Biochemical and Biophysical Research Communications 417 (2012) 287–293

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Theaflavin attenuates ischemia–reperfusion injury in a mouse fatty liver model

Xiao-Yu Luo [a,b,d], Terumi Takahara [c,*], Jiangang Hou [a], Kengo Kawai [c], Toshiro Sugiyama [c], Kazuhiro Tsukada [d], Masumi Takemoto [e], Masao Takeuchi [f], Liang Zhong [b,*], Xiao-Kang Li [a,*]

[a] Division of Radiation Safety and Immune Tolerance, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan
[b] Department of Gastroenterology, Huashan Hospital, Fudan University, 12 Wulumuqi Middle Road, Shanghai 200040, China
[c] Third Department of Internal Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
[d] Second Department of Surgery, University of Toyama, Toyama, Japan
[e] School of Pharmaceutical Sciences, Ohu University, Fukushima, Japan
[f] Japan Institute for the Control of Aging, Shizuoka, Japan

ARTICLE INFO

Article history:
Received 10 November 2011
Available online 29 November 2011

Keywords:
Fatty liver
Ischemia–reperfusion injury
Macrophages
NAFLD
Oxidative stress
Theaflavin

ABSTRACT

The incidence of non-alcoholic fatty liver disease (NAFLD) has been increasing, and there is a shortage of liver donors, which has led to the acceptance of steatotic livers for transplantation. However, steatotic livers are known to experience more severe acute ischemia–reperfusion (I/R) injury than normal livers upon transplantation. In the present study, we investigated the role of theaflavin, a polyphenol substance extracted from black tea, in attenuating acute I/R injury in a fatty liver model. We induced I/R in normal and steatotic livers treated with or without theaflavin. We also separated primary hepatocytes from the normal and steatotic livers, and applied RAW264.7 cells, a mouse macrophage cell line, that was pre-treated with theaflavin. We observed that liver steatosis, oxidative stress, inflammation and hepatocyte apoptosis were increased in the steatotic liver compared to the normal liver, however, these changes were significantly decreased by theaflavin treatment. In addition, theaflavin significantly diminished the ROS production of steatotic hepatocytes and TNF-α production by LPS-stimulated RAW264.7 cells. We concluded that theaflavin has protective effects against I/R injury in fatty livers by anti-oxidant, anti-inflammatory, and anti-apoptotic mechanisms.

1. Introduction

Prevalence estimates of non-alcoholic fatty liver disease (NAFLD) that have been determined using a variety of laboratory and imaging assessments suggest that NAFLD may be the most common form of chronic liver disease of adults in developed countries [1], and is still increasing rapidly worldwide. Liver steatosis is a universal disease, and is a characterized manifestation in the spectrum of NAFLD. The lack of available organs for liver transplantation has led to the acceptance of the use of steatotic livers, which are most frequently used in extended criteria donor grafts [2]. However, the use of steatotic livers contributes to a higher risk of graft dysfunction because they usually have poorer tolerance to acute injury resulting from hepatic ischemia–reperfusion (I/R), which causes the production of reactive oxygen species (ROS). ROS can damage most organic compounds, including lipids, proteins and nucleic acids, subsequently leading to mitochondrial dysfunction [3,4]. ROS can also cause cell injury indirectly by promoting the expression of various transcription factors, which lead to the synthesis of pro-inflammatory cytokines and adhesion molecules [5]. Among trials of new agents with potential applications against I/R injury, tea-derived polyphenol substances have recently attracted considerable interest because they possess a broad spectrum of biological functions, such as anti-oxidant, anti-tumor, anti-inflammatory, and cardioprotective effects [6,7]. A previous study by Fiorini et al. reported that the administration of (−)-epigallocatechin-3-gallate (EGCG), a major flavonoid in green tea, significantly protected steatotic livers from I/R injury [8]. Black tea is the most widely consumed tea in the world [9]. Of interest, Huang and co-workers reported that theaflavin in black tea possessed the same antioxidant activity as EGCG [10], and Lin and co-workers reported that black tea leaves were more effective on hypolipidemia compared to green tea leaves [11]. Therefore, the present study was undertaken to judge the protective potential of theaflavin, a major polyphenol derived from black tea, against I/R injury in steatotic livers.

Abbreviations: I/R, ischemia–reperfusion; MCDHF, methionine and choline-deficient high fat; NAFLD, non-alcoholic fatty liver disease; qRT-PCR, quantitative real-time reverse transcriptase-polymerase chain reaction; ROS, reactive oxygen species.

* Corresponding authors. Fax: +86 76 434 5027 (T. Takahara), +86 21 6248 9191 (L. Zhong), +81 3 3417 2864 (X.-K. Li).
E-mail addresses: terutaka-tym@umin.ac.jp (T. Takahara), zhongniping@163.com (L. Zhong), sri@nch.go.jp (X.-K. Li).

0006-291X/$ - see front matter Crown Copyright © 2011 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.11.102

2. Materials and methods

2.1. Preparation of theaflavin

We have developed a novel fermentation method for the selective production of theaflavin from the four green tea catechins using enzymatic peroxidase. Briefly, 100 g of raw tea leaves (Japanese Yabukita-cha) are added to 2000 ml water and crushed in a blender. The mixture is then agitated for 8 h, filtered, and the filtrate is freeze-dried to afford a theaflavin mixture. The theaflavin mixture is then subjected to column chromatography on a Sephadex LH-20 column with ethanol to yield theaflavin.

