Int J Clin Exp Med 2017;10(6):9180-9188
www.ijcem.com /ISSN:1940-5901/IJCEM0048750

Original Article

Administration of iguratimod suppresses development of arteritis in a murine model of Kawasaki disease

Like Zhao[1*], Feng Yang[2*], Rongwei Zhou[1], Bingyao Mu[1], Cibo Huang[1]

[1]*Department of Rheumatology, Beijing Hospital, National Center of Gerontology, Dong Dan, Beijing, China;* [2]*Department of Respiratory and Critical Care Medicine, Bo'ai Hospital, China Rehabilitation Research Center, Beijing, China.* [*]*Equal contributors.*

Received January 12, 2017; Accepted April 9, 2017; Epub June 15, 2017; Published June 30, 2017

Abstract: Objective: To investigate the inhibitory effect of iguratimod on the development of coronary arteritis in a murine model of vasculitis induced with a Candida albicans water-soluble fraction (CAWS). Methods: CAWS was intraperitoneally injected to C57BL/6 mice for 5 days, followed by administration of iguratimod or PBS. At day 3, day 10 and day 28, the mice were sacrificed. The status of vasculitis in the coronary arteries and the aortic root was investigated histologically, and plasma cytokines and chemokines were measured. Results: CAWS injection induced active inflammation in the aortic root and coronary arteries which were similar to the coronary arteritis in Kawasaki disease (KD). The vasculitis can be reduced by iguratimod at day 3 and this effect persisted for 28 days. The severity of the inflammation of the aortic root and the coronary arteries were reduced in iguratimod group ($P<0.05$). The levels of cytokines including granulocyte colony-stimulating factor (G-CSF), interleukin (IL)-10, IL-6, tumor necrosis factor-α and matrix metalloproteinase (MMP)-9 were increased in CAWS induced groups, while the level of IL-6 were suppressed dramatically by iguratimod ($P<0.05$). Furthermore, there is a strong correlation between the severity of the vasculitis and the plasma interleukin-6 levels. Conclusion: Iguratimod was effective in suppressing CAWS-induced coronary arteritis and expression level of IL-6, which suggests that iguratimod could potentially be an effective therapeutic strategy for arteritis in KD, and serum IL-6 could be a marker for the biological effects of iguratimod in KD.

Keywords: Candida albicans, iguratimod, Kawasaki disease, vasculitis

Introduction

Kawasaki disease (KD), an acute childhood inflammatory systemic disease in which mainly affects medium- to small-sized vessels, especially the coronary arteries, was the most prevalent cause of acquired heart disease in children in industrialized nations. Although many studies have been performed, the etiology of KD is not well understood. The most common and important complication involves the coronary arteries, leading to coronary artery aneurysm formation, thrombosis, stenosis, myocardial infarction, or sudden death [1]. To characterize and develop a therapeutic strategy for KD, various basic and clinical studies have been performed and are currently underway all over the world. At present, administration of intravenous immunoglobulin (IVIG) is the gold standard therapy for coronary arteritis in the acute phase of KD [2, 3]. Although standard therapy with IVIG and aspirin reduces the prevalence of coronary arterial lesions (CALs) in acute KD from 25-30% in untreated patients to 3-5% in treated patients, approximately 10% of patients with KD who fail to respond to the IVIG therapy show a high prevalence of CAL [4, 5]. To treat these IVIG nonresponders, alternative treatments, such as corticosteroids, cyclosporine-A, methotrexate, infliximab, etanacept and plasma pheresis have been tested, which may potentially reduce CAL in KD [6-12]. Despite these clinical trials, the proportion of KD patients with giant coronary aneurysms has remained steady since 2009 [13]. It is necessary to explore new treatment for the KD patients with CALs.

Although KD has unknown etiology, it is probably a cytokine associated disease. Many stud-

ies of cytokine networks in KD have focused on inflammatory cytokines such as tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1), IL-2, IL-6, IL-8, IL-10 and chemokines such as monocyte chemotactic protein-1 (MCP-1). These inflammatory cytokines and chemokines are elevated during the acute phase of KD [14-16]. In recent years, iguratimod (IGU, T-614, 3-formylamino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one), a small molecule, has been considered to be a novel immunomodulator and can improve the symptom of RA patients significantly [17, 18]. Existing studies showed that IGU suppressed the production of some inflammatory cytokines, including IL-1, IL-4, IL-6, IL-17, TNF-α, nuclear factor-kappa B, and interferon in vitro (synovial cells and some cell lines) and in vivo (mouse models) [19, 20]. IGU also reduced immunoglobulin production by acting directly on human B lymphocytes without affecting B lymphocyte proliferation [21]. Till now, no study was focus on whether IGU can be an effective treatment for KD patient with CAL.

The objective of this study was to investigate, whether administration of IGU can suppress the progression of inflammation by detect the histological features and the expression of various plasma cytokines. Since limited clinical specimens are available for the study of KD, animal models are indispensable tools to investigate the molecular mechanisms underlying the disease. Candida albicans water-soluble extract (CAWS)-induced vasculitis in mice is one of the most established animal models for KD because of the many histological similarities observed in this mouse model and KD, including prominent vasculitis in the aortic root and coronary arteries. Intraperitoneal injection of CAWS can induce coronary arteritis in both C57BL/B6 (B6 hereafter) and DBA/2 mice [22-24]. In this study, we evaluate the efficacy of IGU in a CAWS-induced vasculitis in C57BL/B6 mice and the effect on the cytokines/chemokines levels.

