金花清感は新型コロナに感染した際に上昇する炎症性サイトカイン **IL-6** と **IFN-γ** を下げる働きがある。従って金花清感は新型コロナ感染中等症以下の方に改善効果が期待できる。

Jinhua Qinggan granule, a Chinese herbal medicine against COVID-19, induces rapid changes in the plasma levels of IL-6 and IFN-γ

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Abstract

Background: Currently, effective vaccines or specific therapeutic agents against COVID-19 are not available. However, in China, traditional Chinese herbal medicines have provided therapeutic benefit to patients with COVID-19. Jinhua Qinggan granule (JHQGG) is a Chinese multi-herbal formula previously developed for the treatment of H1N1 influenza and has been encouraged for patients clinically suspected of COVID-19 during medical observation. However, the immunological mechanism for the efficacy of JHQGG has not been confirmed.

Objectives: We thus examined whether the administration of JHQGG affects hematological and immunological measures in healthy individuals.

Method: We enrolled 18 healthy volunteers, all of whom tested negative for antibodies to SARS-CoV-2. Peripheral blood samples were collected 1 h after oral administration of JHQGG and subjected to hematological, biochemical, and cytokine tests.

Results: JHQGG rapidly induced a significant decrease in the plasma level of IL-6 and an increase in the plasma level of IFN-γ.

Conclusions: Our finding suggests that the therapeutic efficacy of JHQGG against COVID19 is, in part, associated with its rapid immunomodulatory activity.

Introduction

There have been no specific vaccines or drugs proven to be clinically effective against COVID-19. However, in China, the majority of COVID-19 patients have been treated with a combination of traditional Chinese and modern Western medicine [1–3]. The use of several Chinese herbal formulas is encouraged for the treatment of COVID-19 in the latest version of the diagnosis and treatment protocol released by the National Health Commission of China. One of them is Jinhua Qinggan granule (JHQGG), which was formulated specifically for the symptoms of H1N1 influenza [4]. Previous preclinical studies showed that JHQGG reduces pulmonary lesions and mortality in mice infected with the H1N1 influenza virus [4]. A clinical study also demonstrated that JHQGG reduces the duration of fever and alleviates respiratory symptoms of patients with H1N1 influenza [4].

On the basis of its therapeutic efficacy for influenza, JHQGG has been recommended for patients clinically suspected of COVID-19 during medical observation. In a randomized controlled trial using mild cases in Wuhan, combined administration of JHQGG with Western medicine significantly ameliorated respiratory symptoms and relieved psychological anxiety compared with the administration of Western medicine alone [5–7]. However, the pharmacological mechanism underlying the efficacy of JHQGG has not been confirmed. We thus examined whether JHQGG administration affects hematological and immunological measures in healthy individuals.

Materials and Methods Subjects

This is an open-label, single-arm study to obtain a clue to the pharmacological action of JHQGG. We enrolled a total of 18 healthy volunteers (5 males, 13 females; ages 22–58 years; mean age [SD], 33.8 [10.7] years), all of whom tested negative for IgM and IgG antibodies to SARS-CoV-2. Individuals were excluded if they had current infectious, inflammatory, or immune-related diseases.

Administration of JHQGG

JHQGG was kindly provided by Hugh Wang, Juxiechang (Beijing) Pharmaceutical Co., Ltd. The subjects were instructed to take a packet (5 g) orally 40 min after lunch. This dose is known to be effective for the treatment of influenza [8]. Peripheral blood samples were obtained 1 h after the administration.

Hematological, biochemical and cytokine analyses

Hematological and biochemical tests were outsourced to SRL, Inc. (Tokyo, Japan). Plasma cytokines were quantified using V-PLEX Proinflammatory Panel 1 Human Kit (Meso Scale Diagnostics) and Human IL-18 ELISA Kit (Abcam). All collected data (n = 18) were subjected to the statistical analysis using a two-tailed paired *t*-test.

Results

Hematocrit and mean corpuscular volume changed marginally within the normal ranges (Table 1), but there were no significant differences in other measures of complete blood count and blood biochemistry between pre- and post-administration. Notably, in blood cytokine analysis, the plasma levels of IL-6 and IFN-γ were significantly decreased and increased, respectively, compared with those in pre-administration (IL-6, 2.75 vs. 1.63, 95% CI: -1.90 to -0.326, $P = 0.00837$; IFN-γ, 6.20 vs. 7.17, 95% CI: 0.193–1.73, $P = 0.0172$). The plasma IL-6 was decreased in 14 (77.8%) out of 18 subjects, whereas the plasma IFN-γ was increased in 13 (72.2%) out of 18 subjects (Fig. 1). We also found a relatively large decrease in the IL-18 level; however, the difference was not statistically significant (266 vs. 193, 95% CI: -154–7.69, $P = 0.0732$).