2.2. Animals and experimental protocol

Male C57BL/6 mice, 6–8 weeks old, were housed in temperature- and light-controlled chambers. To develop a steatotic liver model, the mice were fed a methionine and choline-deficient high fat (MCDHF) diet, which contained corn oil and sucrose [40% (w/w) fat and 40% (w/w) carbohydrates] or a normal diet for 14 days [12,13]. Animals were randomized into 3 groups as follows (n = 7/each group): (1) normal group: fed a normal diet and treated with saline before I/R; (2) MCDHF group: fed the MCDHF diet and treated with saline before I/R; (3) MCDHF + theaflavin group: fed the MCDHF diet and treated with theaflavin before I/R. The mice received 30 mg/kg theaflavin solution by intraperitoneal (i.p.) injections at 48, 24, and 2 h before I/R.

2.3. Hepatic ischemia/reperfusion

The model of hepatic I/R was as described in a previous study [8]. In detail, the portal vein was occluded with a microvascular clamp for 15 min, after which the incision was closed. The mice were sacrificed after 3 h, and the liver and blood were collected. The animal protocols and procedures were reviewed and approved by the Committee of Care and Use of Laboratory Animals in our Institute.

2.4. Histopathological examination

Liver tissue samples were fixed in 10% formalin solution. Paraffin sections were stained with hemotoxylin–eosin (HE). The severity of steatosis (steatosis area percentage) was detected by using the WinRoof V6.1 software program (Mitani Corporation, Tokyo, Japan).

2.5. Immunohistochemical examination

A rat monoclonal antibody against F4/80, a surface marker of mouse monocytes/macrophages, and an antibody against myeloperoxidase (MPO), a surface marker of mouse neutrophils (Serotec, Oxford, UK) were applied to paraffin sections.

2.6. TUNEL assay

To detect apoptosis, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling (TUNEL) was performed on paraffin sections using the ApopTag Plus peroxidase *in situ* apoptosis detection kit (CHEMICON, Billerica, MA) according to the manufacturer's instructions.

2.7. Determination of the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels

Whole blood was collected and the serum was then evaluated for ALT and AST, expressed as U/L.

2.8. Thiobarbituric acid-reactive substances (TBARS)

The level of hepatic TBARS, considered to be indicators of oxidative stress, was measured using the Lab Assay™ TBARS kit (Cay Chemical Co., Greensboro, NC) according to the manufacturer's instructions.

2.9. Isolation of mouse hepatocytes and cell culture

Primary normal and steatotic hepatocytes were prepared from normal mice by collagenase perfusion as previously described [14]. The hepatocytes were seeded on plates coated with collagen I and incubated in DMEM containing 10% FCS.

2.10. Measurement of intracellular ROS

We used 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA; Invitrogen, CA), a cell-permeant indicator, to detect reactive oxygen species [15].

2.11. RNA preparation and quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

The total RNA was extracted from frozen liver tissue samples by using ISOGEN (NipponGene, Tokyo, Japan). Each 0.8 μg aliquot of RNA was reverse transcribed to cDNA using oligo (dT) primers and Super Script™ reverse transcriptase (Invitrogen). The sequences are listed in Table 1. Quantitative RT-PCR was performed using a TaqMan system on the Applied Biosystem PRISM7700 (ABI Japan, Co., Ltd., Tokyo, Japan) and were conducted in 0.9 mM of each primer in a final reaction volume of 25 μl of Premix Ex Taq™ (Takara Bio Inc., Shiga, Japan). The PCR cycling conditions were as follows, 50 °C for 2 min, 95 °C for 15 min and 50 cycles of 95 °C for 30 s, 60 °C for 1 min, and 25 °C for 2 min. The normalized Ct value of each gene was obtained by subtracting the Ct value of 18s rRNA.

2.12. Determination of the TNF-α concentration

The murine monocyte/macrophage cell line, RAW264.7, obtained from the ATCC (Rockville, Maryland). The levels of TNF-α in the medium of the RAW264.7 cells were detected by ELISA (R&D Systems, Minneapolis, MN).

2.13. Statistical analysis

The data were presented as means ± SEM and were analyzed statistically using a one-way ANOVA followed by Fisher's protected least-significance difference test or the Mann–Whitney U test. A value of $p < 0.05$ was considered to be statistically significant.

Table 1
The primers and probes used in this study.

Genes	Forward (5′–3′)	Reverse (5′–3′)	Probe
IL-6	CTGCAAGTGC	TGTCTATACCAC	CAGAATTGCCATTGC
	ATCATCGTTGT	TTCACAAGTCGGA	ACAACTCTTTTCTCA
TNF-α	TGTCTACTGAAC	AACTGATGA	TCCCCAAAGGGAT
	TTCGGGTGAT	GAGGGAGGCCAT	GAGAAGTTCCCAA
iNOS	CAGTGGAGAGA	CCCCAAGCAA	CCACAAGGCCAC
	TTTTGCATGACA	GACTTGGACTT	ATCGGATTTCACTT
IFN-γ	CAAGGCGAAAAA	CGGATGAGCTC	TGCCAAGTTTGAGG
	GGATGCA	ATTGAATGCT	TCAACAACCCACA
OPN	CCCGGTGAAA	GATTCTGCTTCT	AGCTCAGAGGAGA
	GTGACTGATTCT	GAGATGGGTCA	AGCTTTACAGCCTGCA
18s	ATGAGTCCACTT	CTTTAATATACGC	ATCCATTGGAGG
	TAAATCCTTTAACGA	TATTGGAGCTGGAA	GCAAGTCTGGTGC

3. Results

3.1. Theaflavin suppressed steatosis and steatotic liver I/R injury

The histological examinations of the livers showed that there was no steatosis in the normal diet group (Fig. 1A), whereas fatty droplets formed after 2 weeks of the MCDHF diet, consisting of liver cell ballooning, microvesicular steatosis and macrovesicular steatosis (Fig. 1B). Among the fatty livers, the theaflavin-treated mice had much less average steatosis and less inflammatory cell infiltration than the vehicle-treated mice (Fig. 1C and D). However, there was no obvious fibrosis in any of the livers of the mice that received the MCDHF diet for 2 weeks. The serum ALT and AST levels were also increased by the steatotic liver I/R, and the administration of theaflavin effectively reduced the serum ALT levels in comparison with the vehicle (Fig. 1E and F).