Materials and methods

Animals

All experimental procedures were approved by the Committee for Animal Research, Capital Medical University. A total of 54 male C57BL/6N mice at five-week-old were purchased from Chinese VitalRiver Laboratory Animal Technology CO. (Beijing, China) and were randomly and equally divided into three group: Control group, CAWS group and IGU group (n=18 each). All mice were kept under specific pathogen-free (SPF) conditions.

Organisms

Candida albicans strain IFO1594 was purchased from the Institute for Fermentation, Guangzhou (IFO), stored at 25°C on Sabauroud's agar (Difco, USA), and passaged once every 3 months.

Preparation of CAWS and IGU

CAWS was prepared from Candida albicans strain IFO1594 in accordance with the reported method [25]. Briefly, 5 L of medium (C-limiting medium) was maintained in a glass incubator for 2 days at 27°C while air was supplied at a rate of 5 L/min and the mixture was swirled at 400 rpm. Following culture, an equal volume of ethanol was added. After allowing the mixture to stand overnight, the precipitate was collected. The precipitate was dissolved in 250 mL of distilled water, and ethanol was added. The mixture was then allowed to stand overnight. The precipitate obtained was collected and dried with acetone to obtain CAWS. IGU were purchased from Simcere Pharmaceutical Co., Ltd. (Nanjing, China).

Administration of CAWS and IGU

Mice in control group were injected with 0.2 mL of phosphate buffered saline intraperitoneally for 5 days, and then normal saline in 0.2 mL was given by intragastric administration daily for 4 weeks (Control group). CAWS in a volume of 0.2 mL (0.5 mg/mouse/day) were injected intraperitoneally to C57BL/6N mice (both in CAWS group and IGU group) on 5 consecutive days. After administration of CAWS, IGU (90 mg/kg/d) was taken by intragastric administration daily for 4 weeks (IGU group). For comparison, mice were injected with 0.2 mL of normal saline for 4 weeks (CAWS group). At day 3, day 10 and day 28 of treatment, the mice were sacrificed by an overdose intraperitoneal injection of pentobarbital. Autopsies were performed to obtain plasma and the hearts were fixed in 10% neutralized formalin.

Histological evaluation

The fixed hearts were embedded in paraffin and sectioned. Hematoxylin and eosin (HE)-

Table 1. Body weight (BW), heart weight (HW), spleen weight (SW), kidney weight (KW) and lung weight (LW) in each group

		Ctrl	CAWS	IGU
3 day (n=6)	BW	18.00 ± 3.03	20.00 ± 1.79	19.00 ± 1.10
	HW	0.14 ± 0.03	0.15 ± 0.01	0.14 ± 0.02
	LW	0.18 ± 0.04	0.23 ± 0.03	0.21 ± 0.02
	SW	0.15 ± 0.04	0.21 ± 0.03*	0.22 ± 0.05*
	KW	0.26 ± 0.05	0.35 ± 0.05*	0.33 ± 0.05*
10 day (n=6)	BW	25.33 ± 1.03	23.83 ± 0.75*	23.00 ± 1.67*
	HW	0.19 ± 0.02	0.16 ± 0.02*	0.13 ± 0.02*
	LW	0.24 ± 0.05	0.23 ± 0.04	0.27 ± 0.07
	SW	0.10 ± 0.02	0.27 ± 0.13*	0.21 ± 0.06*
	KW	0.37 ± 0.02	0.34 ± 0.03	0.34 ± 0.04
28 day (n=6)	BW	27.50 ± 1.76	25.50 ± 0.84*	24.33 ± 1.21*
	HW	0.22 ± 0.02	0.19 ± 0.03*	0.18 ± 0.02*
	LW	0.25 ± 0.05	0.26 ± 0.07	0.30 ± 0.06
	SW	0.12 ± 0.02	0.17 ± 0.02*	0.18 ± 0.03*
	KW	0.44 ± 0.07	0.44 ± 0.05	0.38 ± 0.03

*P<0.05, versus Ctrl; CAWS versus IGU, P>0.05. Control (Ctrl), CAWS with no drug treatment (CAWS), iguratimod treatment (IGU).

stained sections were prepared using routine techniques for examination by light microscopy. Verhoeff-van Gieson (EVG) staining was performed to further investigate the elastic tissue of vascular architecture. First, we investigated the vasculitis in each group. Second, quantitative evaluation of vascular inflammation was performed as previously described. We divided the areas of the aortic root and coronary arteries into 5 segments (left coronary cusp; right coronary cusp; noncoronary cusp; left coronary artery; and right coronary artery) and graded the intensity of inflammation in each segment as follows: score 3, panvasculitis; score 2, inflammation involving the tunica intima and media but not spreading through the adventitia; score 1, inflammation localized to the tunica intima; and score 0, no inflammatory cell infiltration in the vascular wall.

Measurement of cytokines and chemokines

Plasma cytokines and chemokines were measured using a Bio-Plex system. An aliquot of serum (30 μL) was collected from peripheral blood and diluted 4-fold with the dilution solution. The diluted sample was then analyzed for the concentration of cytokines by the 15-Plex kit using mouse luminex screening assay (R&D, LXSAMS-15) according to the manufacture's protocol and the Bio-Plex Luminex 100 XYP instrument (Bio-Rad, Hercules, CA, USA). We assayed the following 15 cytokines and chemokines: granulocyte-colony stimulating factor (G-CSF); granulocyte macrophage colony stimulating factor (GM-CSF); interferon-gamma (IFN-γ), IL-1β, IL-2, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IL-23p19, IL-33, matrix metalloproteinases-9 (MMP-9); TNF-α and vascular endothelial growth factor (VEGF). We used a single assay to a single standard curve provided by the manufacturer. Concentrations of cytokines and chemokines were calculated using the Bio-Plex Manager 3.0 software (Bio-Rad, Tokyo) with a 5-parameter curve fitting algorithm that is applied for standard curve calculations.

