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Epidermal growth factor improved alcohol-induced inflammation in rats

Ya-Ling Chen, Hsiang-Chi Peng, Yi-Ching Hsieh, Suh-Ching Yang*

School of Nutrition and Health Sciences, Taipei Medical University, 250 Wu-Hsing Street, Taipei 110, Taiwan, ROC

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ABSTRACT

The purpose of this study was to investigate the effects of an epidermal growth factor (EGF) intervention on improving the inflammatory response of rats fed an ethanol-containing diet. Eight-week-old male Wistar rats were divided into ethanol (E) and control (C) groups. Rats in the E group were fed an ethanol liquid diet, while rats in the C group were pair-fed an isoenergetic diet without ethanol. After a 4-week ethanol-induction period, both the C and E group were respectively subdivided into 2 groups: a normal liquid diet without (C group, $n = 8$) or with EGF supplementation (C + EGF, $n = 8$), and the ethanol-containing diet without (E group, $n = 8$) or with EGF supplementation (E + EGF group, $n = 8$). The EGF (30 μg/kg body weight/day) intervention period was carried out for the following 8 weeks. At the end of the experiment, activity of aspartate transaminase (AST) and alanine transaminase (ALT) and hepatic levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-10 in group E were significantly higher than those in group C. In addition, alterations in the gut microbiota profile were found in group E. In contrast, activity of AST and ALT and levels of TNF-α, IL-1β, and IL-6 in group E + EGF were significantly lower than those in group E. Significantly lower intestinal permeability and lower numbers of *Escherichia coli* in the fecal microbial culture were also found in group E + EGF. These results suggest that EGF improved the intestinal integrity by decreasing *E. coli* colonization and lowering intestinal permeability, which then ameliorated the inflammatory response under chronic ethanol exposure.

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Introduction

Chronic alcohol consumption triggers complex pathophysiologic mechanisms that lead to alcoholic liver diseases (ALD); however, significant liver disease only occurs in a subset (30%) of alcoholics (Gramenzi et al., 2006). It is believed that gut-derived endotoxin is important for ALD development, since antibiotics prevent ALD by removing the gut flora (Criado-Jiménez, Rivas-Cabañero, Martín-Oterino, López-Novoa, & Sánchez-Rodríguez, 1995; Hunt & Goldin, 1992; McClain & Cohen, 1989). Bode and Bode (2005) found that alcohol leads to a marked increase in the permeability of the gut mucosa, which causes endotoxemia. The resulting endotoxemia then leads to activation of Kupffer cells and other macrophages. Proinflammatory mediators secreted by activated Kupffer cells ultimately cause liver damage. Moreover, Keshavarzian et al. (1999) reported that gut leakiness occurred only in alcoholics with liver injury but not in those without liver disease. Accordingly, maintaining the intestinal integrity is important for ameliorating liver injury in ALD.

Epidermal growth factor (EGF), a potent 53-amino acid cytoprotective peptide, exhibits trophic and healing effects on the intestinal mucosa (Playford & Wright, 1996). EGF is produced by submaxillary salivary glands, duodenal Brunner's glands, and mammary glands (Heitz et al., 1978; Kasselberg, Orth, Gray, & Stahlman, 1985; Poulsen, Nexø, Olsen, Hess, & Kirkegaard, 1986), and its biological action is mediated via binding to the EGF receptor (EGF-R). Previous studies showed that EGF provided protection against lipid-induced mucosal injury in rats (Ishikawa, Cepinskas, Specian, Itoh, & Kvietys, 1994). In addition, EGF reduced the development of necrotizing enterocolitis in a neonatal rat model (Dvorak et al., 2002). In clinical trials, EGF not only contributed to the *in vivo* repair of peptic ulcer disease (Zandomeneghi, Serra, Baumgartl, & Poppi, 1991), but was also considered a kind of useful and safe anti-ulcer drug (Itoh & Matsuo, 1994). Although a few studies have reported that EGF has a protective function against acetaldehyde in human colonic mucosa cells (Sheth, Seth, Thangavel, Basuroy, & Rao, 2004; Suzuki, Seth, & Rao, 2008), there is limited evidence about how it works in ALD *in vivo*. Therefore, we investigated whether EGF

Funding source: National Science Council of Taiwan.
* Corresponding author. Tel.: +886 2 2736 1661x6553; fax: +886 2 2737 3112.
E-mail address: sokei@tmu.edu.tw (S.-C. Yang).

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Table 1
Initial body weight (BW), final BW, and relative liver weight of rats in each group.[a]

Group	Initial BW (g)	Final BW (g)	Relative liver weight ([g/BW] × 100)
C	311 ± 10	459 ± 22	1.9 ± 0.1
C + EGF	310 ± 9	450 ± 26	2.0 ± 0.1
E	311 ± 6	426 ± 37[b]	2.6 ± 0.2[b,c]
E + EGF	311 ± 7	408 ± 23[b,c]	2.2 ± 0.1[d]

C, control group; C + EGF, control diet with epidermal growth factor (EGF) supplementation (30 μg/kg/day); E, ethanol group; E + EGF, ethanol feeding with EGF supplementation (30 μg/kg/day).
[a] Data are the mean ± SD for $n = 8$.
[b] Significant difference ($p < 0.05$) vs. C group.
[c] Significant difference ($p < 0.05$) vs. C + EGF group.
[d] Significant difference ($p < 0.05$) vs. E group.

supplementation can ameliorate ALD in rats by improving intestinal integrity and inflammation.

