Demonstration of Antihistamine Properties with AST-1: A Bioactive Extract from Garden Slugs (*Diplosolenodes occidentalis*)

A Jacob[1], O Simon[1], P Reese[2], P Singh[1]

ABSTRACT

Parched and ground whole garden slugs are claimed in rural Jamaican folklore practices to have useful effects in the treatment of bronchial asthma. Since this claim may be associated with respiratory dysfunction due to histamine from allergic sensitization, the authors investigated the effects of a semi-pure alcoholic extract (AST-1) on histamine-induced contraction of the guinea pig in vitro *tracheal muscle preparation and cutaneous allergic responses in ovalbumin sensitized guinea pigs. Chemical analysis of AST-1 by column chromatography and thin layer chromatography indicated two compounds in the composition, but the molecular structures were not determined. Pharmacological evaluation of AST-1 produced a concentration-dependent inhibition of histamine-induced contraction of the guinea pig tracheal muscle preparation. AST-1 also inhibited contraction of the tracheal muscle produced by selective H_1 receptor stimulation with HTMT dimaleate. H_2 receptors were not involved, as indicated by the absence of contraction with dimaprit hydrochloride, a selective H_2 agonist.*

Also, in ovalbumin sensitized guinea pigs, AST-1 and diphenhydramine, a selective H_1 antagonist, inhibited the cutaneous responses due to intradermal injection of histamine and ovalbumin. These results suggest that AST-1 has H_1 anti-histamine properties which can inhibit histamine-induced tracheobronchial muscle contraction and cutaneous responses due to allergy.

Demostración de las Propiedades Antihestamínicas con AST-1, un Extracto Bioactivo de las Babosas de Jardín (Diplosolenodes Occidentalis)

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RESUMEN

En el contexto de las prácticas folclóricas de la Jamaica rural, se afirma que las babosas de jardín tostadas y molidas, tienen efectos útiles en el tratamiento del asma bronquial. Como que esta afirmación puede estar asociada con una disfunción respiratoria debida a la histamina de la sensibilización alérgica, los autores se dieron a la tarea de investigar los efectos de un extracto alcohólico semi-puro (AST-1) sobre la contracción inducida por histamina de un preparado in vitro de músculo de tráquea de cobayo, y las respuestas cutáneas alérgicas en cobayos sensibilizados con ovalbúmina. El análisis químico de AST-1 mediante cromatografía en columna y cromatografía en capa fina, indicaron dos compuestos en la composición, pero no se determinaron las estructuras moleculares. La evaluación farmacológica de AST-1 produjo una inhibición – dependiente de la concentración – de la contracción, inducida por histamina, del preparado de músculo de tráquea de cobayo. El AST-1 también inhibió la contracción del músculo traqueal producida por la estimulación de receptor selectivo H_1 con dimaleato de HTMT. No se involucraron receptores H_2, como lo indicó la ausencia de contracción con hidrocloruro de dimaprit – un agonista selectivo H_2. Asimismo, en cobayos sensibilizados con ovalbúmina, el AST-1 y la difenidramina – un antagonista selectivo H_1 – inhibieron las respuestas cutáneas debido a la inyección intradérmica de histamina y ovalbúmina. Estos resultados sugieren que el AST-1 tiene propiedades antihestamínicas H_1, las cuales pueden inhibir la contracción muscular traqueobronquial inducida por la histamina y las reacciones cutáneas debido a la alergia.

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INTRODUCTION

The use of folklore remedies from plants for treatment of illnesses is quite common in Jamaica, but occasionally animal materials are also used. The common garden slug (*Diplosolenodes occidentalis)* is an animal source from which a folklore remedy is derived for use in some rural Jamaican agricultural communities to control respiratory difficulties associated with asthmatic attacks. The animal is a terrestrial mollusc from the phylum *Gastropoda* (1). It is nocturnal and is commonly found crawling among garden shrubs on which it feeds.

