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Short communication

Suppressive effect of kamebakaurin on acetaminophen-induced hepatotoxicity by inhibiting lipid peroxidation and inflammatory response in mice

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ABSTRACT

Background: Kamebakaurin (KA) is an *ent*-kaurane diterpenoid known to have anti-inflammatory potential. In the current study, we investigated whether pretreatment with KA could ameliorate acetaminophen (APAP)-induced hepatotoxicity by inhibiting the anti-inflammatory response in mice.
Methods: Seven-week-old C57BL/6J mice were orally administered KA or olive oil emulsion for seven days. Twenty-four hours after the last KA or olive oil administration, the mice were intraperitoneally injected with 400 mg/kg APAP or saline under feed deprived condition. The mice from each group were euthanized and bled for plasma analysis 24 h after the injection.
Result: APAP increased plasma levels of hepatic injury markers (*i.e.*, alanine aminotransferase and aspartate aminotransferase), lipid peroxidation, and pro-inflammatory cytokines. Pretreatment with KA reduced the magnitude of APAP-induced increases in plasma levels of hepatic injury markers, lipid peroxidation, and inflammatory response. In addition, KA exhibited antioxidant capacity in a dose-dependent manner, with slight reactive oxygen species scavenging activity.
Conclusion: Our results indicate that KA has the ability to protect the liver from APAP-induced hepatotoxicity, presumably by both inhibiting the inflammatory response and oxidative stress.

Introduction

Acetaminophen (APAP) is a safe and effective analgesic at therapeutic doses. However, an overdose of APAP can cause severe hepatic injury in humans and animals [1]. About 90% of a therapeutic dose of APAP is conjugated through glucuronidation or sulfation, and then excreted into urine and bile. The other (only 5–10%) is oxidized by cytochrome P450s (CYPs) to form *N*-acetyl-*p*-benzoquinone imine (NAPQI: a causal substance for APAP-induced hepatotoxicity). In mice, phase I biotransformation is mostly limited to CYP1a2 and CYP2e1 [2]. CYP2e1 is the main bio-activator when APAP administration is at low doses. When high concentrations of APAP are present, CYP1a2 is also contributed. At a therapeutic dose, reduced glutathione (GSH) inactivated by conjugation with NAPQI and formed a 3-*S*-glutathionyl conjugate of APAP. In contrast, in a case of overdose, glucuronidation and sulfation pathways become saturated, and more NAPQI is formed, resulting in rapid depletion of hepatic GSH. Once GSH is depleted, excess NAPQI binds to cellular proteins and initiates lipid peroxidation, leading to hepatic injury [3].

Currently, the best therapeutic option to prevent APAP-induced hepatic injury is injection of *N*-acetylcysteine (NAC). NAC effectively minimizes APAP-induced toxicity when injected within a short time after APAP intoxication [4]. However, clinical studies have revealed that injection of NAC often causes untoward side effects [5]. Therefore, a niche exists for protective compounds and/or chemicals that offer maximum protection against APAP-induced hepatotoxicity without NAC's untoward side effects. Nowadays, the search for new drugs and novel therapeutic intervention strategies increasingly includes testing plant extracts and other natural

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products. It is generally accepted that natural products have multiple functions such as anti-cancer and anti-oxidative. Hence, there is a critical and urgent need to explore the therapeutic potential of natural products and other compounds for prevention and treatment of hepatotoxicity.

Kamebakaurin (KA) is an *ent*-kaurane diterpenoid that was isolated from *Rabdosia excisa*. KA has been used in Asian folk medicine to treat arthralgia and fever [6]. Several investigations have indicated that KA inhibits lipopolysaccharide-induced production of nitric oxide and prostaglandin E2 (PGE2) by preventing nuclear factor-kappa B signaling *in vitro* [7,8]. In addition, Lee et al. showed that KA prevents neutrophil recruitment and PGE2 and tumor necrosis factor alpha (TNFα) production in an air-pouch animal model [9]. Since APAP-induced hepatotoxicity is frequently associated with infiltration of inflammatory factors, we hypothesized that KA might inhibit APAP-induced hepatotoxicity by suppressing the inflammatory response.

Therefore, in the present study, we investigated whether pretreatment with KA would be sufficient to attenuate APAP-induced hepatotoxicity *in vivo* model.

Material and methods

Animal treatment

Six-week-old female C57BL/6J mice were purchased from CLEA Japan (Tokyo, Japan). Following arrival at our facility, mice were maintained under standard conditions of controlled temperature (24 ± 1 °C), humidity (55 ± 5%), and light (12:12-h light/dark cycles) with free access to water and food. Experimental treatments were used seven-week-old animals. Following the experiment, any surviving mice were sacrificed using pentobarbital. These experiments were approved by the Institutional Animal Care and Experiment Committee of our institution (No. 126).

