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Protective effect of bioactive ceramics on liver injury: regulation of pro-inflammatory cytokins expression

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Abstract *Aim of study* A bioactive ceramics has been reported to regulate the expression of inflammatory cytokines in macrophage cells activated by lipopolysaccharides (LPS). In present study, we investigated the anti-inflammatory effect of bioactive ceramics using liver injury model in mouse. *Materials and Methods* Mice were divided into three groups: Normal group, LPS group (LPS and no ceramics treatment), Ceramics group (LPS and ceramics treatment). *Results* LPS administration induced the increase of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in mouse. The losses of cytoplasm of hepatocytes due to LPS caused the increase of AST and ALT in mouse plasma. In Ceramics group, however, the concentration of AST and ALT were much lower than LPS group until 6 weeks. And the losses of cytoplasm were rarely seen in Ceramics group. RT-PCR results showed that the decrease of proinflammatory cytokines such as IL-1β and IL-6 was observed in Ceramics group. Moreover, TGF-β1 and VEGF expression was increased in Ceramics group. *Conclusion* Bioactive ceramics effectively protected endotoxin-induced liver injury by attenuation of inflammatory processes in mice.

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1 Introduction

The word ceramics is derived from the Greek word *keramos,* which covers inorganic non-metallic materials. Until the 1950s, the most important of these were the traditional clays, made into pottery, bricks, and tiles. However, ceramics has been recently used as biomedical materials (biomaterials or bioactive materials) in dental and orthopedic implants [1]. In 1960s and 1970s, first-generation biomaterials were developed to be biological inertness for reduction of the immune response to the foreign body [2]. Bioactive materials were advanced to second-generation biomaterials which could elicit a controlled action and reaction in the physiological environment [3]. In 2002, Hench and Polak reported the development of third-generation biomedical materials that are designed to stimulate specific cellular responses at the molecular level [4].

On the other hand, there are a few reports in terms of alternative application of bioactive materials. For instance, bioactive materials were used in medical supplements such as glove, called as thermoglove, which has clinical effect on Raynaud's syndrome [5].

We previously reported that a bioactive ceramics suppressed inflammatory cytokines such as IL-1β, IL-10 and IL-6 in LPS-induced macrophages [6]. The ceramics inhibited LPS-induced NF-κB activity in macrophages. Moreover, the ceramics attenuated not only pro-inflammatory cytokines (IL-1β, IL-6, IL-10) expression but also the expression and activity of COX2 in rat vascular smooth muscle cells [7].

The purpose of this work was to evaluate effects of the bioactive ceramics in vivo using liver injury mouse model. The composition of ceramics used in this study was same as that of the ceramics previously reported [6, 7]. As mentioned above, the ceramics has been reported anti-inflammatory effect on macrophage through the expression of cytokines.

We examined whether bioactive ceramics inhibited the expression of cytokine, leading to liver injury and failure.

2 Materials and methods

2.1 Bioactive ceramics

Bioactive ceramics used in present study consisted of three kind of oxidative compounds: Silicon dioxide (SiO_2), Aluminum oxide (Al_2O_3), Titanium (IV) dioxide (TiO_2) purchased from Sigma-Aldrich Co. Each C57BL/6 mouse was anaesthetized with the mixture of sodium pentobarbital (2.5 mg/kg, *i.p.*) and xylazine (0.1 mg/kg, *i.p.*). We constructed patches containing 0.2 g of bioactive ceramics, fixed it to dorsal area of mouse by sutures.

2.2 Liver injury model

Male C56/BL6 mice, weighing 20–25 g at the start of the experiment, were used in this study. The mice were then challenged with LPS (2 mg/kg, *i.p.*) every day for 6 weeks. To investigate the biological effect of bioactive ceramics on mouse liver injury, mice were divided to three groups: Normal group, which was neither administrated LPS nor exposed to bioactive ceramics; LPS group, which was administrated with LPS but was not exposed to the ceramics; Ceramics group, which was constantly exposed to the ceramics and LPS. Mice were sacrificed 2, 4, and 6 weeks. All animal use procedures were approved by the Institutional Animal Care and Use Committee and were accomplished in accordance with the provisions of the NIH "Guide for the Care and Use of Laboratory Animals."

