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DHA attenuates hepatic ischemia reperfusion injury by inhibiting pyroptosis and activating PI3K/Akt pathway

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ABSTRACT

Hepatic ischemia reperfusion (I/R) injury is very common in liver transplantation and major liver surgeries and may cause liver failure or even death. Docosahexaenoic acid (DHA) has displayed activities in reducing oxidative stress and inflammatory reaction in many disorders. In the present study, we investigated the protective effects of DHA against I/R-induced injury and the underlying mechanisms. Here, we show that DHA protected hepatic I/R injury by reducing aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels and decreasing the oxidative stress in liver tissues. The viability of Buffalo rat liver (BRL) cells was reduced by hypoxia/restoration (H/R) but restored by DHA. DHA significantly downregulated the expression of pyroptosis-related proteins including NLR pyrin domain containing 3 (NLRP3), apoptotic speck-like protein containing CARD (ASC) and cleaved caspase-1 and reduced the secretion of pro-inflammatory cytokines. The above results were supported by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. However, incubation with LY294002, a specific inhibitor of phosphatidylinositol-3-kinase (PI3K), abolished the effects of DHA, since it increased the expression of cleaved caspase-1 and the production of inflammatory cytokines. The present results have demonstrated that DHA ameliorated I/R-induced injury by inhibiting pyroptosis of hepatocytes induced in liver I/R injury *in vivo* and *in vitro* through the PI3K/Akt pathway, providing a potential therapeutic option to prevent liver injury by I/R.

1. Introduction

Hepatic (I/R) is very common in liver transplantation and major liver surgeries. It causes the injury to livers, liver failure, or even death. The main mechanisms contributing to the pathophysiology of hepatic (I/R)-induced injury include induction of reactive oxygen species, increased inflammatory responses and production of cytokines, and activation of apoptosis (Malhi and Gores, 2008; Vardanian et al., 2008; Haga et al., 2009). Hepatic I/R causes liver microcirculation disturbance, which induces the production of endogenous reactive oxygen species, and in turn recruits innate immune cells into the liver (Sosa et al., 2016). When they are activated, innate immune cells, particularly neutrophils and macrophages, produce inflammatory cytokines and aggravate inflammatory response, leading to the death of hepatocytes (Yu et al., 2016).

Pyroptosis is a highly inflammatory form of non-apoptotic and caspase-1-dependent programmed cell death (Gaidt and Hornung, 2016). Pyroptosis is regarded as a general and natural immune effector mechanism in vertebrates (Jorgensen and Miao, 2015), and contributes to inflammatory responses in bacterial infection and many non-infectious diseases including various types of liver injury (Miao et al., 2011; Geng et al., 2015; Chen et al., 2016). Pyroptosis is characterized by cellular extensive plasma-membrane swelling and rupture in a caspse-1-dependent way, distinct from apoptosis (Fernandes-Alnemri et al., 2007; Bergsbaken et al., 2009; Zha et al., 2016). DNA damage taking place in pyroptotic cells can also be positively stained by TUNEL (Fink and Cookson, 2006). Activation of caspase-1 is the feature of pyroptosis, and inflammasomes are responsible for this activation, which lead to the maturation of cytokines IL-1β and IL-18 (Fernandes-Alnemri et al., 2007; Lamkanfi and Dixit, 2014). NLRP3 inflammasome