In folklore practices, whole slugs are parched, ground and eaten with food to treat asthmatic attacks. However, there have only been anectdotal claims of therapeutic effectiveness of the use of slug material to control respiratory difficulties associated with asthmatic attacks. There is no clear indication of the type of asthmatic attack that would benefit from the folklore remedy but it is mostly used in rural agricultural communities where individuals are constantly exposed to plant materials that can make them susceptible to atopic allergy which can precipitate extrinsic asthma (2). This usage of the remedy provided a basis for this study to investigate the effects of the parched and ground slug material on responses due to histamine, a major mediator of allergy (3, 4).

The histamine-mediated responses selected for investigation included the allergic cutaneous responses and contractions of the tracheal muscle which can increase airway resistance and precipitate an asthmatic attack in susceptible individuals (4). With this experimental approach, it was possible to determine whether the slug preparation has antihistamine properties which can inhibit respiratory smooth muscle contraction and allergic responses that have the potential to precipitate an asthmatic attack.

MATERIALS AND METHODS

Preparation of AST-1

Garden slugs were collected from vegetations and identified by the Department of Life Sciences, University of the West Indies, Jamaica as *Diplosolenodes occidentalis*. The slugs were washed and parched in an oven (Bockel Industries) at 160°C. The parched material was milled to a fine powder and extracted in 95% ethanol at 4°C for 72 hours. The resulting ethanolic mixture was filtered via gravity flow and the filtrate was evaporated under vacuum at 70°C to remove the ethanol. The solid residue was not soluble in water; therefore, it was dissolved in 95% ethanol and eluted on a silica gel column with a mixture of chloroform and ethyl acetate (10:1 V/V). Fractions were collected and tested for biological activity on the tracheal muscle preparation. The most active fraction was designated AST-1 and it was subjected to further separation by Thin Layer Chromatography (TLC) (with a solvent system of chloroform and ethyl acetate at 10:1, V/V) for determination of the number of constituent compounds which were identified with phosphomolybdic acid/ceric sulphate reagent. The ethanol was obtained from Industrial and Technical Supplies, Jamaica, and the other chemicals were obtained from British Drug House.

Pharmacological procedures

These procedures conformed to the guidelines of the National Research Council (USA) for the use and care of laboratory animals (5). The protocol for the study was presented and approved by the board of the Department of Basic Medical Sciences.

i) Investigation on guinea pig isolated tracheal muscle preparation

Adult male guinea pigs (Dunkin Hartley) weighing 350–400 g were sacrificed and the trachea was excised from each animal and placed in Krebs Hensleit solution. Adherent connective tissue was removed from the trachea and a length consisting of four cartilage bands was cut through the cartilage side of the trachea to form a broad muscle band between the two halves of the cut cartilage. The tissue was then suspended in a 20 ml bath containing Krebs Hensleit solution maintained at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2. A loop of thread attached to one half of the cut cartilage anchored the tissue at the bottom of the bath. The other half of the cartilage (with the band of muscle between) was attached via a length of thread to a force displacement transducer (Grass, Model FT03) connected to a polygraph (Grass, Model 79E) for recording isometric muscle tension as indicator of the magnitude of contraction. The tissue was then subjected to one gram resting tension and allowed to equilibrate for one hour before investigation of pharmacological effects.

In control experiments, contractions of the tracheal muscle preparation were produced with histamine (0.63 μg/ml – 40 μg/ml) in a cumulative dosing schedule in a tissue bath of 2.9% alcoholic (bath concentration of AST-1 solvent) Krebs Hensleit solution. In three test experiments, contractions of the muscle preparation were produced with a similar dosing schedule for histamine (0.63 μg/ml – 40 μg/ml) in the presence of three concentrations of AST-1 (0.1 mg/ml, 0.2 mg/ml and 0.4 mg/ml) dissolved in a bath of 2.9% alcoholic Krebs Hensleit solution. During each series of treatment, changes in isometric tension developed by the contractions were recorded. At the end of each treatment schedule (35 minutes drug contact time), the drug was washed from the tissue with fresh Krebs Hensleit solution. The maximum changes in isometric tension in the control and test experiments were compared for determination of the pharmacological effect of AST-1.