3.2. Theaflavin suppressed oxidative stress during I/R

To assess the hepatic oxidative stress during I/R, we examined the expression of TBARS, a reliable marker of the lipid peroxidation levels, which is commonly used to evaluate oxidative stress and cellular injury. Low expression of TBARS was detected in the normal livers subjected to I/R, and the level was significantly higher in the steatotic livers subjected to I/R, while the administration of theaflavin dramatically decreased the levels of TBARS in the steatotic livers subjected to I/R (Fig. 1G).

3.3. Steatotic livers exhibited increased hepatocyte apoptosis and macrophage infiltration during I/R, which were decreased by theaflavin

To examine hepatocyte apoptosis, we performed the TUNEL assay. Following I/R, the steatotic livers showed a significant increase in the number of TUNEL-positive cells compared to the normal livers (Fig. 2A, B and G). The administration of theaflavin significantly decreased the number of TUNEL-positive cells (Fig. 2C and G). We also performed an immunohistochemical analysis of F4/80 and MPO to identify the inflammatory cell types. F4/80-positive cells were more prevalent in the steatotic liver following I/R than in normal liver (Fig. 2D, E and H). Moreover, theaflavin effectively reduced the number of F4/80-positive cells (Fig. 2F and H). In contrast, the MPO immunostaining was not significantly different among the groups (data not shown).

3.4. Theaflavin suppressed the expression of inflammatory cytokine mRNA expression in steatotic livers subjected to I/R

The injury occurring as a result of steatotic liver I/R has also been demonstrated to be associated with various inflammatory cytokines [16]. The mRNA expression levels of inflammatory cytokine products were quantified by qRT-PCR, and the expression levels of TNF-α, IL-6, iNOS, OPN and interferon gamma (IFN-γ) were evaluated. Compared to normal livers subjected to I/R, there were higher levels of all of above mRNA except IFN-γ in the steatotic livers following I/R (Fig. 3). These increases were all reduced by the theaflavin treatment.

3.5. Theaflavin reduced ROS production in steatotic hepatocytes

To further investigate the role of theaflavin in protecting steatotic livers from oxidative stress, we quantified the intracellular ROS production in hepatocytes *in vitro* by using H_2DCFDA. Steatotic and normal hepatocytes were incubated with H_2DCFDA and then seeded onto 96 well collagen-coated plates. Fluorescence was measured at 3, 6, 9, 12, 15, 18, 21 and 24 h after incubation. The fluorescence of hepatocytes was also measured after incubation with theaflavin at concentrations of 1, 3 and 10 μg/ml for 12 h. The steatotic hepatocytes produced significantly more ROS than normal hepatocytes at each time point (Fig. 4A). However, the ROS production in steatotic hepatocytes was significantly decreased after incubation with theaflavin, especially at the 10 μg/ml concentration (Fig. 4B). The production of ROS was dose-dependently decreased in steatotic hepatocytes incubated with theaflavin.

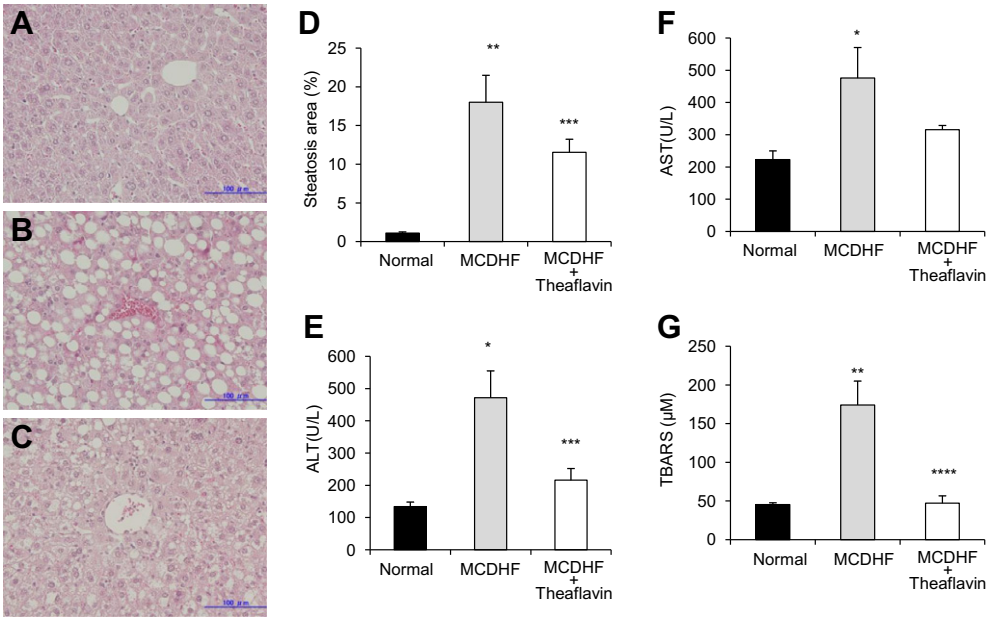

Fig. 1. Theaflavin attenuated the steatosis, hepatic injury and oxidative stress during I/R. The mice were fed (A) a normal diet, (B) a MCDHF diet, (C) a MCDHF diet + theaflavin. The normal diet did not cause steatosis. The MCDHF diet caused hepatic steatosis and infiltration of inflammatory cells, which were decreased by the administration of theaflavin. *Scale bars* represent 100 μm. (D) The measured steatotic area in the livers of each group. (E) The serum ALT levels. (F) The serum AST levels. (G) The level of hepatic TBARS (means ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$ versus the normal group; $^{***}p < 0.05$, $^{****}p < 0.01$ versus the MCDHF group).