Statistical analysis

The data on the animal weight, the scope and severity of the arteritis and cytokine/chemokine levels were analyzed using the two-sample *t*-test. For all statistical analyses, a value of $P<0.05$ was considered statistically significant.

Results

Animal data

Animal data are summarized in **Table 1**. The mice with administration of CAWS (both in CAWS group and IGU group) showed reductions in the volume of the food and water consumption, and body weight reduce comparing with control group (P<0.05). At day 3, Spleen weight and kidney weight increased in CAWS-induced mice than those in control group (P<0.05). At day 10 and day 28, heart weight decreased and spleen weight increased in CAWS-induced mice than those in the control group (P<0.05). There was no statistical difference between CAWS group and IGU group.

Induction of vasculitis by CAWS administration

The histopathological staining of the aortic root and coronary arteries of the control group revealed no abnormalities or inflammatory cells

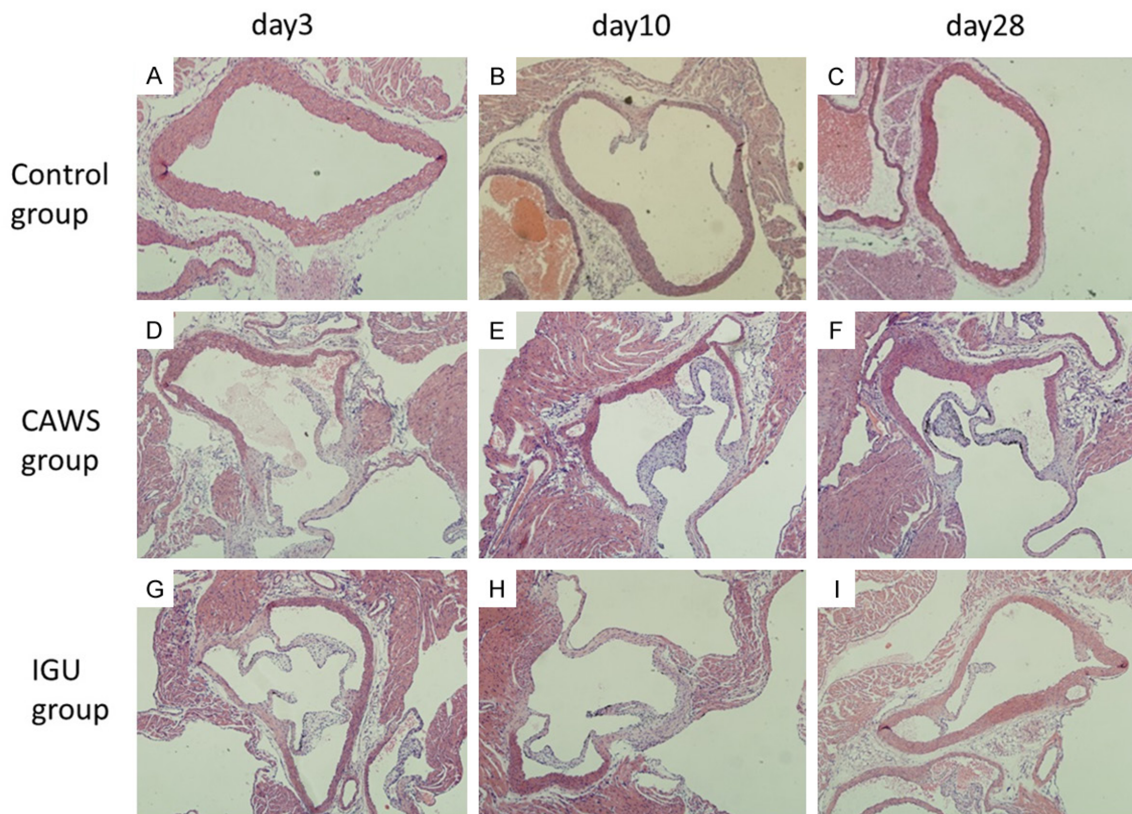

Figure 1. Histoligical features of murine heart by Hematoxylin and eosin (HE) stain (40 ×). A-C. Normal coronary arteries, no significant histological abnormalities or inflammatory cells in the aortic root and coronary arteries in control group at day 3, day 10 and day 28. D, G. At day 3, endothelial swelling and neutrophils infiltration were observed in the aortic root and coronary arteries both in CAWS group and IGU group. E. The active inflammatory infiltrates, composed of numerous neutrophils and lymphocytes, involved the aortic root, coronary arteries, endocardium, epicardium and myocardial interstitium of mice were pronounced in CAWS group at day 10. F. The inflammatory infiltration was decreased in coronary arteries at day 28. H, I. At day 10 and day 28, rare significant inflammatory changes and less inflammatory cell infiltration were observed in IGU mice.