In each of two other series of experiments with the tracheal muscle preparation, histamine (Puriss) was replaced either by HTMT dimaleate (Tocris), a selective H_1 agonist, or dimaprit hydrochloride (Tocris), a selective H_2 agonist. In

one series of experiments, one concentration of HTMT dimaleate (20 µg/ml) was used as previously described to produced contractions of the muscle preparation in the absence and presence of AST-1 (0.2 mg/ml and 0.4 mg/ml) to determine the effect on H_1 receptor stimulation. The experiment was repeated with dimaprit hydrochloride instead of HTMT dimaleate, but the tracheal muscle preparation did not contract with the H_2 agonist treatment.

ii) Investigation in Ovalbumin sensitized guinea pigs

Dunkin Hartley guinea pigs (12) weighing 350–400 g were sensitized with ovalbumin (Sigma) in accordance with the procedure described by Weinreich and Undem (6). Sensitization was developed over a period of 30 days following intraperitoneal injections of ovalbumin (10 mg/Kg) on alternate days for three days. After this period, the sensitized animals were separated into two groups (six each) and used in control experiments. Each animal in the two groups was given an intraperitoneal injection of either 0.3 ml of 2.9% alcoholic solution (AST-1 solvent) or 0.3 ml of 0.9% saline (diphenhydramine solvent). Fifteen minutes after these injections, histamine (0.1 ml of 1 mg/ml solution) and ovalbumin (0.1 ml of 1 mg/ml solution) were injected intradermally at separate demarcated sites on the shaven flank of each animal. The resulting cutaneous responses were assessed in terms of the diameters of the encircled area of vasodilation produced at the injection sites at zero time and at half hour intervals for four hours.

One week after the control experiments, the two groups of sensitized guinea pigs were retested with the same concentrations and volumes of histamine and ovalbumin. These agents were given intradermally 15 minutes after intraperitoneal injection of either AST-1 (0.3 ml of 8 mg/ml in 2.9% alcoholic solution) or diphenhydramine (0.3 ml of 300 µg/ml in 0.9% saline). After these treatments, the diameters of the cutaneous responses were measured and compared with the control experiments for determination of the effects of AST-1 and diphenhydramine (Sigma). The animals were then allowed a further one week period of recovery before testing for the return of the cutaneous response due to intradermal injections of histamine and ovalbumin in the absence of AST-1 and diphenhydramine.

Statistical Analysis

The data are presented as means ± SEM. The student's *t* test (for two samples) at $p \# 0.05$ was used for determination of statistically significant difference between treatments. Graphs were prepared with the Sigma Plot graphing programme for Windows 2000.

RESULTS

Preliminary chemical analysis of AST-1

An alcoholic extract (AST-1) prepared from parched and ground whole slugs was shown on TLC to consist of two compounds (R_f values = 0.4 and 0.6) after separation with a solvent system of chloroform/ethyl acetate (10/1, V/V) and development with phosphomolybdic acid/ceric sulphate reagent. These compounds were not alkaloids since the extract did not give a positive test with Dragendorff's reagent.

Effects of AST-1 on contractions produced by histamine and HTMT dimaleate

In pharmacological studies, 0.1 mg/ml (bath concentration) AST-1 inhibited histamine-induced increase in tension of the guinea pig tracheal muscle preparation (Fig. 1) from a

