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Anti-anaphylactic action of nordihydroguaiaretic acid in antigen sensitized guinea pigs

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ABSTRACT

Therapeutic natural products and medicinal herbs has gained popularity. The anti-antigenic action of the plant alkaloid nordihydroguaiaretic acid (NDGA) was studied in ovalbumin (OA)-sensitized guinea pigs. In one series of experiments conscious, non-sedated guinea pigs were challenged with OA aerosol. Specific airway resistance (SR_{AW}) was monitored using a two-chambered whole-body plethysmograph. OA aerosol increased SR_{AW} above that produced by vehicle administration. Prior NDGA administration by a 1 min 0.9% aerosol (w/vol) attenuated the increase in SR_{AW} resulting from OA challenge. In the anesthetized guinea pig pretreated with indomethacin, pyrilamine and propranolol, intravenous OA injection increased intra-tracheal pressure above vehicle injection. Intravenous NDGA administration (5 mg/kg) reduced the intra-tracheal pressure increases. In a third series of experiments plasma leukotriene C_4 was measured by radio-immunoassay in 3 groups challenged with OA aerosol: vehicle-treated OA-sensitized, OA-sensitized receiving NDGA and vehicle treated guinea pigs. NDGA pretreatment reduced plasma LTC_4 in response to OA challenge in OA sensitized guinea pigs. This study demonstrates that NDGA is an effective antigenic agent when given by aerosol or intravenous injection in either conscious or anesthetized guinea pigs, respectively. The mechanism of action of NDGA is presumed primarily be due to the blockage of 5-lipoxygenase and therefore the synthesis of leukotrienes.

1. Introduction

Interest in the therapeutic activity of the plant alkaloid nordihydroguaiaretic acid (NDGA) has increased recently particularly with regards to cancer therapy (Kimura and Huang, 2016; Yarla et al., 2016). In addition NDGA has anti-oxidant properties, blocks lipoxygenase (5-LOX) receptors, antagonizes pulmonary hypertension (Kriska et al., 2012; Shen et al., 2015) and recently has been shown to activate human transient receptor potential cation channel subfamily A, member 1 (hTRPA-1) receptors (Redmond et al., 2014). These actions may be of therapeutic value in attenuating airway hyperresponsiveness.

Airway reactivity in ovalbumin (OA)-sensitized guinea pigs results from the release of bronchoconstrictive substances including histamine, prostaglandins and leukotrienes (Adams and Lichtenstein, 1979; Burka and Paterson, 1981). Anti-histamine and cyclooxygenase inhibitors alone are not effective antigenic agents (Adams and Lichtenstein, 1979; Burka and Peterson, 1981; Dahlen et al., 1983; Hand et al., 1980). Airway responsiveness not attributable to histamine, bradykinin and prostaglandin $F_2\alpha$ after the immediate phase of bronchoconstriction are block by antagonism of leukotrienes (Hand et al., 1984, 1986).

This later phase of bronchoconstriction response is mediated at least in part by leukotrienes (Bach, 1982; Chand and Eyre, 1978; Hand et al., 1980). The leukotrienes are synthesized from arachidonic acid by a complex of enzymes including 5-LOX (Piper, 1984).

The contribution of leukotrienes to asthma and anaphylaxis is substantial. Certain asthmatic individuals receive benefit from either blocking lipoxygenase or through blockade of leukotriene receptors. Leukotriene antagonism is effective in the treatment of exercise-induced bronchoconstriction (Philteos et al., 2005).

Lipoxygenases such as 5-LOX catalyze leukotriene formation (Chen and Funk, 2001; Peters-Golden and Brock, 2001). Upon activation 5-LOX transforms arachidonic acid to leukotriene A_4 (LTA_4). LTA_4 undergoes transformation to produce bioactive eicosanoids LTB_4, C_4, D_4 and E_4 (Nair and Funk, 2009).

Abbreviations: LTC_4, leukotriene C_4; 5-LOX, 5 lipoxygenase; NDGA, nordihydroguaiaretic acid; OA, ovalbumin; P_{Tr}, intra-tracheal pressure; SR_{AW}, specific resistance of the airways.

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NDGA is a plant alkaloid having clinical potential as a lipoxygenase inhibitor and is derived from the leaves of Larrea divaricate (Valentine et al., 1984). NDGA reduces antigen induced contraction of guinea pig tracheal strips (Armour et al., 1981; Franchi-Micheli et al., 1986; Hand et al., 1986) and also intra-tracheal pressure (P_{Tr}) in anesthetized guinea pigs (Anderson et al., 1983). One mechanism of action of NDGA is to block the conversion of arachidonic acid to leukotrienes products by inhibiting 5-LOX (Kelley et al., 1979). Although this action has been recognized for some time, there is a renewed interest in NDGA clinical usage (Lü et al., 2010). We hypothesize that NDGA has anti-antigenic action during OA-induce anaphylaxis by either aerosol or intravenous administration.

2. Methods

2.1. Animals

Hartley strain male guinea pigs approximately three months of age weighing from 645 to 740 g were purchased from a commercial vendor (Charles River). The animals were housed in the Creighton University animal resource facility with food and water ad libitum. Guinea pigs were treated as recommended by the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, Office of Laboratory Animal Welfare.