at day 3, day 10 and day 28 (**Figure 1A-C**). However, 3 days after injection of CAWS, endothelial swelling and neutrophils infiltration were observed in the aortic root and coronary arteries both in CAWS group and IGU group (**Figure 1D**, **1G**). At day 10, panvasculitis was developed in CAWS group. The active inflammatory infiltrates, composed of numerous neutrophils and lymphocytes, involved the aortic root, coronary arteries, endocardium, epicardium and myocardial interstitium of mice were pronounced in CAWS group at day 10. Inflammatory cell infiltration spreading through the adventitia was observed (**Figure 1E**). In IGU group, to varying degrees, less panvasculitis were developed and less inflammatory infiltration was observed (**Figure 1H**). At day 28, although there were thickened walls of blood vessels, significant inflammatory changes and inflammatory infiltration were decreased in the CAWS-induced mice (**Figure 1F**, **1I**). EVG stain results showed that elastic fibers in control groups were in order at day 3, day 10 and day 28 (**Figure 2A-C**). The layers of vascular walls were in disorder (diffusely distorted or misshapen) at day 3 and day 10 in CAWS group (**Figure 2D**, **2E**). The vascular architecture was diffusely lost, and disruption and breakage of elastic fiber in the coronary arteries were also observed in CAWS group at day 28 (**Figure 2F**). In IGU group, layers of vascular walls were diffusely distorted or misshapen. However, no disruption was observed (**Figure 2G-I**).

Effects of IGU on development of vasculitis

The severity scores of inflammation increased significantly both in CAWS group and in IGU

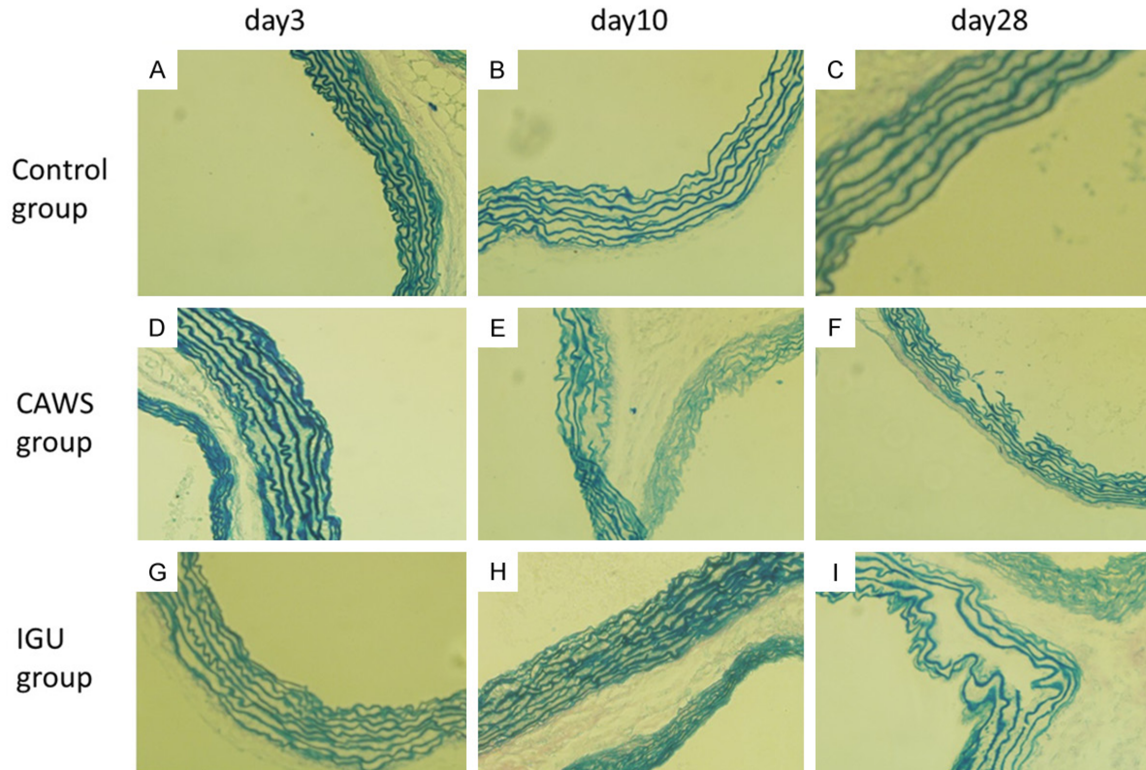

Figure 2. Verhoeff-van Gieson (EVG) staining of elastic fiber (100 ×). A-C. Layers of vascular walls were in order in control group. D-F. Layers of vascular walls were in disorder in day 3 and day 10 in CAWS group, and disruption and breakage of elastic fiber was observed at day 28. G-I. Layers of vascular walls were diffusely distorted or misshapen without disruption or breakage in IGU group.

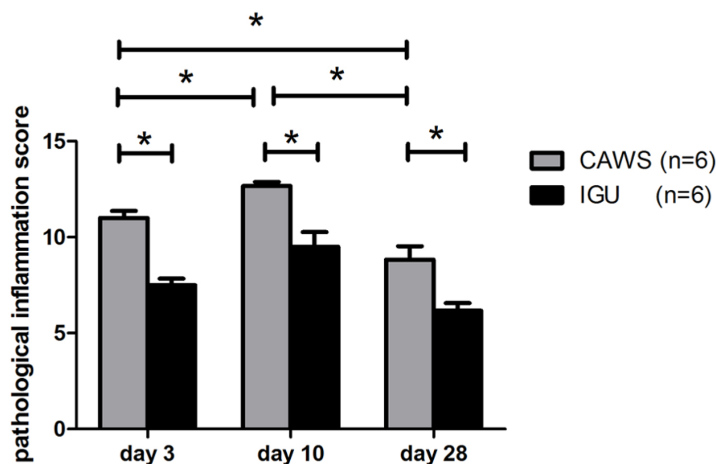

Figure 3. Inflammation severity scores at aortic root and coronary arteries in each group. The inflammation severity score of vasculitis was evident both in CAWS group and IGU group, and the inflammation was attenuated at day 28. IGU treatment reduced the inflammation severity score at day 3, day 10, day 28 compared with CAWS group. *P<0.05 versus CAWS group. Abbreviations: CAWS with no drug treatment (CAWS), iguratimod treatment (IGU).