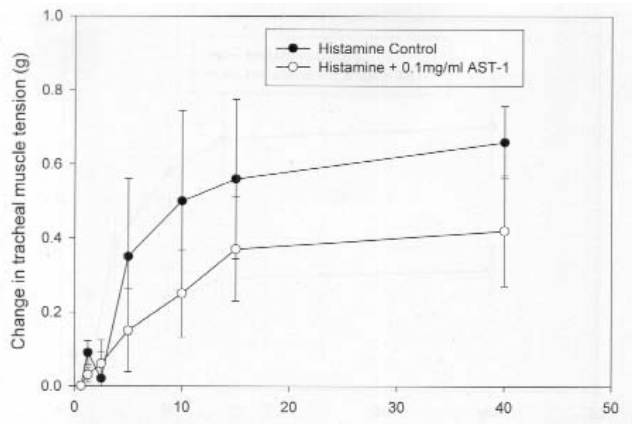

Fig. 1: Effect of 0.1mg/ml AST-1 on muscle tension produced by histamine induced contraction of the guinea pig *in vitro* tracheal muscle preparation. Each data point is the mean ± SEM value from six experiments. The maximum change in tension at 40 mg/ml histamine is significantly different ($p < 0.05$) with AST-1.

maximum of 0.66 ± 0.01 g to 0.42 ± 0.15 g ($p < 0.05$, n = 6). Larger concentrations of AST-1 (0.2 mg/ml and 0.4 mg/ml – bath concentration) also inhibited histamine-induced increase in muscle tension (Figs. 2, 3, respectively) from maxima of

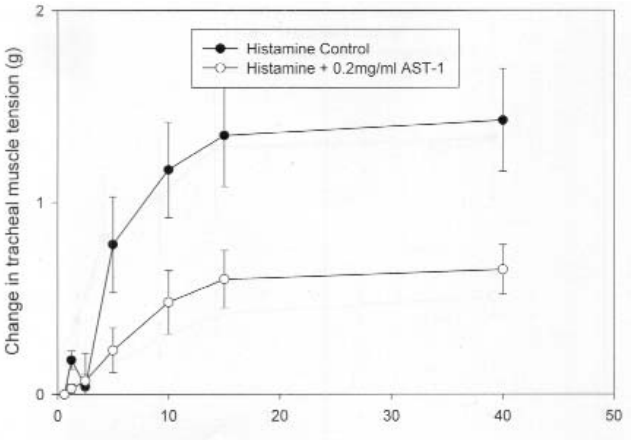

Fig. 2: Effect of 0.2 mg/ml AST-1 on muscle tension produced by histamine induced contraction of the guinea pig *in vitro* tracheal muscle preparation. Each data point is the mean ± SEM value from six experiments. The changes in tension at 5–40 µg/ml histamine are significantly different ($p < 0.02$) with AST-1.

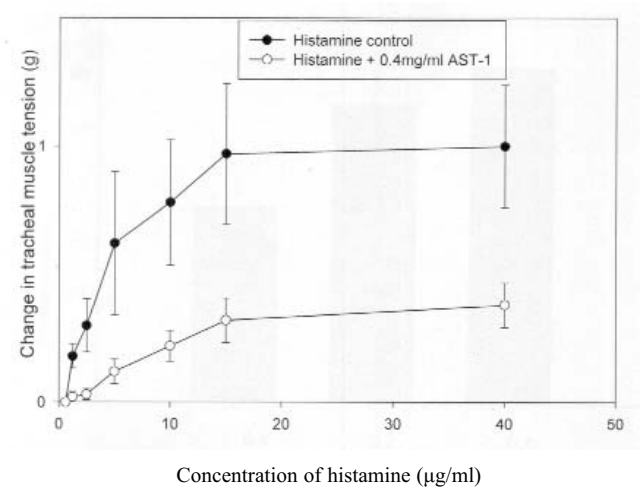

Fig. 3: Effect of 0.4 mg/ml AST-1 on muscle tension produced by histamine induced contraction of the guinea pig *in vitro* tracheal muscle preparation. Each data point is the mean ± SEM value from six experiments. The changes in tension at all histamine concentrations are significantly different ($p < 0.02$) with AST-1.