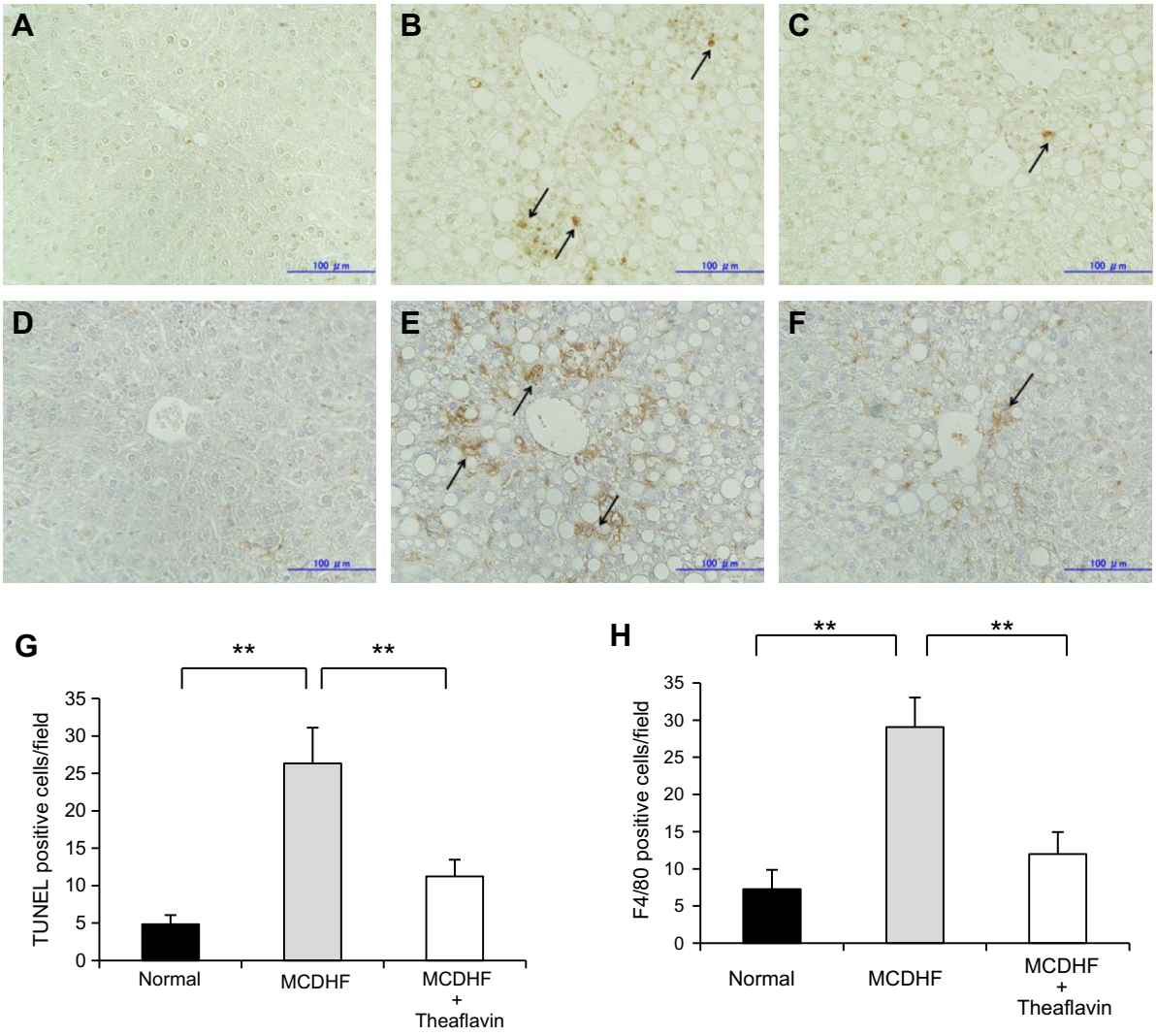

Fig. 2. The steatotic livers had increased hepatocyte apoptosis and macrophage infiltration during I/R, which were decreased by theaflavin. (A and D) Normal livers, (B and E) livers from mice fed the MCDHF diet, (C and F) livers from mice fed the MCHHF diet + theaflavin. (A–C) TUNEL assay. (D–F) F4/80 immunostaining. TUNEL-positive cells (arrows) were prevalent in the MCDHF group, but were seldom seen in the mice pretreated with theaflavin. F4/80-positive cells (arrows) were evident in the steatotic livers, and were suppressed by pretreatment with theaflavin. (G) The number of TUNEL positive cells. (H) The number of F4/80-positive cells. *Scale bars* represent 100 μm (means ± SEM; $^{**}p < 0.01$).