Preparation of KA

KA was isolated from *Rabdosia excisa* as previously reported [6]. An ethanol solution was employed to the following assay (100 mg/mL).

Experimental protocol

Mice were divided into four groups of six mice, respectively. Two groups were orally pretreated for seven days once per day with 100 mg/kg KA (10 mL/kg body weight) in an olive oil emulsion and the other groups were orally administered olive oil emulsion as a negative control. Before APAP injection, mice from each group were fasted for 16-h. Then, one KA-treated group and one negative control group of mice received 400 mg/kg APAP (10 mL/kg body weight) intraperitoneally in a saline and polyethylene glycol emulsion. The other two groups received saline and polyethylene glycol emulsion as a control (10 mL/kg body weight). Twenty-four hours post administration, mice were euthanized and bled to obtain plasma. The resulting plasma and liver samples were stored at −80 °C.

Plasma biochemical analysis

Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using the Transaminase CII kit (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's instructions, as previously described [10]. Plasma TNFα levels were determined using commercially available ELISA kits (eBioscience, San Diego, CA, USA), according to the manufacturer's instructions.

Measurement of malondialdehyde (MDA) levels and total GSH in the liver

Total MDA levels in the liver examined with a colorimetric thiobarbituric acid reactive substances (TBARS) microplate assay kit (FR40, Oxford Biochemical Research, Oxford, MI, USA) according to the manufacturer's protocol and as previously described [11]. Hepatic GSH levels measured using a GSSG/GSH quantification kit (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions and as previously described [12].

Isolation of total RNA and qRT-PCR assay

Total RNA were extracted from liver sections using ISOGEN II (Nippon Gene, Tokyo, Japan). qRT-PCR was performed with One Step SYBR PrimeScript PLUS RT-PCR kit (Perfect Real Time) (Takara Bio, Shiga, Japan) using an Applied Biosystems 7300 (Applied Biosystems, Foster City, CA). PCR condition was previously described [11]. Gene expression was normalized to *GAPDH* mRNA levels. The oligonucleotide sequences of the primers were as follows: sense, 5′-TGGTGAAGGTCGGTGTGAAC-3′, and antisense, 5′-GTCGTTGATGGCAACAATCTCC-3′ for the mouse GAPDH (NM_001289726); sense, 5′- GAACTTCGGGGTGATCGGTC-3′, and antisense, 5′- GTGAGGGTCTGGGCCATAG-3′ for the mouse TNFα (NM_013693); sense, 5′-GAAATGATGGATGCTACCAAACTG-3′, and antisense, 5′-TACTCCAGGTAGCTATGGTACTC-3′ for the mouse IL-6 (NM_031168); sense, 5′-CATTCCTGTGTTCCAGGAGTACAAG-3′, and antisense, 5′-GATACTTAGGGAAAACCTCCGCAC-3′ for the mouse CYP2e1 (NM_021282); sense, 5′-GACACCTCACTGAATGGCTTC-3′,

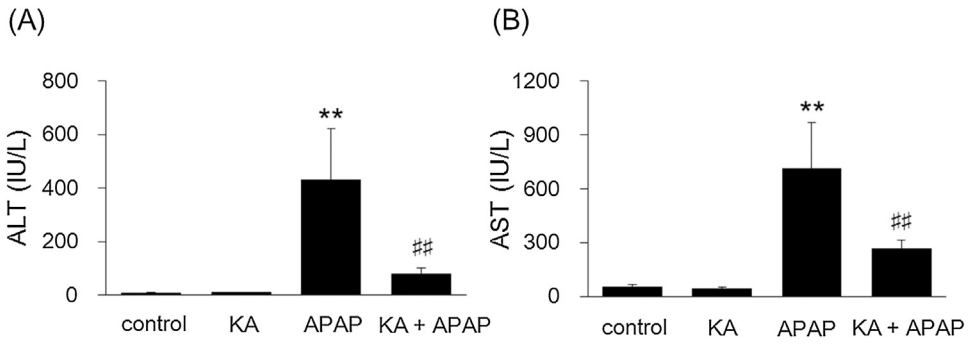

Fig. 1. Effect of pretreatment with KA on levels of hepatic injury markers. Mice received KA or vehicle (olive oil) by oral gavage once daily for seven days. Before APAP treatment on day seven, mice were fasted for 16-h. Mice were injected intraperitoneally with 400 mg/kg APAP. Plasma levels of hepatic injury markers were determined 24-h after administration of APAP. Panels (A) and (B) indicate ALT and AST, respectively. Data are plotted as mean ± SD for groups of six-mice each. $^{**}p < 0.01$ *versus (vs.)* control group, and $^{\#\#}$ $p < 0.01$ *vs.* APAP group.