2.3 Liver function test

Blood was collected from each mouse by vena cava puncture at the time they were killed. Serum samples were analyzed for plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by automatic analysis.

2.4 Histology

Formalin fixed, paraffin-embedded liver sample were cut into 4-μm-thick sections, deparaffinized in xylene, and rehydrated through a series of decreasing concentration of ethanol. Sections were stained with hematoxylin-eosin.

2.5 Real-time PCR

Total RNA from liver was extracted using Trizol according to the manufacturer's protocol (Invitrogen, USA). Target RNA (0.5 μg) was reverse-transcribed using 100 units Superscript II reverse transcriptase (RT) (Invitrogen, USA) at 42°C for 50 min and 5 μmol/l oligo$(dT)_{16}$. Real-time PCR was performed with SYBR Green Master Mix (Applied Biosystems, USA). Mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene for normalization. The validity of GAPDH as a reference gene was confirmed experimentally by measuring the slope of the plot of log input RNA amount versus DCT, the difference in thermal cycle, as suggested by the user manual (MJ, USA). The primers for real-time PCR of target genes are shown in Table 1. PCR amplification was performed in triplicate, using the following cycle conditions: 40 cycles of 90°C for 30 s, 55–60°C for 45 s, 72°C for 1 min.

Table 1 Primer sequences for real-time PCR

Amplified RNA	Primer sequences (5′–3′)
GAPDH	Forward: GTCTTCACCACCATGGAGAAGGC
	Reverse: ATGCCAGTGAGCTTCCCGTTCAGC
IL-1β	Forward: ATGGCAACTGTTCCTGAACTCAACT
	Reverse: CAGGACAGGTATAGATTCTTTCCTTT
TNF-α	Forward: CCTGTAGCCCACGTCCGTAGC
	Reverse: TTGACCTCAGCGCTGAGTTG
IL-6	Forward: TGCTGGTGACAACAACGGCC
	Reverse: GTACTCCAGAAGACCAGAGG
TGF-β1	Forward: GAAGCCATCCGTGGCCAGAT
	Reverse: GACGTCAAAAGACAGCCACT
iNOS	Forward: ATGGAACAGTATAAGGCAAAC
	Reverse: GTTTCTGGTCGATGTCATGAG
VEGF	Forward: CCATGAACTTTCTGCTGTCTTGGGT
	Reverse: CTGCATGGTGATGTTGCTCTCTGAC

GAPDH; Glyceraldehyde-3-phosphate Dehydrogenase, IL-1β; Interlukin-1β, TNF-α; Tumor necrosis factor-α, IL-6; Interlukin-6, TGF-β1; Transforming growth factor-β1, iNOS; Inducible nitric oxide synthase, VEGF; Vascular endothelial growth factor

2.6 Statistical analysis

Data are expressed as the mean ± SD. Statistical analyses were performed using one-way ANOVA and Duncan method for multiple comparision. $P < 0.05$ was considered to be statistically significant. SPSS Win.Ver 12.0 was used for statistical analysis.

3 Results

As shown in Fig. 1, lipopolysaccharides administration resulted in significant rises in serum ALT and AST levels in LPS group. However, the increases were blunted by the bioactive ceramics treatment from 2 weeks to 6 weeks.