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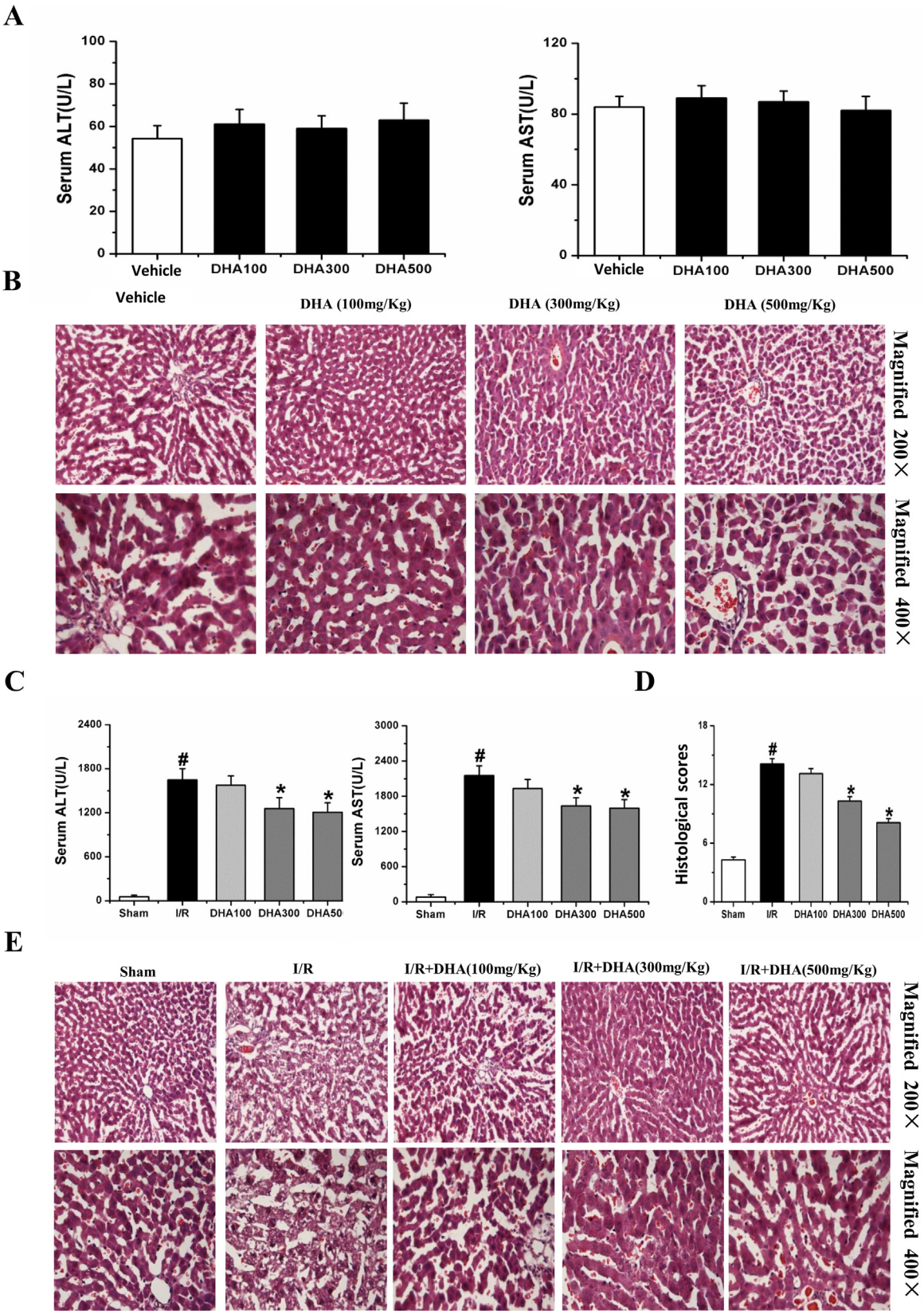

Fig. 1. DHA inhibits hepatic injury induced by ischemia reperfusion in rats. (A, B) Sham-operated rats were administered with vehicle or different doses of DHA as indicated. (C-E) Rats underwent ischemia reperfusion (I/R) and were administered with vehicle or different doses of DHA as indicated. Sham-operated rats served as controls. (A, C) The levels of ALT and AST in sera were measured. (B, E) Representative images were from HE-stained liver sections examined under microscopy (Upper panel: 200 × magnification; lower panel: 400 × magnification). (D) Histological scores were quantified from (E). # ($P < 0.05$) indicates significant difference from the sham group; and *($P < 0.05$), from the I/R group.