group (**Figure 3**, P<0.05 versus control group). The vasculitis was the most remarkable at day 10 compared with that at day 3 and day 28. And the severity score was decreased at day 28. Compared to CAWS group, IGU treatment at day 3, day 10, day 28 reduced the inflammation severity score (**Figure 3**, P<0.05). The decrease in severity score was significant, suggesting that a strong suppressive effect was exerted by IGU.

Effects of different biological agents on proinflammatory cytokine and chemokine levels in plasma

The results of IGU on cytokine and chemokine levels are summarized in **Table 2**. At day 3, there was significant elevation of G-CSF, IL-6, IL-10 and TNF-α levels in the CAWS group and IGU group comparing with control group (P<0.001). The expression levels of IL-6 and TNF-α were

Table 2. Cytokines and chemokines level in plasma at different time point. At day 3, plasma levels of G-CSF, IL-6, IL-10 and TNF-α were increased in the CAWS group and IGU group, compared to negative control group. IL-6 and TNF-α was suppressed in IGU group. At day 10, the levels of G-CSF, IL-6, IL-10 and MMP-9 was increased both in CAWS group and IGU group, while IL-6 and MMP-9 were reduced by IGU. At day 28, the increased levels of G-CSF, IL-6 and IL-10 persisted in the CAWS group and IGU group, and IL-6 was supresssed in IGU group

		Ctrl	CAWS	IGU
Day 3 (n=6)	GCSF	20.67 ± 5.50	222.19 ± 96.93*	191.57 ± 121.98*
	IL-10	17.83 ± 4.22	176.31 ± 122.07*	96.24 ± 78.70*
	IL-6	4.20 ± 2.09	45.65 ± 19.54*	17.56 ± 4.23*,#
	TNF-α	1.48 ± 0.64	22.96 ± 13.38*	3.31 ± 0.79*,#
Day 10 (n=6)	GCSF	25.37 ± 9.34	191.00 ± 105.86*	260.13 ± 123.17*
	IL-10	6.29 ± 4.58	48.60 ± 21.69*	45.30 ± 21.70*
	IL-6	6.05 ± 2.22	83.94 ± 38.94*	26.94 ± 1.96*,#
	MMP-9	69.26 ± 17.89	481.46 ± 158.97*	217.01 ± 71.66*,#
Day 28 (n=6)	GCSF	25.76 ± 8.18	180.64 ± 103.73*	135.25 ± 39.40*
	IL-10	4.87 ± 2.46	47.79 ± 27.64*	30.85 ± 17.63*
	Il-6	10.55 ± 2.45	30.85 ± 12.49*	20.46 ± 4.86*,#

*P<0.05 versus Control group, #P<0.05 versus CAWS group. Abbreviations: Control (Ctrl), CAWS with no drug treatment (CAWS), iguratimod treatment (IGU).

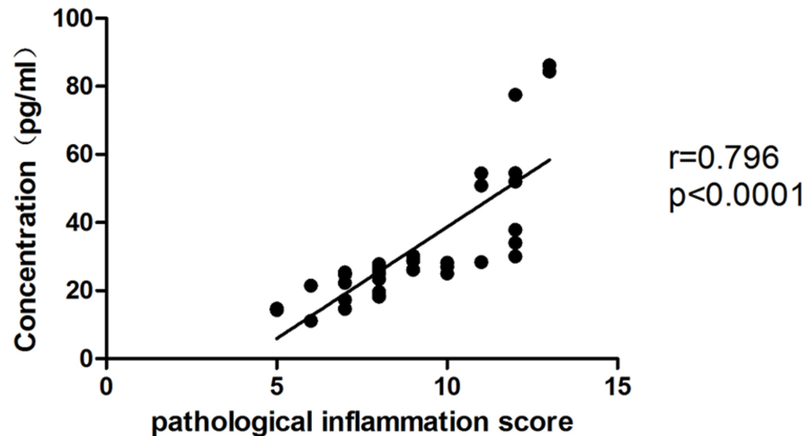

Figure 4. Correlation between Inflammation severity scores and plasma cytokine levels. Relationship between the Inflammation severity scores and plasma levels (pg/mL) of IL-6.

reduced in IGU group (P<0.001) comparing with CAWS group. At day 10, the levels of G-CSF, IL-6, IL-10 and MMP-9 were increased both in CAWS group and IGU group, while IL-6 and MMP-9 were reduced by IGU (P<0.001). At day 28, the increased levels of G-CSF, IL-6 and IL-10 still persisted and were higher than those in the control group (P<0.001). IGU significantly suppressed IL-6 persisting from day 3 till day 28 (P<0.05).

Correlation between plasma cytokine levels and histological changes

To evaluate the effects of cytokines on the development of vasculitis, we examined the statistical correlation between the inflammation severity score and levels of IL-6. The severity of vasculitis, as indicated by histological inflammation scores, correlated with serum IL-6 levels (r=0.796, P<0.0001) (**Figure 4**).