3.6. Theaflavin inhibited the TNF-α secretion of the LPS-stimulated RAW264.7 cells

To investigate theaflavin's role in inflammation, we evaluated the effects of theaflavin on RAW264.7 macrophages *in vitro*. We first detected the levels of TNF-α in the RAW264.7 cell culture when the cells were treated with various concentrations of theaflavin. We found that, when the cells were treated with 0.1 or 0.3 μg/ml theaflavin, there was very similar low production of TNF-α (data not shown). Thus, we chose 0.1 μg/ml as a suitable concentration for further studies. After incubation for 12 h, the cells were subsequently treated with 0.1 ng/ml LPS with or without 0.1 μg/ml theaflavin for 10 h. The results showed that treatment with 0.1 μg/ml theaflavin effectively reduced the TNF-α levels compared to the no-treatment control group (Fig. 4C).

4. Discussion

Although fatty livers are currently being used for transplantation due to the donor shortage, clinical data have shown that these livers are more susceptible to I/R injury, leading to increased plasma ALT levels and diminished liver function. In this regard, we have aimed to ameliorate the fatty condition to produce better outcomes following I/R. A previous study demonstrated that feeding black tea leaves or theaflavin can directly reduce plasma triglyceride and cholesterol levels by suppressing lipogenesis [17,18], and in an *in vitro* experiment, decreases the lipid accumulation in HepG2 cells by activating AMP-activated protein kinase (AMPK) and suppressing fatty acid synthetase [19]. In our present study, we found that even short term intraperitoneal administration of theaflavin can significantly reduce hepatocyte steatosis, confirming theaflavin's role in diminishing the fat content in steatotic livers.

It is well known that ROS, such as superoxide anion, hydrogen peroxide and the hydroxyl radical, play critical roles in I/R injury. Furthermore, there is known to be increased generation of ROS in fatty livers after I/R, and greater lipid peroxidation is thought to be facilitated by the accumulation of lipids, which provides a greater amount of substrate for the initiation and subsequent amplification of the free radical attack [5]. The detection of TBARS in this study confirmed that the poor tolerance in the steatotic livers was associated with a greater extent of oxidative injury during

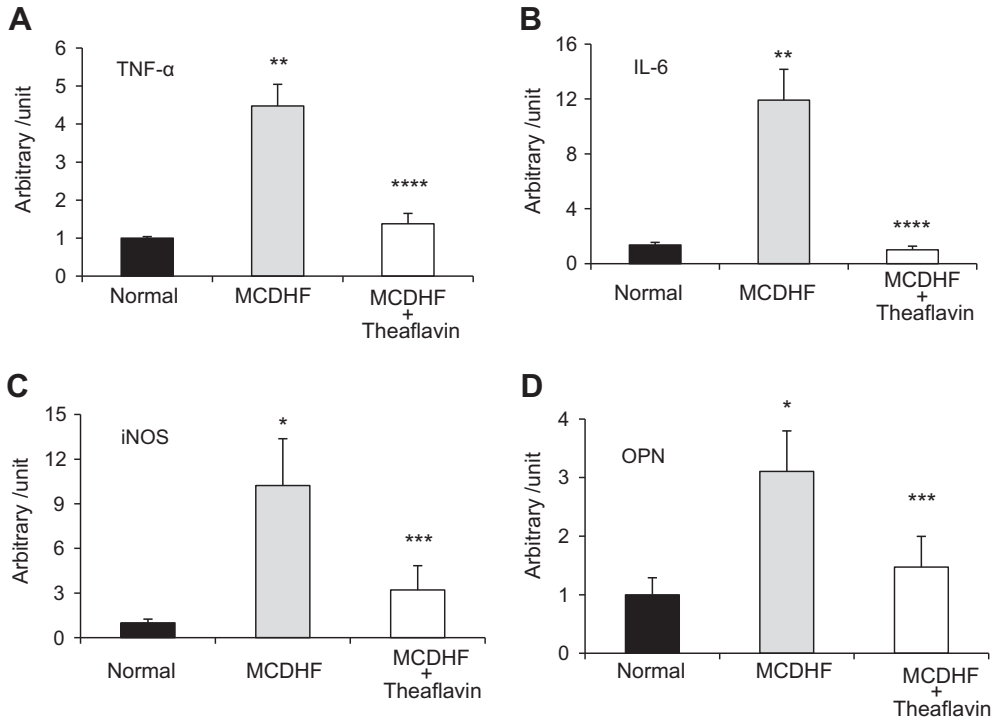

Fig. 3. Theaflavin suppressed the expression of inflammatory cytokines in steatotic livers following I/R. The data are representative of 3–5 animals for each group (means ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$ versus normal group; $^{***}p < 0.05$, $^{****}p < 0.01$ versus MCDHF group).

