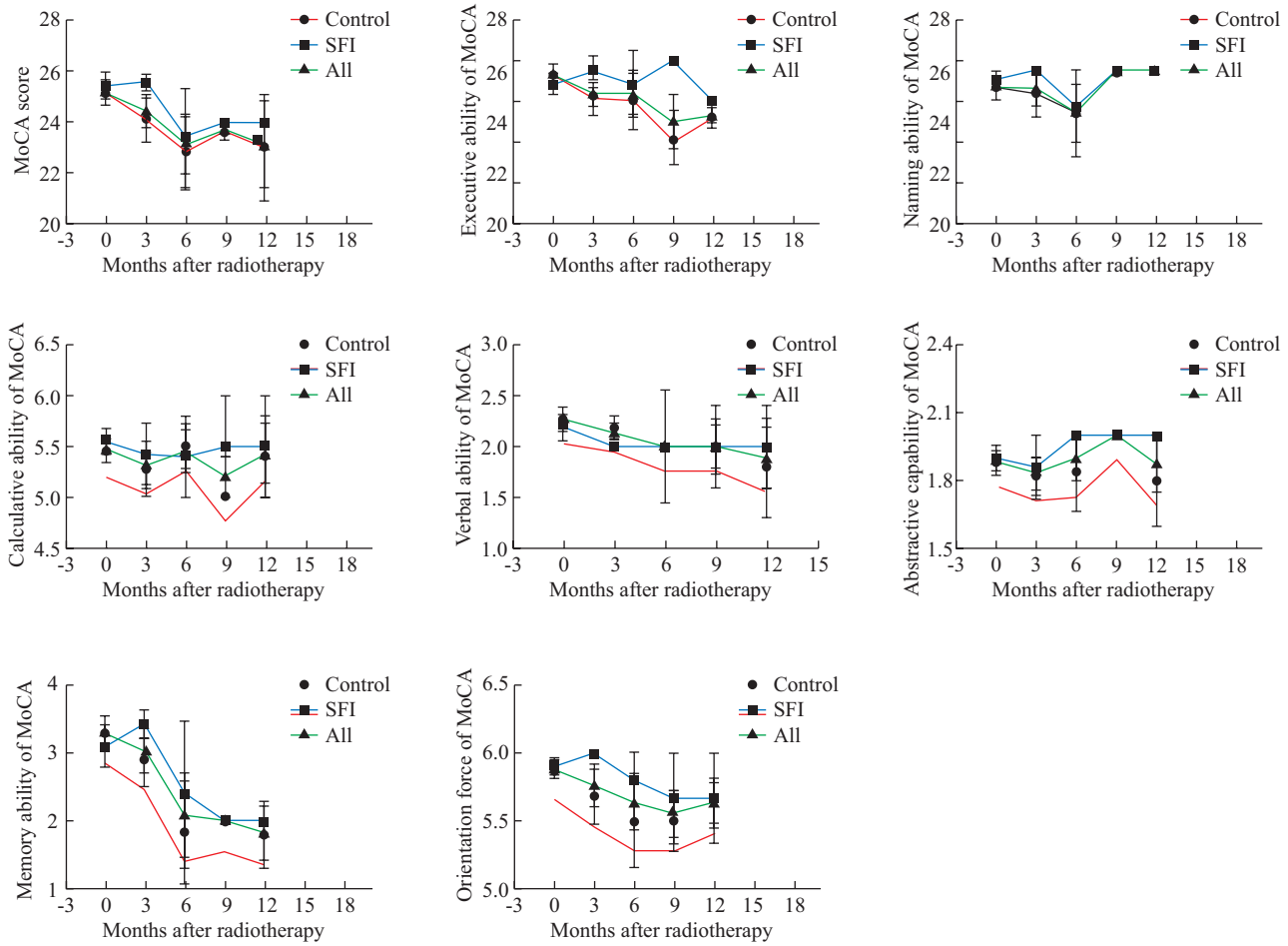

Fig. 2 Changes in mean score and each item of the MoCA scale

radiation primarily affected memory ability.

Emotional assessment against SDS scale showed that the SDS score declined during the 6 months after cranial radiation and then rose. The SAS score dropped slightly at the 9th month. But both curves presented a gentle slope, suggesting the impact of cranial radiation for emotions such as depression or anxiety was not obvious (fig. 3).

2.3 Changes in Inflammatory Factors after Radiation and Effect of SFI

Venous blood samples were taken respectively in the two groups before, during and one month after radiation (15 Gy/5 F). The changes in inflammatory factors were detected by ELISA. The concrete results of TGF-β1, TNF-α and IL-10 were shown in table 2.