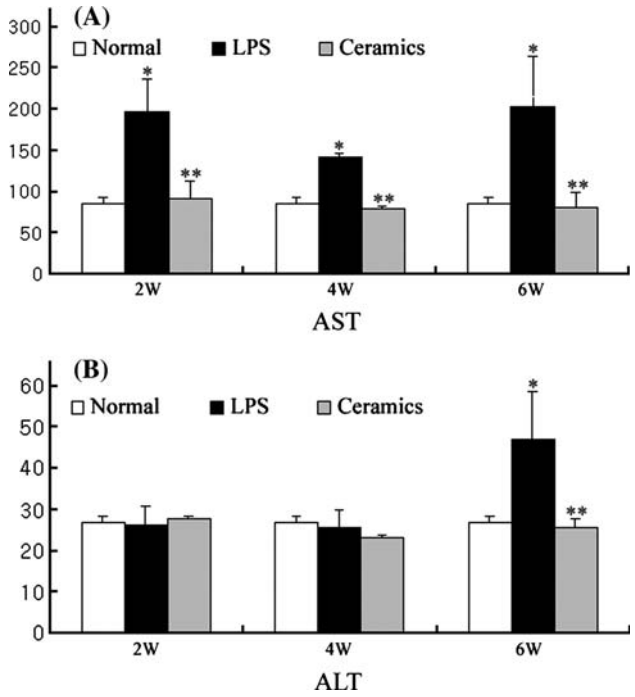

Fig. 1 Effect of bioactive ceramics on serum AST and ALT levels in LPS treated mouse Blood was collected from each mouse by vena cava puncture at the time they were killed (2, 4, 6 weeks). AST and ALT levels were determined as described in Materials and Methods. Data represent means ± SD ($n = 5$). $*P < 0.05$ compared with Normal group and $**P < 0.05$ compared with LPS group by ANOVA

Serum ALT and AST in Ceramics group were comparable to those of Normal group (Fig. 1).

Liver histological studies revealed that cytoplasm losses of hepatocytes were seen in LPS group from 2 weeks (data not shown). Most of hepatocytes were shown the loss of cytoplasm at 4 weeks after LPS administration in LPS group (Fig. 2). Accumulation of blood cells in the sinusoidal lining can be seen. Those cellular damages were consistent with increase of serum aminotransferase activities after LPS challenge. These changes caused by LPS were markedly reduced in the animals treated with bioactive ceramics (Fig. 2). Cytoplasm loss of hepatocytes and accumulation of blood cells in Ceramics group were detected, but much less than those in LPS group (Fig. 2).

The loss of cytoplasm was caused by damages from LPS-induced inflammation in hepatocyte. To determine whether alteration of cytokines expression occurred in response to long-term injection of LPS, RNA was isolated from liver and the changes in mRNA transcripts were determined by real-time RT-PCR. As shown in Fig. 3, the expressions of cytokines were detected in LPS group and Ceramics group from 2 weeks to 6 weeks. There was a substantial increase of cytokines such as IL-1β and IL-6 at 4 and 6 weeks in LPS group, respectively. In contrast, treatment of the ceramics resulted in significant decline in the expression of IL-1β and IL-6 induced by LPS. The expression of TGF-β1 and VEGF was also increased in LPS group and Ceramics group. There was no big difference of TGF-β1 expression between LPS and Ceramics group. However, VEGF level in Ceramics group was gradually increased until 6 weeks, whereas its expression was gradually decreased in LPS group. Inducible NOS (iNOS) level were also gradually increased in LPS and Ceramics group from 2 to 6 weeks. However, iNOS level of ceramics group was higher than that of LPS one.

4 Discussion

Ceramics is originated from clay or mud which has been known as one of the oldest forms of natural therapies to inflammatory disease including rheumatic diseases and other musculoskeletal conditions [8]. This ancient method of therapy is still widely used in central Europe. As mentioned previously, we reported that a bioactive ceramics showed an anti-inflammatory effect on LPS-induced macrophage ells by attenuation of inflammatory cytokine expressions [6].