Discussion

In this study, we carried out vasculitis induction experiments by using CAWS in C57BL/6N mice, and evaluated the efficacy of IGU on vasculitis. To explore the underlying mechanisms, we measured the plasma cytokine levels using a Bio-Plex system. We further examined the relationship between IL-6 levels and inflammation severity. We found that IGU was effective in attenuating the arteritis by reducing cytokine levels of IL-6.

In KD, acute vascular injury involves the coronary arteries and is characterized by acute inflammatory cell infiltration and subsequent aneurysmal formation. A histological study on the coronary arteries from KD patients during the acute phase revealed prominent infiltration of macrophages, and numerous neutrophils in the arterial walls [26]. The animal model in our research showed that CAWS caused similar histological features of vasculitis in the aortic root and coronary arteries, and EVG results showed that the vascular elastic fibers were diffusely lost, broken and disrupted in CAWS group. The rup-

ture of an aneurysm evolves from primary intimal breakage. Elastin breakdown in coronary arteries is a hallmark of aneurysm formation, which corresponds to the subacute phase of human KD. Macrophages, T lymphocytes and myofibroblasts secrete matrix metalloproteinases, and other cytokines and enzymes within the arterial wall. Secreted enzymes destroy collagen and elastin fibers, and the structural support of the arterial wall weakens, resulting in dilatation or aneurysm formation [27]. There was no disruption or breakage of the coronary arteries in IGU group, which indicate that IGU might be beneficial in reducing the development of CAL.

It is clear that various inflammatory cytokines and chemokines, MMP, nitric oxide production, autoantibody production, and adhesive molecule expression are also over activated in the acute stage of KD, which is considered to facilitate vascular endothelial inflammation and then participate in the pathogenesis of KD and development of coronary arteritis [28]. Aberrant production of IL-1, IL-6, IFN-γ, TNF-α and other inflammatory cytokines purportedly induced a series of inflammatory reactions, leading to endothelial cell injury and inflammation of small and medium-sized arteries, especially coronary arteries [29]. The primary therapeutic endpoint in acute KD is prompt control of vasculitis to reduce the risk of CALs. In animal model, CAWS injection caused neutrophil activation, followed by complement activation and production of proinflammatory cytokines, chemokines and G-CSF [30]. IL-1, IL-13, GM-CSF and TNF-α are increased in a CAWS model in C57BL/6N mice [31]. In our study, the increased expression level of G-CSF, IL-6 and IL-10 were observed from day 3 till day 28; And the increase in IL-6 levels was the most obvious of the changes observed among all cytokines tested. Meanwhile, the effect of IGU treatment was pronounced. Although the median level of IL-6 was more than 10-fold higher than that in control group, the IL-6 level decreased significantly after the treatment of IGU. The effect of IGU appeared as early as day 3 and persisted until day 28. Moreover, the significant correlation between IL-6 level and the inflammation severity of coronary arteries, suggests a direct link between serum IL-6 and inflammatory development. Thus, our findings demonstrated the critical role of IL-6 in the development of vasculitis in KD, which indicate IL-6 suppression implies a mechanism explaining the effects of IGU.

IL-6 is an interleukin that acts as both a proinflammatory and anti-inflammatory cytokine. It can be secreted by T cells and macrophages to stimulate an immune response leading to inflammation and has been shown to correlate with the acute phase of KD [32]. In KD, IL-6 has been reported to rise more than any other cytokine, and correlates with coronary complication [33]. High levels of IL-6 could inhibit the differentiation of Th1 cells while promoting Th2 cells activation and consequently increasing the production of Th2 cytokines. IL-6 also induces activated B cells to differentiate into antibody producing cells and promotes the production of vascular endothelial growth factor, which plays an important role in angiogenesis [34]. Studies have shown that the levels of IL-4, IL-6, IL-10, and IFN-γ were significantly higher in KD patients with CALs. Furthermore, the levels of IL-6 and IL-10 were significantly higher in IVIG nonresponders. Therefore, IL-6 is a critical cytokine in the KD pathogenesis of autoimmune vasculitis [35]. IGU was originally developed as an anti-inflammatory and analgesic drug. The inhibitory effects of IGU on the production of IL-1 and IL-6 were considered to be important for its efficacy. Our study has shown that IGU treatment effectively reduced persisting serum IL-6 in CAWS-induced arteritis, which explained the therapeutic effect of IGU in KD. Since previous studies have shown that IL-6 was dramatically increased in IVIG-resistant KD patients, IGU might be an option for those patients.

Conclusion

In conclusion, we demonstrated the efficacy of IGU in suppressing the vasculitis occurring in C57BL/6N mice induced by CAWS. We found that IGU was effective not only in suppressing vascular inflammation, but also in decreasing plasma cytokine levels. The treatment goal for KD patients is to suppress systemic inflammation as early as possible because a prolonged elevation of serum inflammatory cytokines is considered to cause CALs. IGU treatment is beneficial in attenuating CALs and it suppressed the expression of IL-6 as early as day 3. Furthermore, there is a strong correlation between the extent of the vasculitis and the plasma IL-6 levels, which confirmed that IL-6

has an important role in the pathogenesis of vasculitis in KD, and serum IL-6 could be a marker for the biological effects of IGU in KD. Therefore, IGU could potentially be an effective therapeutic strategy for arteritis in KD, especially for the IVIG-resistant KD patients. Further studies are required to confirm the efficacy and safety of IGU therapy and establish its usefulness in the treatment of KD.

Disclosure of conflict of interest

None.