TGF-β1 serves as a bidirectional regulator in pro- or anti-inflammation. In our study, TGF-β1 increased during radiation, but decreased sharply one month after radiation in both the control group and SFI group. The increase during radiation in the control group was more significant but it went down more slowly. Nonetheless, no significant difference was found between the two groups (fig. 4A).

TNF-α is one of the most crucial pro-inflammatory cytokines. Our study showed TNF-α also increased during radiation, but decreased gently one month after radiation in both groups. Further analysis showed that the control group had a more notable increase during radiation, but the value dropped slowly after radiation. And no significant difference was found between the two groups (fig. 4B).

IL-10 is an anti-inflammatory factor and can suppress inflammatory reaction. IL-10 increased during radiation and still stayed at a high level after radiation in both groups. The average level of IL-10 in the SFI group increased during radiation and rose steadily after radiation, with significant differences among the time points (P<0.0001). In the control group it increased continuously after radiation, being statistically significant differences among the three time points (P<0.0001). The increase during radiation in the SFI group was more evident, with the difference between the two groups being significant. After radiation, IL-10 increased more dramatically in the SFI group with significant difference as compared with the control group (P<0.01) (fig. 4C).

3 DISCUSSION

Standard treatment of brain tumors whether primary or metastatic includes high-dose radiation to the cranial vault. About half of patients can survive more than 6 months, and many attain long-term control or cure. However, 50%–90% of survivors develop RIBI, including cognitive changes[25], predominantly manifested as impairment in learning, memory, and spatial information processing ability[26]. As to brain cancer survivors, cognitive competence and quality of life are as important as life span. The Response Assessment in Neuro-Oncology (RANO) working group recommended that neurocognitive outcome be considered as one of the primary endpoints in brain tumor clinical trials[27].

The pathophysiology of RIBI remains poorly understood. Some studies provided evidence of acute radiation-triggered central nervous system inflammation, injury to neuronal lineages, accessory cells and their progenitors, and loss of supporting structure integrity[25]. Early inflammation influences microenvironment of the hippocampus and can intensify dysregulated neurogenesis. Widespread inflammation can affect the integrity and function of white matter. Thus, our study investigated the changes of cognitive status of patients and inflammatory factors before, during and after cranial radiation. We also tried to study whether SFI could alleviate RIBI and the possible mechanism.

Our study showed that after cranial radiation, the total MMSE and MoCA scores decreased, including calculative ability, executive capability and orientation force. Especially memory ability had the most evident change in 6 months after radiation, suggesting that memory ability was susceptible to radiation.

This study exhibited that 4 weeks of treatment with SFI exerted a protective effect on the cognitive functions, especially memory ability in post-radiation brain tumor survivors. The total MMSE and MoCA scores decreased in these patients, but patients in the control group experienced a more obvious decline. Memory ability whether on MoCA scale or MMSE scale in the control group showed a more evident change than in the SFI group, suggesting SFI had a protective role and could alleviate the cognitive impairment.

Radiation may directly damage glial cells and endothelial cells of the brain, leading to hyalinization and demyelination of vessels and followed inflammation, ischemia, and delayed radiation necrosis. Many

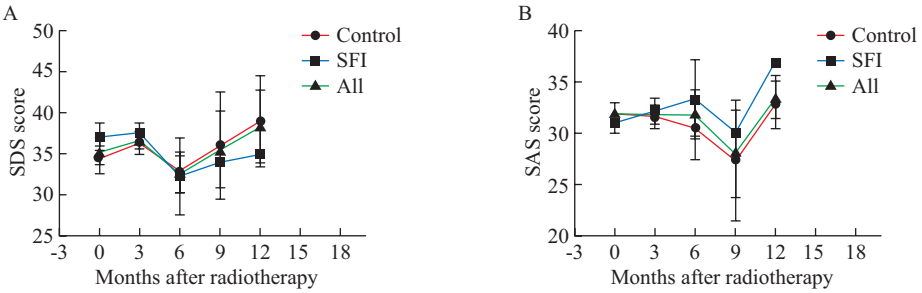

Fig. 3 Changes in mean score of the SDS (A) and SAS (B) scale

Table 2 Changes in inflammatory factors during radiation in different groups (mean±SEM)

Inflammatory factors	Control group			SFI group		
	Pre-radiation	Inter-radiation	Post-radiation	Pre-radiation	Inter-radiation	Post-radiation
TGF-β1 (ng/mL)	41.21±7.35	47.10±8.36	34.04±9.11	38.33±7.96	41.89±9.86	32.81±3.52
TNF-α (pg/mL)	13.02±1.56	17.22±1.79	16.62±1.83	18.54±2.01	19.45±1.34	17.52±0.95
IL-10 (pg/mL)	9.34±1.53	10.84±2.16	32.41±10.95	15.38±5.28	37.61±11.96	61.70±2.56