Methods and materials

Animals

Eight-week-old male Wistar rats (BioLasco Taiwan, Ilan, Taiwan) weighing about 280 g were used in this experiment. All rats were housed in individual stainless-steel cages in an animal room maintained at 22 ± 2 °C with ~50–70% humidity and a 12-h light/dark cycle. They were allowed free access to a standard rodent diet (LabDiet 5001 Rodent Diet; PMI Nutrition International, St. Louis, MO, USA) and water during acclimation before the study. All procedures were approved by the Institutional Animal Care and Use Committee of Taipei Medical University.

Study protocol

After 1 week of acclimation, rats were divided into 2 groups, an ethanol (E) group ($n = 16$) and a control (C) group ($n = 16$). The plasma aspartate transaminase (AST) and alanine transaminase (ALT) activity levels were measured before the first week of the experiment, and then rats were assigned to groups based on their level of plasma AST and ALT activity to be sure there would be no significant differences between groups in their liver function at the beginning of the experiment. Rats in group E were fed an ethanol-containing liquid diet, while rats in group C were pair-fed an isoenergetic diet without ethanol. After an ethanol-induction period (the 1st to 4th week of the experiment), both groups C and E were respectively subdivided into 2 groups: a normal liquid diet without (C group, $n = 8$) or with EGF supplementation (C + EGF, $n = 8$), and the ethanol-containing diet without (E group, $n = 8$) or with EGF supplementation (E + EGF group, $n = 8$). The EGF (30 μg/kg body weight/day) intervention period was carried out for the following 8 weeks (the 5th to 12th weeks of the experiment). Recombinant human (rh) EGF, purchased from Apeloa® (Shandong, China), was mixed in the liquid diet during the intervention period. The ethanol liquid diet in this study, which provided 35% of its energy through ethanol, was modified from Lieber and DeCarli's (Lieber & DeCarli, 1994) ethanol liquid diet, while pair-fed control rats (C and C + EGF group) received an equal amount of calories as their alcohol-fed counterparts (E and E + EGF) by substituting the alcohol-derived calories with maltodextrin. A pair-feeding procedure was conducted in this study, that is, the amounts of liquid diet consumed by rats of group E were measured, and then the equivalent amount of diet was provided to rats of the other 3 groups on the next day.

After conducting the intestinal permeability test and microbial culture of feces at the end of the study, all rats were sacrificed at the end of the 12th week of the experiment. Blood samples were collected in heparin-containing tubes and centrifuged (1200 × *g* for 15 min at 4 °C) to obtain plasma samples. All plasma samples were stored at −80 °C until being assayed. Liver tissues were rapidly excised and stored at −80 °C for further analysis.

Measurements and analytical procedures

Liver function

Plasma AST and ALT activity was measured with a Beckman Synchron LXTM system (GMI, Ramsey, MN, USA) at 340 nm.

Inflammatory response

Hepatic supernatants were prepared by the method of Rüdiger and Clavien (2002), and supernatants were collected for subsequent analysis. Hepatic cytokine concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits. Tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-10 concentrations were respectively determined with a rat TNF-α/TNFSF1A kit (R&D Systems, Minneapolis, MN, USA), a rat IL-1β/IL-1F2 kit (R&D Systems), a rat IL-6 kit (R&D Systems), and a rat IL-10 kit (R&D Systems). The optical density was read at 450 nm for all cytokines using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Histopathological analysis

Liver tissues were fixed in 10% buffered formaldehyde and processed for the histological examination with hematoxylin-eosin (H&E) stain, silver stain, and Masson stain. H&E stain was used to evaluate chronic liver damage including gross hepatocyte necrosis, fatty change, and fibrosis. Masson stain was for collagenous fibers, and silver stain was for reticular fibers. A semiquantitative histological evaluation was carried out by a pathologist blinded to the treatment groups to assess the degree of fatty change, inflammation, and fibrosis. The grading of H&E stain ranged from 0 to 4 where 0 = absent, 1 = trace, 2 = mild, 3 = moderate, and 4 = severe. The scale for semiquantitation of hepatic tissue fibrosis (Masson

Table 2
Effects of epidermal growth factor (EGF) on plasma AST and ALT activity in rats under long-term ethanol feeding.[a]

Group	Initial AST activity (U/L)	Initial ALT activity (U/L)	Final AST activity (U/L)	Final ALT activity (U/L)	Change of AST activity (U/L)	Change of ALT activity (U/L)
C	81.6 ± 6.8	38.3 ± 5.9	67.6 ± 6.8	23.6 ± 3.5	−14.0 ± 8.8	−14.6 ± 4.1
C + EGF	85.5 ± 6.1	39.3 ± 5.2	61.9 ± 5.8	22.4 ± 3.5	−23.6 ± 8.7	−16.9 ± 7.3
E	80.4 ± 11.6	38.3 ± 5.8	100.6 ± 13.6[b,c]	50.5 ± 11.3[b,c]	20.3 ± 16.7[b,c]	12.3 ± 14.6[b,c]
E + EGF	85.1 ± 16.5	37.1 ± 5.2	81.9 ± 12.0[b,c,d]	38.6 ± 9.8[b,c,d]	−3.3 ± 21.2[c,d]	1.5 ± 10.9[b,c,d]

AST, plasma aspartate transaminase; ALT, alanine transaminase; C, control group; C + EGF, control diet with EGF supplementation (30 μg/kg/day); E, ethanol group; E + EGF, ethanol feeding with EGF supplementation (30 μg/kg/day).
[a] Data are the mean ± SD for $n = 8$.
[b] Significant difference ($p < 0.05$) vs. C group.
[c] Significant difference ($p < 0.05$) vs. C + EGF group.
[d] Significant difference ($p < 0.05$) vs. E group.

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