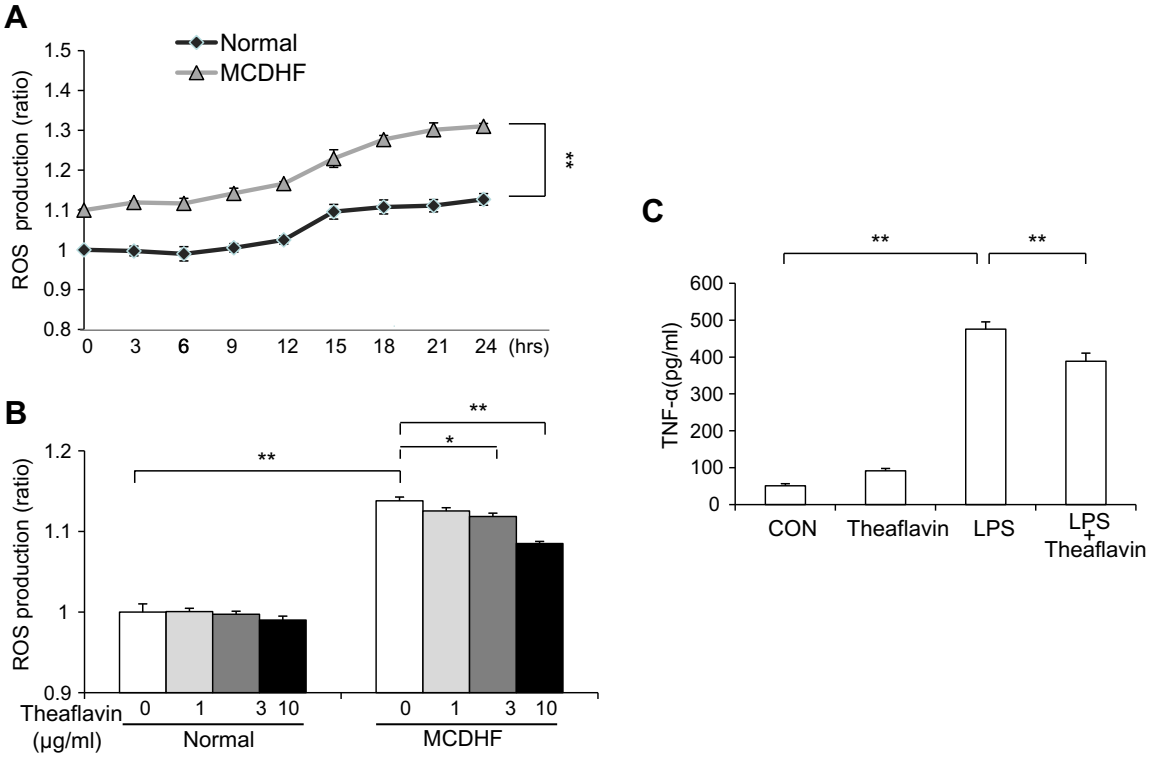

Fig. 4. Theaflavin decreased the ROS levels in steatotic hepatocytes and the TNF-α levels in LPS-stimulated RAW264.7 cells. (A) ROS were measured at various time points. Steatotic hepatocytes generated more ROS than normal hepatocytes at each time point. (B) The administration of theaflavin significantly reduced the ROS production in steatotic hepatocytes, especially at the 10 μg/ml concentration. (C) The administration of theaflavin effectively reduced the TNF-α production in LPS-stimulated RAW264.7 cells (means ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$).

I/R. However, the oxidative stress of steatotic liver was effectively inhibited by theaflavin. In this study, we successfully provided the first demonstration that theaflavin has anti-oxidant effects on steatotic hepatocytes *in vitro*.

In addition, we found that activated macrophages play an important role in steatotic liver I/R injury. Although macrophage activation was related to the progression of steatohepatitis, as we have already shown in the MCDHF diet model [12], we supposed that the interaction between steatosis and I/R further promoted the activation and accumulation of macrophages, and that it might have induced the recruitment of macrophages from the hepatic microcirculation during the I/R process. Indeed, these findings were similar to those of the study by Clouse and co-workers who reported that steatotic livers showed an increase in the number and phagocytic activity of macrophages, with a further increase in phagocytosis after transplantation [20]. Moreover, a previous study showed that in a NAFLD model, altered macrophage function influenced the neighboring cells, such as hepatocytes, stellate cells and endothelial cells, because products of macrophage, including H_2O_2, nitric oxide, and various cytokines, can exert noxious effects on these cells [16]. In addition, these factors will further impair the viability of steatotic livers following I/R. In this study, increased levels of inflammatory cytokines, including TNF-α, IL-6, iNOS, and OPN, were coincident with macrophage recruitment. OPN, an extracellular structural protein, acts as a cytokine which can interact with multiple cell surface receptors in many physiological and pathological processes including wound healing, bone turnover, tumor genesis, inflammation and ischemia [21]. The inflammatory cytokines TNF-α, IL-6 and iNOS, which are important and typical cytokines elevated in both the NAFLD and I/R models, showed more prominent and specific elevation, and this might be related to the fact that these three inflammatory cytokines can be directly expressed by activated macrophages [22,23]. Of interest, the increase in the expression of these inflammatory cytokines was suppressed by pretreatment with theaflavin in our present study.

To further investigate the anti-inflammatory effects of theaflavin, we subsequently performed an *in vitro* experiment, and found that the administration of theaflavin could reduce the TNF-α levels of LPS-stimulated macrophages. Associated with the F4/80 immunostaining examination, we hypothesized that theaflavin not only suppressed the recruitment of macrophages, but also participated in regulating the cytokines produced from macrophages, and maybe even endothelial cells and adipocytes by some signaling pathways. This hypothesis will need further investigation in the future. In other experiments, theaflavin was reported to inhibit IL-8 gene expression in lung adenocarcinoma cells [24], and inhibited intercellular adhesion molecule 1 (ICAM-1), cyclooxygenase-2 (COX-2), and iNOS expression in brain tissue during I/R injury [6]. Our present study provided evidence to support that theaflavin was a novel anti-inflammatory compound, which inhibited macrophage accumulation and suppressed the production of inflammatory cytokines in steatotic livers following I/R.

The data from previous studies indicated that both graft steatosis and I/R contributed to the extent of neutrophil-mediated I/R injury [25]. However, in our study, neither pathological HE staining nor MPO immunostaining showed evidence of increased neutrophils in steatotic livers following I/R. This was likely due to the fact that the length of the MCDHF diet (2 weeks) and the I/R time (15 min/3 h) were too short to increase the neutrophil infiltration. Serviddio et al. noted similar findings with us in his recent study [26].