Discussion

Dysregulated cytokine production is a hallmark of patients with COVID-19. Elevated blood levels of IL-2, soluble IL-2 receptor (sIL-2R), IL-4, IL-6, IL-10, and TNF-α have been observed especially in severe cases [9–12]. In particular, IL-6 plays pivotal roles in the exacerbation of COVID-19. Evidence is accumulating that an aberrant increase in IL-6 leads to complex immune dysregulation caused by SARS-CoV-2 infection. The blood IL-6 level is positively correlated with the severity and mortality in COVID-19 patients [13–15].

IFN-γ is a central mediator of antiviral immunity with an ability to directly interfere with viral replication. In addition, IFN-γ is indirectly involved in viral clearance through potentiating the action of IFN-α/β, activating Th1–dependent immune responses, and enhancing the MHC class I pathway [16]. Compared to healthy individuals, COVID-19 patients have significantly lower numbers of $CD4^+$ T, $CD8^+$ T, and NK cells with reduced capacity to produce IFN-γ, which causes the slightly decreased expression of IFN-γ [10,17]. Notably, the reduced cytotoxic potential of $CD4^+$ T, $CD8^+$ T, and NK cells is IL-6–dependent [17] and severe COVID-19 cases have a higher IL-6/IFN-γ ratio than moderate cases [18].

In conclusion, JHQGG can down- and up-regulate the plasma levels of IL-6 and IFN-γ, respectively, immediately after oral administration. The rapid immunomodulatory effects of JHQGG may be able to remedy the immune dysregulation observed in COVID-19 patients and thus provide therapeutic benefit to mild to severe cases as well as asymptomatic or suspected cases.

Statements Acknowledgement

We thank Hugh Wang [Juxiechang (Beijing) Pharmaceutical Co., Ltd.] for generously providing us with JHQGG. **Statement of Ethics**

This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All procedures were approved by the Ethics Committees of the Takanawa Clinic (approval number: 2020-2). A signed informed consent form was obtained from each participant prior to inclusion in this study. All experimental procedures and data analyses were conducted by investigators who were blinded to the subjects' clinical information using a de-identified dataset. This study has been registered on University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR) under the trial number UMIN000040407.

Conflict of Interest Statement

Yasunari Kageyama, Koichi Aida, Kimihiko Kawauchi, Masafumi Morimoto, and Tomoka Ebisui are employees of Takanawa Clinic. Tetsu Akiyama and Tsutomu Nakamura have advisory roles in conducting clinical research in Takanawa Clinic and receive advisory fees from Takanawa Clinic.

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Author Contributions

Yasunari Kageyama, Koichi Aida, Kimihiko Kawauchi, Masafumi Morimoto, and Tomoka Ebisui contributed to the conception and design of the study, contributed to data acquisition, analysis, and interpretation, and critically revised the manuscript for important intellectual content. Tetsu Akiyama contributed to the conception and design of the study and critically revised the manuscript for important intellectual content. Tsutomu Nakamura contributed to the conception and design of the study, contributed to data analysis and interpretation, and

drafted the manuscript. All authors gave final approval of the submitted version of the manuscript and agree to be accountable for all aspects of the work.

References

1. Ren JL, Zhang AH, Wang XJ. Traditional Chinese medicine for COVID-19 treatment. Pharmacol Res. 2020;155:104743.
2. Yang Y, Islam MS, Wang J, Li Y, Chen X. Traditional Chinese medicine in the treatment of patients infected with 2019-new coronavirus (SARS-CoV-2): A review and perspective. Int J Biol Sci. 2020;16(10):1708–17.
3. Cao P, Wu S, Wu T, Deng Y, Zhang Q, Wang K, et al. The important role of polysaccharides from a traditional Chinese medicine-Lung Cleansing and Detoxifying Decoction against the COVID-19 pandemic. Carbohydr Polym. 2020 July;240:116346.
4. Tao Z, Yang Y, Shi W, Xue M, Yang W, Song Z, et al. Complementary and alternative medicine is expected to make greater contribution in controlling the prevalence of influenza. Biosci Trends. 2013 Oct;7(5):253–6.
5. Ang L, Song E, Lee HW, Lee MS. Herbal medicine for the treatment of coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis of randomized controlled trials. J Clin Med. 2020;9(5):1583.
6. Liu M, Gao Y, Yuan Y, Yang K, Shi S, Zhang J, et al. Efficacy and safety of integrated traditional Chinese and Western medicine for corona virus disease 2019 (COVID-19): a systematic review and meta-analysis. Pharmacol Res. 2020;104896.
7. Zhang D, Zhang B, Lv JT, Sa RN, Zhang XM, Lin ZJ. The clinical benefits of Chinese patent medicines against COVID-19 based on current evidence. Pharmacol Res. 2020;157:104882.
8. Li GQ, Zhao J, Tu ZT, Li JB, Liu QQ, Shi LQ, et al. Treating influenza patients of windheat affecting fei syndrome by Jinhua Qinggan granule: A double-blinded randomized control trial. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2013 Dec;33(12):1631–5. [Article in Chinese]
9. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb;395(10223):497–506.
10. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest. 2020 May;130(5):2620–9.

11. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine. 2020 May;55:102763.
12. Cao X. COVID-19: immunopathology and its implications for therapy. Nat Rev Immunol. 2020 May;20(5):269–70.
13. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N, et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. Cell Host Microbe. 2020 Apr:S1931-3128(20)30236-5.
14. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell. 2020 May:S0092-8674(20)30489-X.
15. Zhang X, Tan Y, Ling Y, Lu G, Liu F, Yi Z, et al. Viral and host factors related to the clinical outcome of COVID-19. Nature. 2020 May. doi: 10.1038/s41586-020-2355-0.
16. Zhou F. Molecular mechanisms of IFN-γ to up-regulate MHC class I antigen processing and presentation. Int Rev Immunol. 2009;28(3-4):239–60.
17. Mazzoni A, Salvati L, Maggi L, Capone M, Vanni A, Spinicci M, et al. Impaired immune cell cytotoxicity in severe COVID-19 is IL-6 dependent. J Clin Invest. 2020 May:138554.
18. Lagunas-Rangel FA, Chávez-Valencia V. High IL-6/IFN-γ ratio could be associated with severe disease in COVID-19 patients. J Med Virol. 2020 Apr:10.1002/jmv.25900.

Figure Legends

Fig. 1. Changes in the plasma levels of IFN-γ and IL-6 before and after oral administration of JHQGG.

Table 1. Hematological and cytokine changes in JHQGG-administered healthy individuals.

Parameters	Pre-administration		Post-administration		Post – Pre	95% CI	*P*-value
	Mean	SD	Mean	SD			
Complete blood count							
Red blood cell count (x 10^4/μl)	448	50.3	445	52.1	-2.94	-7.58–1.69	0.198
Hemoglobin (g/dl)	13.4	1.79	13.3	1.81	-0.100	-0.229–0.0288	0.120
Hematocrit (%)	40.5	4.72	40.1	4.91	-0.450	**-0.851 to -0.0495**	**0.0298***
MCV (fl)	90.7	6.50	90.3	6.28	-0.456	**-0.837 to -0.0744**	**0.0219***
MCH (pg)	30.0	2.79	30.0	2.65	-0.0444	-0.257–0.168	0.665
MCHC (%)	33.0	1.31	33.2	1.24	0.139	-0.0542–0.332	0.147
White blood cell count (/μl)	6170	797	6320	835	144	-103–392	0.235
Platelet count (x 10^4/μl)	25.5	4.84	25.6	5.17	0.0722	-0.555–0.699	0.811
Blood biochemistry							
AST (U/l)	17.1	3.95	17.1	3.72	0.00	-0.482–0.482	1.00
ALT (U/l)	16.3	8.46	16.4	8.44	0.111	-0.266–0.488	0.542
γ-GT (U/l)	26.9	44.6	27.2	44.8	0.278	-0.0961–0.652	0.135
LDH (U/l)	150	30.0	147	26.0	-3.00	-7.67–1.67	0.193
Albumin (g/dl)	4.63	0.345	4.63	0.366	0.00	-0.0782–0.0782	1.00
Urea nitrogen (mg/dl)	13.1	2.51	12.7	3.07	-0.333	-1.17–0.506	0.414
HDL cholesterol (mg/dl)	72.0	18.4	71.6	17.2	-0.444	-1.70–0.812	0.466
LDL cholesterol (mg/dl)	107	31.8	106	32.4	-0.444	-2.24–1.35	0.607
Triglycerides (mg/dl)	80.3	47.2	83.6	54.2	3.28	-3.20–9.75	0.301
CRP (mg/dl)	0.102	0.200	0.106	0.209	0.00444	-0.00127–0.0102	0.119
Cytokines							
IFN-γ (pg/ml)	6.20	11.1	7.17	11.4	0.963	**0.193–1.73**	**0.0172***
IL-12p70 (pg/ml)	0.320	0.266	0.348	0.223	0.0279	-0.0670–0.123	0.543
IL-6 (pg/ml)	2.75	3.02	1.63	1.82	-1.11	**-1.90 to -0.326**	**0.00837****
TNF-α (pg/ml)	3.08	1.88	3.16	1.71	0.0849	-0.152–0.322	0.460
IL-1β (pg/ml)	2.77	7.09	1.83	3.03	-0.942	-3.27–1.39	0.405
IL-18 (pg/ml)	266	273	193	160	-73.3	-154–7.69	0.0732
IL-2 (pg/ml)	0.532	0.763	0.643	0.795	0.111	-0.0429–0.264	0.147
IL-8 (pg/ml)	734	808	1010	1420	274	-123–671	0.163
IL-10 (pg/ml)	0.360	0.220	0.368	0.225	0.00792	-0.0744–0.0902	0.841

MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γ-GT: γ-glutamyltransferase; LDH: lactate dehydrogenase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein; IFN: interferon; IL: interleukin; TNF-α: tumor necrosis factor-α; SD: standard deviation; CI: confidence interval. *$P < 0.05$, **$P < 0.01$.