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Analytical Methods

The influence of durian (*Durio zibethinus* Murray cv. Monthong) on conditioned taste aversion to ethanol

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ABSTRACT

The adverse reaction to 1.25 g/kg ethanol was monitored in male Fischer rats given durian or cabbage (2.4 g FW/100 g BW/day), administered intragastrically. During the first ethanol challenge, a reduced rate of blood acetaldehyde clearance and hypothermia, which is associated with the disulfiram-ethanol reaction, was observed in rats given durian or cabbage. Blood ethanol levels and rate of acetaldehyde elimination were lowest 30 min after the first ethanol challenge in rats given cabbage, while a similar but more exacerbated trend was observed at 60 min in rats given durian. When subjected to conditioned taste aversion using saccharine solution (0.2% v/v) paired with ethanol administration, the rats given durian or cabbage exhibited aversion, with the former showing the earliest and most pronounced response, persisting through to the last ethanol challenge. Rats given cabbage exhibited delayed aversion, which progressively increased to the same level as that observed in rats given durian.

1. Introduction

Robust scientific evidence promotes increased fruit and vegetable consumption combined with moderate ethanol intake on account of its pro-health effects (Tunstall-Pedoe et al., 1999). The Mediterranean diet, that includes high fruit and vegetable ingestion, followed by moderate alcohol drinking has been admonished for its beneficial role in increasing longevity and in lowering the incidence of cardiovascular disease (Buckland et al., 2009; Keys et al., 1986; Trichopoulou et al., 2005). However, unsafe interactions with alcohol of certain fresh produce may negate the health benefits of this admonished combination.

Durian (*Durio zibethinus* Murray) has had a long history of safe use as food, and novel findings on its bioactive constituents point to its functionality in disease-preventive diets (Haruenkit et al., 2010; Leontowicz et al., 2008; Mahattanatawee et al., 2006; Verhoeven, Verhagen, Goldbohm, van den Brandnt, & van Poppel, 1997). However, anecdotal accounts that persist in durian-producing and -consuming countries warn against the adverse reaction of alcohol ingestion with the fruit. The unpleasant, or at times lethal, effect of drinking alcohol with durian is reminiscent of the clinical manifestations seen in patients that binge on alcohol while under antidipsotropic medication with disulfiram (tetraethylthioram disulphide), a sulphur-containing drug. Disulfiram is known to inhibit aldehyde dehydrogenase (ALDH), causing the accumulation of ethanol-derived acetaldehyde (Hart & Faiman, 1992). Rather than ethanol itself, acetaldehyde appears to have a central role in mediating the adverse reactions referred to as the disulfiram-ethanol reaction or DER (Adinoff, Iranmanesh, Veldhuis, & Fisher, 1998; Brien & Loomis, 1985; Duranceaux et al., 2006; IARC, 1999; Tottmar & Hellstrom, 1979). The DER turns the penchant for alcohol drinking into aversive distaste. Excessive blood acetaldehyde level has been implicated in a myriad of serious health conditions ranging from vasopastic anginal attack induction (Seki et al., 1999) to cytotoxic, immunotoxic and carcinogenic consequences (IARC, 1999).

In mammalian models, conditioned taste aversion (CTA) has been proven as the most reliable and sensitive behavioural index of illness-taste associations (Davis & Riley, 2010; Freeman & Riley, 2009; Pautassi, Nizhnikov, & Spear, 2009). By pairing the consumption of a distinct taste (conditioned stimulus, CS) with the administration of ethanol, subsequent avoidance of the CS serves as a behavioural cue of an ill postingestive effect caused by the drug (unpaired stimulus, US). Amongst the mechanisms that likely underlie the aversive effect of the DER, hypothermia has received

Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; DER, disulfiram-ethanol reaction; CTA, conditioned taste aversion; PEITC, phenethyl isothiocyanate.

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compelling empirical support (Cunningham, Niehus, & Bachtold, 1992; Jensen & Faiman, 1986).

We have previously reported, from *in vitro* studies, that the sulphur constituents in the durian fruit inhibit ALDH, which may explain the unpleasant durian–ethanol interaction (Maninang, Lizada, & Gemma, 2009). Sulphur-containing compounds from natural products have already been previously implicated in giving rise to DER-like symptoms. For instance, phenethyl isothiocyanate (PEITC), that naturally abounds in cruciferous vegetables, e.g. cabbage, has been reported to potently inhibit ALDH *in vivo* (Lindros, Badger, Ronis, Ingelman-Sundberg, & Koivusalo, 1995). However, evidence that links concurrent administration of ethanol and durian or cabbage with DER-like effects has yet to be presented. Using the biobehavioural manifestations associated with the DER (namely acetaldehydemia, hypothermia and aversion), we hereby demonstrate that durian elicits unsafe combination with ethanol *in vivo*.

2. Materials and methods

2.1. Materials

Table-ripe durian cv. Monthong was imported from Thailand (Phol Intergrower Co. Ltd, Bangkok, Thailand); cabbage was sourced from a local supermarket in Tsukuba City, Japan.