Address correspondence to: Cibo Huang, Department of Rheumatology, Beijing Hospital, National Center of Gerontology, No. 1 Dahua Road, Dong Dan, Beijing 100730, China. Tel: 86-10-85136736; E-mail: huangcibo1208@139.com

References

[1] Burns JC, Shike H, Gordon JB, Malhotra A, Schoenwetter M, Kawasaki T. Sequelae of Kawasaki disease in adolescents and young adults. J Am Coll Cardio 1996; 28: 253-257.
[2] Furusho K, Kamiya T, Nakano H, Kiyosawa N, Shinomiya K, Hayashidera T, Tamura T, Hirose O, Manabe Y, Yokoyama T, Kawarano M, Baba K, Mori C. High-dose intravenous gammaglobulin for Kawasaki disease. Lancet 1984; 2: 1055-1058.
[3] Newburger JW, Takahashi M, Burns JC, Beiser AS, Chung KJ, Duffy CE, Glode MP, Mason WH, Reddy V, Sanders SP, Shulrman ST, Wiggins JW, Hicks RV, Fulton DR, Lewis AB, Leung DY, Colton T, Rosen FS, Melish ME. The treatment of Kawasaki syndrome with intravenous gamma globulin. N Engl J Med 1986; 315: 341-347.
[4] Oates-Whitehead RM, Baumer JH, Haines L, Love S, Maconochie IK, Gupta A, Roman K, Dua JS, Flynn I. Intravenous immunoglobulin for the treatment of Kawasaki disease in children. Cochrane Database Syst Rev 2003; CD004000.
[5] Hamada H, Suzuki H, Abe J, Suzuki Y, Suenaga T, Takeuchi T, Yoshikawa N, Shibuta S, Miyawaki M, Oishi K, Yamaga H, Aoyagi N, Iwahashi S, Miyashita R, Honda T, Onouchi Y, Terai M, Hata A. Inflammatory cytokine profiles during cyclosporin treatment for immunoglobulin-resistant Kawasaki disease. Cytokine 2012; 60: 681-685.
[6] Lee TJ, Kim KH, Chun JK, Kim DS. Low-dose methotrexate therapy for intravenous immunoglobulin-resistant Kawasaki disease. Yonsei Med J 2008; 49: 714-718.
[7] Burns JC, Best BM, Mejias A, Mahony L, Fixler DE, Jafri HS, Melish ME, Jackson MA, Asmar BI, Lang DJ, Connor JD, Capparelli EV, Keen ML, Mamun K, Keenan GF, Ramilo O. Infliximab treatment of intravenous immunoglobulin-resistant Kawasaki disease. J Pediatr 2008; 153: 833-838.
[8] Burns JC, Mason WH, Hauger SB, Janai H, Bastian JF, Wohrley JD, Balfour I, Shen CA, Michel ED, Shulman ST, Melish ME. Infliximab treatment for refractory Kawasaki syndrome. J Pediatr 2005; 146: 662-667.
[9] Ohashi R, Fukazawa R, Watanabe M, Tajima H, Nagi-Miura N, Ohno N, Tsuchiya S, Fukuda Y, Ogawa S, Itoh Y. Etanercept suppresses arteritis in a murine model of kawasaki disease: a comparative study involving different biological agents. Int J Vasc Med 2013; 2013: 543141.
[10] Kobayashi T, Saji T, Otani T, Takeuchi K, Nakamura T, Arakawa H, Kato T, Hara T, Hamaoka K, Ogawa S, Miura M, Nomura Y, Fuse S, Ichida F, Seki M, Fukazawa R, Ogawa C, Furuno K, Tokunaga H, Takatsuki S, Hara S, Morikawa A; RAISE Study Group Investigators. Efficacy of immunoglobulin plus prednisolone for prevention of coronary artery abnormalities in severe Kawasaki disease (RAISE study): a randomised, open-label, blinded-endpoints trial. Lancet 2012; 379: 1613-1620.
[11] Mori M, Imagawa T, Katakura S, Miyamae T, Okuyama K, Ito S, Nakamura T, Kimura H, Yokota S. Efficacy of plasma exchange therapy for Kawasaki disease intractable to intravenous gamma-globulin. Mod Rheumatol 2004; 14: 43-47.
[12] Raman V, Kim J, Sharkey A, Chatila T. Response of refractory Kawasaki disease to pulse steroid and cyclosporin a therapy. Pediatr Infect Dis J 2001; 20: 635-637.
[13] Nakamura Y, Yashiro M, Uehara R, Sadakane A, Tsuboi S, Aoyama Y, Kotani K, Tsogzolbaatar EO, Yanagawa H. Epidemiologic features of Kawasaki disease in Japan: results of the 2009-2010 nationwide survey. J Epidemiol 2012; 22: 216-221.
[14] Gupta M, Noel GJ, Schaefer M, Friedman D, Bussel J, Johann-Liang R. Cytokine modulation with immune gamma-globulin in peripheral blood of normal children and its implications in Kawasaki disease treatment. J Clin Immunol 2001; 21: 193-199.
[15] Matsubara T, Ichiyama T, Furukawa S. Immunological profile of peripheral blood lymphocytes and monocytes/macrophages in Kawasaki disease. Clin Exp Immunol 2005; 141: 381-387.
[16] Takahashi K, Oharaseki T, Yokouchi Y. Update on etio and immunopathogenesis of Kawasaki