2.2. Guinea pig sensitization

Guinea pigs were sensitized to OA in accordance with the methods used by Stewart et al. (1984). Each guinea pig was injected intraperitoneally with 1000 mg/kg OA (Sigma) dissolved in 1 ml saline with an equal volume of Freund's complete adjuvant (Difco Labs). Two days thereafter, a second injection of OA (50 mg/kg) dissolve in 0.1 ml saline was administered subcutaneously. The guinea pigs were challenged with OA antigen 16–17 days after the original OA injection.

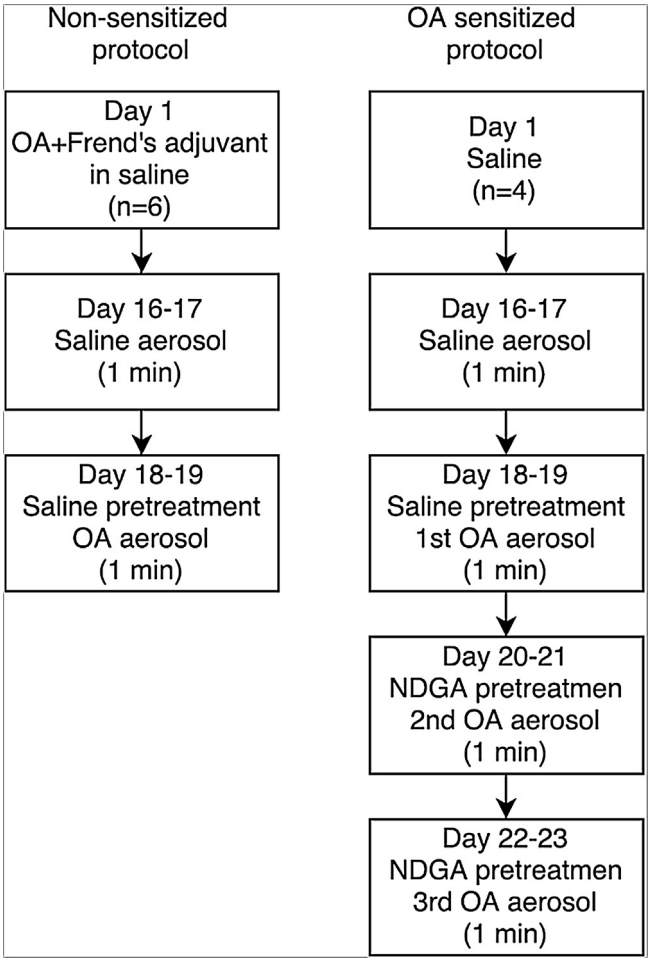

Fig 1. Time line flow chart of first experimental series using whole body plethysmography. Series 1 protocol flow chart.

2.3. Determination of SR_{AW} in conscious guinea pigs

The guinea pigs were placed in a whole-body plethysmograph patterned after that developed by Agrawal (1981). The plethysmograph was composed of two chambers (see Bergren, 1988 for details). An inner chamber housed the guinea pig. The inner chamber is comprised of two cylinders. The inner cylinder housed the guinea pig and held it with a neck restraint which was sealed with latex and modeling clay. The outer cylinder then was placed over the cephalic end of the inner cylinder. Therefore, airflow was directed solely through the nose piece of the outer cylinder. A pneumotachograph (Fleish, Model 000) was placed in the nose piece to measure airflow. The pneumotachograph was attached to a differential pressure transducer (Statham PM15E).

The plethysmograph was then closed and sealed. Box pressure was sensed by a differential pressure transducer (Statham PM15E). The procedure described by Agrawal was modified in that the second port of the box pressure transducer was attached to a second airtight box of nearly equal volume to the plethysmograph (31). A high resistance line (PE90) connected the two boxes. This procedure reduced drift and noise due to atmospheric pressure changes and air currents in the laboratory. The two differential pressure transducers were connected to a respiratory analyzer (Buxco, Model 6). Permanent records of airflows, box pressure, tidal volume, respiratory rate, minute volume and specific airway resistance (SR_{AW}, cmH_20/s) from the respiratory analyzer were recorded on a polygraph (Grass, Model 7D).

2.4. Agent administration

For the studies using the plethysmograph an aerosol was generated from an ultrasonic nebulizer (DeVilbis Model 35B). The average particle size was approximately 5 μm, which insured penetration of the aerosol into the lower airways. Aerosols were delivered into the nose piece of the inner cylinder. Fresh air was also delivered through this line when the aerosols were not being delivered or when resistance recordings were not being recorded. The bias flow into the plethysmograph was 5 l per minute. Aerosol challenges of either saline or OA (10 mg/ml) were delivered for 1 min.

Six guinea pigs were sensitized to OA and four guinea pigs treated with the vehicle (saline) were used as controls. The guinea pigs were challenged with aerosols of the vehicle and pulmonary function monitored for 15 min. Immediately thereafter OA challenge was performed and again pulmonary function was monitored for 15 min. The following day each guinea pig was given a 1 min aerosol of NDGA (0.9% w/vol). Thirty minutes later an OA challenge of equal concentration to the first OA challenge was delivered.

OA challenge was again repeated 24 h after this second OA challenge in five of the six guinea pigs. One guinea pig had little or no reaction to the OA so the 24 h test was not performed. Vehicle and OA challenge was performed in the four saline-treated guinea pigs. SR_{AW} was determined just before aerosol challenge and at Minutes 1 through 5, 10 and 15 min post aerosol challenge. A flow chart of this protocol is presented in Fig. 1.