It is noteworthy that liver steatosis has recently been demonstrated to contribute to I/R injury by a lysosomal pathway of apoptosis [27]. The TUNEL assay in our present experiment supported this conclusion. Interestingly, theaflavin and other tea extracts have been reported to inhibit the proliferation and induce apoptosis in a variety of cancer cells [28]. In contrast, in this study, we found that theaflavin effectively protected hepatocytes from apoptosis in steatotic livers during I/R injury, which was similar to the results reported by Youdim and co-workers wherein green tea polyphenol-epigallocatechin-3-gallate (EGCG) exerted neuro-rescue effects against apoptosis [29]. Kamata et al. showed ROS promoted TNF-α induced cell death via the JNK–ASK pathway [30], which our previous study also confirmed in hepatocytes from a NAFLD model [13,15]. We suppose that the anti-apoptotic effects of theaflavin in our study may have resulted from the significant decrease in ROS and TNF-α. The role of theaflavin in inducing or inhibiting the apoptosis of different kinds of cells needs to be investigated in further studies.

In summary, we herein demonstrated that steatotic livers contributed to increasing the I/R injury compared to normal livers, and this is the first study to report that pretreatment with theaflavin noticeably decreased the level of injury in steatotic livers following I/R. We also proved that the protective effects of theaflavin were associated with its hypolipidemic, anti-oxidant, anti-inflammatory, and anti-apoptotic mechanisms of action. Thus, theaflavin may be a promising candidate for the pretreatment of steatotic livers prior to transplantation.

Acknowledgment

The authors thank Mr. Y. Kuwabara and Ms. T. Kozawa for their technical assistance.

References

[1] D.E. Kleiner, E.M. Brunt, M. Van Natta, C. Behling, M.J. Contos, O.W. Cummings, L.D. Ferrell, Y.C. Liu, M.S. Torbenson, A. Unalp-Arida, M. Yeh, A.J. McCullough, A.J. Sanyal, C. Nonalcoholic Steatohepatitis, Design and validation of a histological scoring system for nonalcoholic fatty liver disease, Hepatology 41 (2005) 1313–1321.

[2] L. McCormack, P. Dutkowski, A.M. El-Badry, P.A. Clavien, Liver transplantation using fatty livers: always feasible?, J Hepatol. 54 (2011) 1055–1062.

[3] A.J. Tompkins, L.S. Burwell, S.B. Digerness, C. Zaragoza, W.L. Holman, P.S. Brookes, Mitochondrial dysfunction in cardiac ischemia–reperfusion injury: ROS from complex I. Without inhibition, Biochim. Biophys. Acta, Mol. Basis Dis. 1762 (2006) 223–231.

[4] Q. Wang, A.Y. Sun, A. Simonyi, T.J. Kalogeris, D.K. Miller, G.Y. Sun, R.J. Korthuis, Ethanol preconditioning protects against ischemia/reperfusion-induced brain damage: role of NADPH oxidase-derived ROS, Free Radic. Biol. Med. 43 (2007) 1048–1060.

[5] B. Nardo, P. Caraceni, P. Pasini, M. Domenicali, F. Catena, G. Cavallari, B. Santoni, E. Maiolini, I. Grattagliano, G. Vendemiale, F. Trevisani, A. Roda, M. Bernardi, A. Cavallari, Increased generation of reactive oxygen species in isolated rat fatty liver during postischemic reoxygenation, Transplantation 71 (2001) 1816–1820.

[6] F. Cai, C.R. Li, J.L. Wu, J.G. Chen, C. Liu, Q. Min, W. Yu, C.H. Ouyang, J.H. Chen, Theaflavin ameliorates cerebral ischemia–reperfusion injury in rats through its anti-inflammatory effect and modulation of STAT-1, Mediat. Inflamm. 2006 (2006) 30490.

[7] J.V. Higdon, B. Frei, Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions, Crit. Rev. Food Sci. Nutr. 43 (2003) 89–143.

[8] R.N. Fiorini, J.L. Donovan, D. Rodwell, Z. Evans, G. Cheng, H.D. May, C.E. Milliken, J.S. Markowitz, C. Campbell, J.K. Haines, M.G. Schmidt, K.D. Chavin, Short-term administration of (−)-epigallocatechin gallate reduces hepatic steatosis and protects against warm hepatic ischemia/reperfusion injury in steatotic mice, Liver Transpl. 11 (2005) 298–308.

[9] N. Chen, R. Bezzina, E. Hinch, P.A. Lewandowski, D. Cameron-Smith, M.L. Mathai, M. Jois, A.J. Sinclair, D.P. Begg, J.D. Wark, H.S. Weisinger, R.S. Weisinger, Green tea, black tea, and epigallocatechin modify body composition, improve glucose tolerance, and differentially alter metabolic gene expression in rats fed a high-fat diet, Nutr. Res. 29 (2009) 784–793.

[10] L.K. Leung, Y.L. Su, R.Y. Chen, Z.H. Zhang, Y. Huang, Z.Y. Chen, Theaflavins in black tea and catechins in green tea are equally effective antioxidants, J. Nutr. 131 (2001) 2248–2251.

[11] K.L. Kuo, M.S. Weng, C.T. Chiang, Y.J. Tsai, S.Y. Lin-Shiau, J.K. Lin, Comparative studies on the hypolipidemic and growth suppressive effects of oolong, black, pu-erh, and green tea leaves in rats, J. Agric. Food Chem. 53 (2005) 480–489.

[12] H. Kudo, Y. Yata, T. Takahara, K. Kawai, Y. Nakayama, M. Kanayama, T. Oya, S. Morita, M. Sasahara, D.A. Mann, T. Sugiyama, Telmisartan attenuates progression of steatohepatitis in mice: role of hepatic macrophage infiltration and effects on adipose tissue, Liver Int. 29 (2009) 988–996.