All reagents of analytical grade and purity were purchased from Sigma–Aldrich (Tokyo, Japan) unless specified.

2.2. Durian and cabbage extraction

For *in vitro* ALDH inhibition assay, cabbage and durian homogenates (80% w/v) were extracted of juice using cheesecloth. The filtrate was centrifuged first at 600*g* and the supernatant was again centrifuged at 2000*g*. The resulting supernatants were used for the assay of the rat liver mitochondrial fraction. All steps of the extraction procedure were done at 4 °C.

2.3. Subjects

A total of 23 alcohol-naïve [28 days, postnatal (PN)] male Fischer rats (SPF/VAF F344/DuCrlCrlj) with average weights of 50 ± 2 g, obtained from Charles River Japan, Inc. (Tsukuba City, Japan), were individually housed in polycarbonate cages covered with metal grills. The temperature- and humidity-controlled room (25 °C, 70% RH) operated on 12 h light/dark cycles (lights on at 0600 h).

2.4. Conditioned taste aversion (CTA) protocol

2.4.1. General

The study design was approved by the Experimental Animal Care Committee of the University of Tsukuba, Japan. Animal care and procedures were also in observance of the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Fischer rats were subjected to a single-bottle CTA protocol modified from previous studies (Camarini & Hodge, 2004; Roma, Flint, Higley, & Riley, 2006) with three stages: initiation, pre-conditioning and conditioning. The subjects were acclimatised for 36 h from arrival to day 1 with *ad libitum* access to MF diet (Oriental Yeast Co., Ltd, Tokyo, Japan) and tap water presented in 100 ml touch-and-drink type plastic bottles. Fig. 1 shows the body weight and MF consumption profile of the subjects at each stage of the protocol that were used for caloric intake computation. The type of drinking liquid provided and access period at each stage are detailed below, while MF was *ad libitum* throughout.

2.4.2. Treatments

The subjects were quasirandomly assigned to treatment groups (Table 1) with MF diet (Oriental Bio-Service Kanto, Inc., Tsukuba City, Japan) as the base food. Homogenates were freshly prepared (1 h prior to administration) using a Polytron PTMR 2100 Homogenizer (Kinematica AG, Switzerland) such that 1 ml contained 0.8 g durian pulp in distiled water or 0.8 g cabbage leaves in 1% carboxymethylcellulose filtered through double-layer cheesecloth. Once-a-day supplementation (between 0600–0800 h) commenced 36 h after acclimatisation 30 days PN at 3 ml/100 g BW. The rats were allowed to taste the treatments for familiarisation, but bulk were administered intragastrically (i.g.) via a ø1.7 × L 90 mm flexible catheter (Oriental Bio-Service Kanto, Inc., Tskuba City, Japan).

2.4.3. Initiation

At 31 days PN, the ethanol initiation procedure commenced by offering the 12 h water-deprived subjects five days of non-contingent pre-exposure to 10% (v/v) ethanol in tap water presented in an identical container for 1 h. The volume of intake was recorded and the tap water-containing bottle was restored after a further hour such that each subject was presented with two drinking liquids. Both bottles were placed equidistant from the feed container, daily interchanged at the beginning of light periods to avoid development of place preference, and made accessible for 24 h until 35 days PN. Two bottles similarly treated were also presented to the C group, but both contained tap water. Volume of intake from each bottle was obtained daily from the change in weight every beginning of the light period with careful account of losses due to evaporation (negligible) and drippings.

2.4.4. Pre-conditioning

At 36 days PN, access of the subjects to tap water was restricted to one hour right after supplement administration. The volume of liquid consumption was recorded at the end of the restricted access period, with the drippings collected and accounted for. A 6-day stabilized water consumption and body weight adaptation suggested coping of the subjects with the restricted access and signified the end of pre-conditioning (48 days PN).

2.4.5. Conditioning

At 49 days PN, the first ethanol challenge of the conditioning stage commenced. At each ethanol challenge, saccharin (Wako Pure Chemical Ind., Ltd., Osaka, Japan) solution (0.2% w/v in tap water) was offered as drinking liquid, for 1 h, instead of water. The subjects were then dosed with 1.25 g/kg BW ethanol (20% in saline) *ig* via catheter (Ch1). The next day, tap water was offered as drinking liquid for 1 h, immediately followed by administration of saline solution via catheter. This 2-day regimen constituted one cycle that was repeated five times over 10 days with the fifth cycle having a reversed regimen – tap water-saline solution combination on the first day, and saccharine-ethanol solution combination on the second day. The same drinking bottle was used throughout the experiment, with thorough rinsing, to avoid bottle preference that was observed in the control group during the initiation period.

2.5. Temperature measurement

Core temperatures were obtained by inserting the probe of a digital thermometer (Thermistor D611, Takara Instruments Co., Ltd., Yokohama, Japan) 4 cm deep into the rectum before and at 30, 60, 90 and 120 min after alcohol administration.

2.6. Blood ethanol (BEL) and acetaldehyde levels (BAL)

During the first (Ch1) and last (Ch5) ethanol challenge, blood samples (0.1 ml) were collected before and 30, 60, and 120 min