In present study, our data demonstrated that bioactive ceramics treatment provided effective protection against

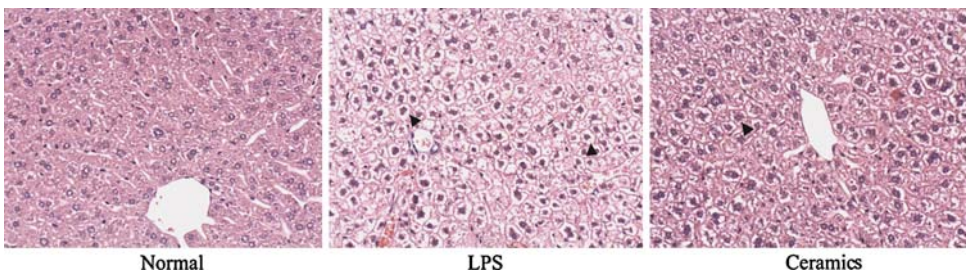

Fig. 2 Representative features of hepatocyte at 4 weeks after LPS administration. Liver section from Normal control; LPS group; and ceramics group are shown. The loss of cytoplasm of hepatocytes was observed in LPS and Ceramics group (▲; arrowhead). (H&E staining, original magnification ×100)

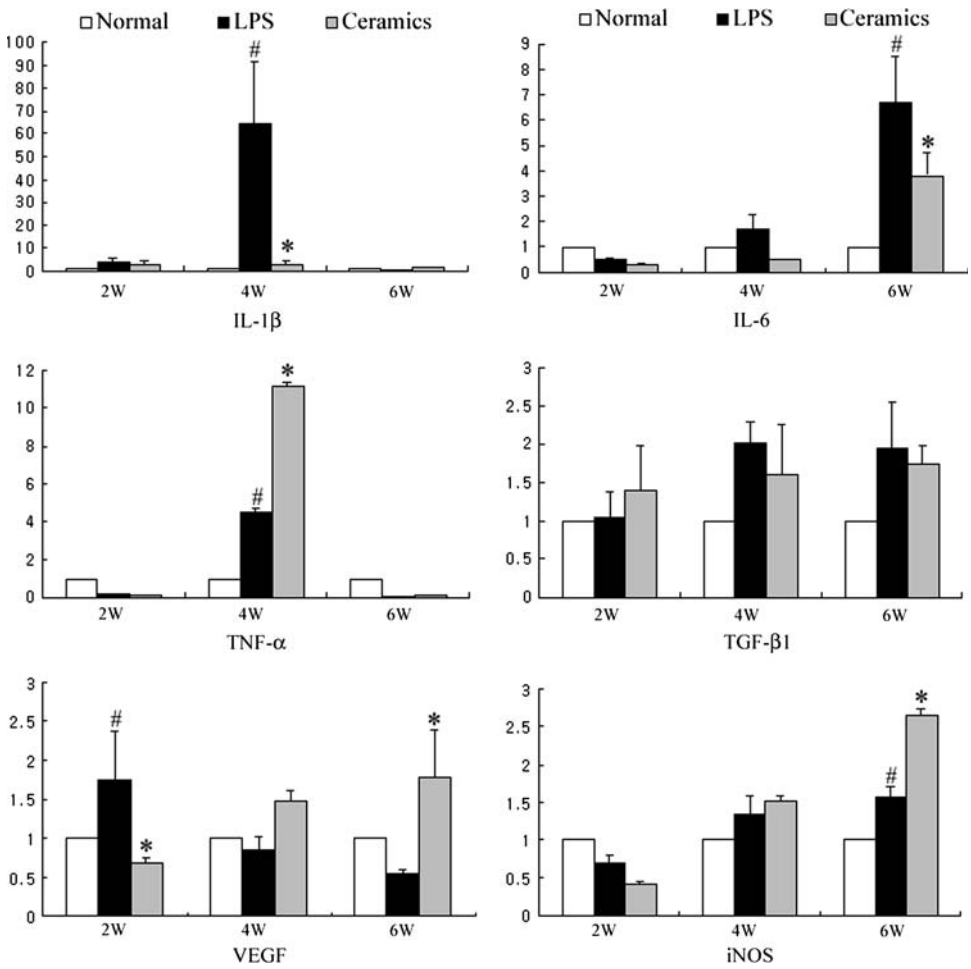

Fig. 3 Effect of bioactive ceramics on expression of inflammatory cytokines induced by LPS. The mRNA transcriptions of IL-1β, TNF-α, IL-6, IL-10, TGF-β1 and VEGF were determined by real-time PCR analysis. The *X*-axis represents the harvest time, the *Y*-axis represents the real-time PCR value to be normalized to the internal control GAPDH. A graph of a representative experiment of three independent studies is presented. All values are mean ± SD of triplicates. # $P < 0.05$ compared to Normal, * $P < 0.05$ compared to LPS by ANOVA