[13] H. Kudo, T. Takahara, Y. Yata, K. Kawai, W. Zhang, T. Sugiyama, Lipopolysaccharide triggered TNF-alpha-induced hepatocyte apoptosis in a murine non-alcoholic steatohepatitis model, J. Hepatol. 51 (2009) 168–175.

[14] T. Takahara, F. Xue, M. Mazzone, Y. Yata, K. Nonome, M. Kanayama, K. Kawai, A.M. Pisacane, S. Takahara, X.K. Li, P.M. Comoglio, T. Sugiyama, P. Michieli,

Metron factor-1 prevents liver injury without promoting tumor growth and metastasis, Hepatology 47 (2008) 2010–2025.
[15] W. Zhang, H. Kudo, K. Kawai, S. Fujisaka, I. Usui, T. Sugiyama, K. Tsukada, N.C. Chen, T. Takahara, Tumor necrosis factor-alpha accelerates apoptosis of steatotic hepatocytes from a murine model of non-alcoholic fatty liver disease, Biochem. Biophys. Res. Commun. 391 (2010) 1731–1736.
[16] A.M. Diehl, Nonalcoholic Steatosis and Steatohepatitis – IV. Nonalcoholic fatty liver disease abnormalities in macrophage function and cytokines, Am. J. Physiol. Gastroint. Liver Physiol. 282 (2002) G1–G5.
[17] J.K. Lin, S.Y. Lin-Shiau, Mechanisms of hypolipidemic and anti-obesity effects of tea and tea polyphenols, Mol. Nutr. Food Res. 50 (2006) 211–217.
[18] M.A. Vermeer, T.P.J. Mulder, H.O.F. Molhuizen, Theaflavins from black tea, especially theaflavin-3-gallate, reduce the incorporation of cholesterol into mixed micelles, J. Agric. Food Chem. 56 (2008) 12031–12036.
[19] C.L. Lin, H.C. Huang, J.K. Lin, Theaflavins attenuate hepatic lipid accumulation through activating AMPK in human HepG2 cells, J. Lipid Res. 48 (2007) 2334–2343.
[20] K. Teramoto, J.L. Bowers, J.B. Kruskal, M.E. Clouse, Hepatic microcirculatory changes after reperfusion in fatty and normal liver-transplantation in the rat, Transplantation 56 (1993) 1076–1082.
[21] Z.X. Zhang, K. Shek, S.A. Wang, X.Y. Huang, A. Lau, Z.Q. Yin, H.T. Sun, W.H. Liu, B. Garcia, S. Rittling, A.M. Jevnikar, Osteopontin expressed in tubular epithelial cells regulates NK cell-mediated kidney ischemia reperfusion injury, J. Immunol. 185 (2010) 967–973.
[22] R. Thieringer, J.E. Fenyk-Melody, C.B. Le Grand, B.A. Shelton, P.A. Detmers, E.P. Somers, L. Carbin, D.E. Moller, S.D. Wright, J. Berger, Activation of peroxisome proliferator-activated receptor gamma does not inhibit IL-6 or TNF-alpha responses of macrophages to lipopolysaccharide in vitro or in vivo, J. Immunol. 164 (2000) 1046–1054.
[23] G. Hasko, C. Szabo, Z.H. Nemeth, V. Kvetan, S.M. Pastores, E.S. Vizi, Adenosine receptor agonists differentially regulate IL-10, TNF-alpha and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice, J. Immunol. 157 (1996) 4634–4640.
[24] R. Aneja, K. Odoms, A.G. Denenberg, H.R. Wong, Theaflavin, a black tea extract, is a novel anti-inflammatory compound, Crit. Care Med. 32 (2004) 2097–2103.
[25] M. Kataoka, H. Shimizu, N. Mitsuhashi, M. Ohtsuka, Y. Wakabayashi, H. Ito, F. Kimura, K. Nakagawa, H. Yoshidome, Y. Shimizu, M. Miyazaki, Effect of cold-ischemia time on C–X–C chemokine expression and neutrophil accumulation in the graft liver after orthotopic liver transplantation in rats, Transplantation 73 (2002) 1730–1735.
[26] G. Serviddio, F. Bellanti, R. Tamborra, T. Rollo, N. Capitanio, A.D. Romano, J. Sastre, G. Vendemiale, E. Altomare, Uncoupling protein-2 (UCP2) induces mitochondrial proton leak and increases susceptibility of non-alcoholic steatohepatitis (NASH) liver to ischaemia–reperfusion injury, Gut 57 (2008) 957–965.
[27] E.S. Baskin-Bey, A. Canbay, S.F. Bronk, N. Werneburg, M.E. Guicciardi, S.L. Nyberg, G.J. Gores, Cathepsin B inactivation attenuates hepatocyte apoptosis and liver damage in steatotic livers after cold ischemia-warm reperfusion injury, Am. J. Physiol. Gastroint. Liver Physiol. 288 (2005) G396–G402.
[28] J.B. Lu, C.T. Ho, G. Ghai, K.Y. Chen, Differential effects of theaflavin monogallates on cell growth, apoptosis, and Cox-2 gene expression in cancerous versus normal cells, Cancer Res. 60 (2000) 6465–6471.
[29] S. Mandel, L. Reznichenko, T. Amit, M.B.H. Youdim, Green tea polyphenol (−)-epigallocatechin-3-gallate protects rat PC12 cells from apoptosis induced by serum withdrawal, Neurotox. Res. 5 (2003) 419–424.
[30] H. Kamata, S. Honda, S. Maeda, L.F. Chang, H. Hirata, M. Karin, Reactive oxygen species promote TNF alpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases, Cell 120 (2005) 649–661.