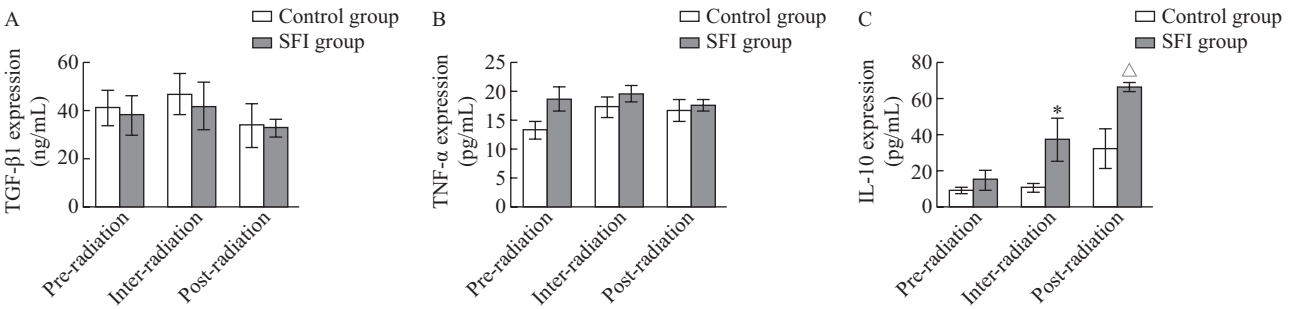

Fig. 4 Influence of SFI on inflammatory factors after radiation
*P<0.05, pre-radiation *vs.* inter-radiation; △P<0.05, inter-radiation *vs.* post-radiation

studies have shown that neuro-inflammatory reaction after radiation was involved in brain injury and cognitive impairment[28–32].

Neuro-inflammation induced by radiation is a crossover network composed of a variety of pro- and anti-inflammatory cytokines. Microglial cells play an main role in neuro-inflammation[10–12, 33–35]. Radioactive rays activate microglial cells through changing morphology and function of the cells[36]. There are two distinct types of microglial cells after activation: a classical M1 and an alternative M2 activation. The M1 type microglial cells may become ameboid/ phagocytic or hyperramified[37] and may synthesize proinflammatory molecules such as IL-1b, TNF-α, IL-6, superoxide radicals and nitric oxide (NO) which may help clear infections and repair tissues. On the other hand, M2 activation type is associated with anti-inflammatory cytokines such as IL-10, insulin-growth factor-1 (IGF-1), and neurotrophic factors[37] which limit neuronal injury and facilitate healing[38]. However, although microglia activation plays a hallmark role of brain pathology, the specific mechanism of microglial activation and polarization, as well as the downstream molecular cascades and how to adjust the process, will still need a further study[39].

Our study showed that TGF-β1[15, 40–42] and TNF-α[13, 43], two pro-inflammatory factors associated with radiation-induced injury, were increased significantly shortly after radiation and decreased quickly one month after radiation. On the other hand, IL-10[14], an anti-inflammatory cytokine[44], was increased both during and after radiation. The change of these inflammatory cytokines further suggested that they were influenced by radiation and might play a significant role in the process of RIBI.

Studies showed that early use of anti-inflammatory agents might prove beneficial in limiting and ameliorating radiation-induced cognitive impairment[16, 17]. Our previous study demonstrated that treatment with Corilagin (a novel member of the tannin family with anti-inflammatory properties) after cranial radiation could inhibit microglial activation and limit the production of inflammatory cytokines, as well as further attenuate structural and biochemical abnormalities of mouse RIBI model[11]. Another previous researches demonstrated that SFI was able to alleviate radiation-induced pneumonitis and RIBI in mice. And SFI also could change the levels of TNF-α, and TGF-β at various stages of radiation[18, 19].

The stratified analysis of this study showed that the increase in TGF-β1 and TNF-α was less but decrease was more after radiation in the SFI group than in the control group, but the increase of IL-10 was greater in the SFI group than in the control group, suggesting SFI may promote the production of anti-inflammatory cytokines and influence the generation of pro-inflammatory factors, which may control radiation-induced neuro-inflammation and alleviate RIBI.

In summary, the study showed that the treatment with SFI during cranial radiation could alleviate brain injury and improve cognitive function. The mechanism of the protective role may lie in the regulation of the neuro-inflammatory process and the inflammatory cytokines. This study showed that SFI has the potential to be used as a neuroprotective agent for alleviating RIBI.

However, uncertainty still remains as to the mechanism by which SFI adjusts the inflammatory cytokines and what is the possible signal pathway, which is the further research direction.

The findings of this study suggest that SFI can reduce cognitive function, especially memory impairment after brain radiation by regulating the expression of inflammatory factors.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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