LPS-induced liver injury as evidenced by the reduction of serum ALT and AST levels, and the improvement of pathological changes.

Bacterial infections may pass into the bloodstream where endotoxin or LPS can provoke a systemic inflammatory response syndrome. LPS pass the mucosal barrier and enter the portal circulation. Circulating LPS forms certain complex with the LPS binding protein (LBP) which has high affinity to the receptor located on Kupffer cells [9]. Activation of the CD14 receptor by LPS-LBP complex initiates the release of cytokines, prostanoids, and nitrogen intermediates, resulting in liver injury and liver failure [10].

Here, LPS administration induced elevation of pro-inflammatory cytokines expression and the severity of liver damage. However, bioactive ceramics decreased the LPS-induced hepatic cytokines expression (IL-1β and IL-6), leading to improve liver damage by LPS. These results were consistent with those of previous study [6], where bioactive ceramics showed the significant suppression of IL-1β and IL-6 in LPS-activated macrophages.

The ceramics promoted iNOS mRNA expression in liver cells treated with LPS. Indeed, iNOS actions in hepatocytes are still controversial as beneficial on one hand and harmful on the other hand depending on the experimental set up conditions. Of some studies, a study demonstrates that iNOS may elicit apoptosis and contradicting results on nitric oxide (NO) [11]. On the other hand, a recent report demonstrates that the inhibition of endogenous iNOS during endotoxemia in rats reduced hepatocyte viability and increased ALT leakage, leading to increase mortality rate [12]. Our present study was consistent with later one. Thus, we assumed that bioactive ceramics may improve LPS-induced hepatocyte damage by increase of iNOS expression.

Taken together, we propose that bioactive ceramics might ameliorate LPS-induced liver injury by down-regulation of inflammatory cytokines and by the increase of iNOS and VEGF expression. Thus, we suggest that bioactive ceramics would represent an alternative strategy or a potential adjunct remedy to manage inflammation process such as liver injury.

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References

1. L.L. Hench, Ann. NY Acad. Sci. **523,** 54 (1988)
2. L.L. Hench, H.A. Paschall, J. Biomed. Mater. Res. Symp. **4**, 25 (1973)
3. L.L. Hench, J. Wilson, Science **226**, 630 (1984)
4. L.L. Hench, J.M. Polak, Sicence **295**, 1014 (2002)
5. G.D. Ko, D. Berbrayer, Altern. Med. Rev. **7**, 328 (2002)
6. M. Hwang, K. Park, Y. Chang, Y. Choo, J. Jeon, I. Shin, T. Lee, J. Biomed. Mater. Res. A **80**, 513 (2007)
7. T. Lee, K. Park, H. Cho, Y. Choo, M. Hwang, *J .Mater. Sci. Mater. Med.* Oct (2007) (on-line)
8. A. Donmez, M.Z. Karagulle, N. Tercan, M. Dinler, H. Issever, M. Karagulle, M. Turan, Rheumatol. Int. **26**, 168 (2005)
9. S.D. Wright, R.A. Ramos, P.S. Tobias, R.J. Ulevitch, J.C. Mathison, Science **249**, 1431 (1990)
10. T.L. Kielian, F. Blecha, Immunopharmacology **29**, 187 (1995)
11. R. Moreau, J. Hepatol. **37**, 678 (2002)
12. B. Chang, M. Nishikawa, E. Sato, M. Inoue, Arch. Biochem. Biophys. **411**, 63 (2003)