disease. Curr Opin Rheumatol 2014; 26: 31-36.
[17] Lu LJ, Bao CD, Dai M, Teng JL, Fan W, Du F, Yang NP, Zhao YH, Chen ZW, Xu JH, He PG, Wu HX, Tao Y, Zhang MJ, Han XH, Li XF, Gu JR, Li JH, Yu H. Multicenter, randomized, double-blind, controlled trial of treatment of active rheumatoid arthritis with T-614 compared with methotrexate. Arthritis Rheum 2009; 61: 979-987.
[18] Wei Y, Sun X, Hua M, Tan W, Wang F, Zhang M. Inhibitory effect of a novel antirheumatic drug T-614 on the IL-6-induced RANKL/OPG, IL-17, and MMP-3 expression in synovial fibroblasts from rheumatoid arthritis patients. Biomed Res Int 2015; 2015: 214683.
[19] Du F, Lu LJ, Fu Q, Dai M, Teng JL, Fan W, Chen SL, Ye P, Shen N, Huang XF, Qian J, Bao CD. T-614, a novel immunomodulator, attenuates joint inflammation and articular damage in collagen-induced arthritis. Arthritis Res Ther 2008; 10: R136.
[20] Kohno M, Aikawa Y, Tsubouchi Y, Hashiramoto A, Yamada R, Kawahito Y, Inoue K, Kusaka Y, Kondo M, Sano H. Inhibitory effect of T-614 on tumor necrosis factor-alpha induced cytokine production and nuclear factor-kappaB activation in cultured human synovial cells. J Rheumatol 2001; 28: 2591-2596.
[21] Tanaka K, Yamamoto T, Aikawa Y, Kizawa K, Muramoto K, Matsuno H, Muraguchi A. Inhibitory effects of an anti-rheumatic agent T-614 on immunoglobulin production by cultured B cells and rheumatoid synovial tissues engrafted into SCID mice. Rheumatol 2003; 42: 1365-1371.
[22] Nagi-Miura N, Adachi Y, Ohno N. Coronary arteritis induced by CAWS (Candida albicans water-soluble fraction) in various strains of mice. Nihon Ishinkin Gakkai zasshi 2008; 49: 287-292.
[23] Nagi-Miura N, Shingo Y, Adachi Y, Ishida-Okawara A, Oharaseki T, Takahashi K, Naoe S, Suzuki K, Ohno N. Induction of coronary arteritis with administration of CAWS (Candida albicans water-soluble fraction) depending on mouse strains. Immunopharmacol Immunotoxicol 2004; 26: 527-543.
[24] Ohno N. A murine model of vasculitis induced by fungal polysaccharide. Cardiovasc Hematol Agents Med Chem 2008; 6: 44-52.
[25] Uchiyama M, Ohno N, Miura NN, Adachi Y, Aizawa MW, Tamura H, Tanaka S, Yadomae T. Chemical and immunochemical characterization of limulus factor G-activating substance of Candida spp. FEMS Immunol Med Microbiol 1999; 24: 411-420.
[26] Takahashi K, Oharaseki T, Naoe S, Wakayama M, Yokouchi Y. Neutrophilic involvement in the damage to coronary arteries in acute stage of Kawasaki disease. Pediatri Int 2005; 47: 305-310.
[27] Takahashi K, Oharaseki T, Yokouchi Y, Miura NN, Ohno N, Okawara AI, Murata H, Naoe S, Suzuki K. Administration of human immunoglobulin suppresses development of murine systemic vasculitis induced with Candida albicans water-soluble fraction: an animal model of Kawasaki disease. Mod Rheumatol 2010; 20: 160-167.
[28] Lv YW, Wang J, Sun L, Zhang JM, Cao L, Ding YY, Chen Y, Dou JJ, Huang J, Tang YF, Wu WT, Cui WR, Lv HT. Understanding the pathogenesis of Kawasaki disease by network and pathway analysis. Comput Math Methods Med 2013; 2013: 989307.
[29] Ahn HM, Park IS, Hong SJ, Hong YM. Interleukin-6 (-636 c/g) gene polymorphism in korean children with Kawasaki disease. Korean Circ J 2011; 41: 321-326.
[30] Ishida-Okawara A, Nagi-Miura N, Oharaseki T, Takahashi K, Okumura A, Tachikawa H, Kashiwamura S, Okamura H, Ohno N, Okada H, Ohno N, Okada H, Ward PA, Suzuki K. Neutrophil activation and arteritis induced by C. albicans water-soluble mannoprotein-beta-glucan complex (CAWS). Expe Mol Pathol 2007; 82: 220-226.
[31] Takahashi K, Oharaseki T, Nagao T, Yokouchi Y, Yamada H, Nagi-Miura N, Ohno N, Saji T, Okazaki T, Suzuki K. Mizoribine provides effective treatment of sequential histological change of arteritis and reduction of inflammatory cytokines and chemokines in an animal model of Kawasaki disease. Pediatr Rheumatol Online J 2011; 9: 30.
[32] Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 2003; 374: 1-20.
[33] Lin CY, Lin CC, Hwang B, Chiang BN. Cytokines predict coronary aneurysm formation in Kawasaki disease patients. Eur J Pediatr 1993; 152: 309-312.
[34] Kuo HC, Liang CD, Wang CL, Yu HR, Hwang KP, Yang KD. Serum albumin level predicts initial intravenous immunoglobulin treatment failure in Kawasaki disease. Acta Paediatr 2010; 99: 1578-1583.
[35] Wang Y, Wang W, Gong F, Fu S, Zhang Q, Hu J, Qi Y, Xie C, Zhang Y. Evaluation of intravenous immunoglobulin resistance and coronary artery lesions in relation to Th1/Th2 cytokine profiles in patients with Kawasaki disease. Arthritis Rheum 2013; 65: 805-814.