1.43 ± 0.27 g to 0.7 ± 0.13 ($p < 0.02$, n = 6) and 1.0 ± 0.24 g to 0.4 ± 0.09g ($p < 0.02$, n = 6). Note that in the experiments with each concentration of AST-1 and the corresponding histamine control, a different set of six tracheal muscle preparations were used to ensure the maintenance of adequate tissue responsiveness. Also, in Figs. 1–3, it should be noted that increasing concentration of histamine only partially surmounted the AST-1 inhibition, but the magnitude of the inhibition was dependent on the concentration of AST-1. This is shown in Fig. 4 in which the percentage inhibition

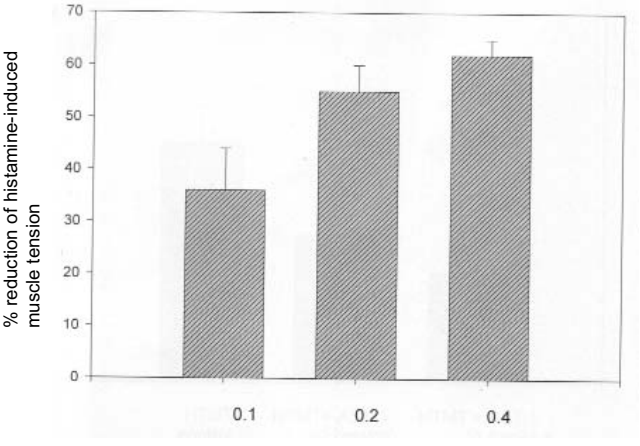

Fig. 4: Bar graph showing significant ($p < 0.05$) concentration-dependent percentage inhibition by AST-1 on the maximum change in muscle tension produced by 40 µg/ml histamine-induced contraction of the guinea pig *in vitro* tracheal muscle preparation. Each bar represents the mean ± SEM value from six experiments.

of the maximum histamine-induced increase in tracheal muscle tension was compared.

Selective H_1 agonist stimulation with HTMT dimaleate (20 µg/ml bath concentration) also contracted the tracheal muscle preparation, but in the presence of AST-1 (0.2 mg/ml and 0.4 mg/ml), the muscle tension was inhibited in a concentration- dependent manner (Fig. 5). H_2 receptors were

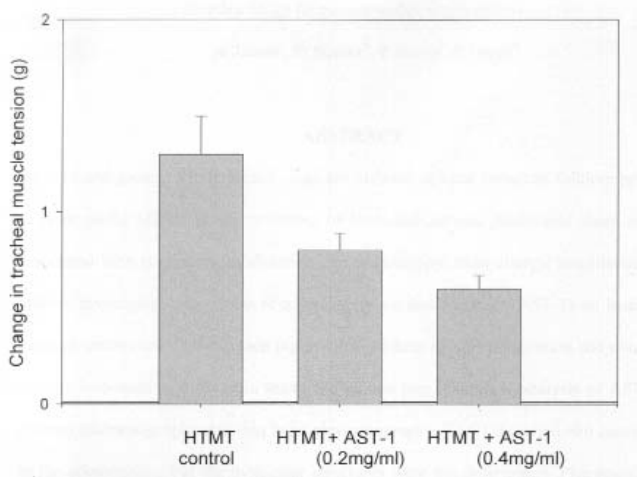

Fig. 5: Bar graph showing significant ($p < 0.05$) concentration-dependent inhibition by AST-1 on muscle tension produced by HTMT dimaleate (selective H_1 agonist) induced contraction of the guinea pig in vitro tracheal muscle preparation. Each bar represents the mean ± SEM value from six experiments.

not involved, as indicated by the absence of contraction (not shown) when the tracheal muscle preparation was treated with dimaprit hydrochloride, a selective H_2 agonist.

Effects of AST-1 and diphenhydramine on responses of sensitized guinea pigs

In control experiments, localized vasodilation (a red spot) was produced within five minutes of intradermal injections of histamine (100 µg in 0.1 ml aqueous solution) and ovalbumin (1 mg in 0.1 ml aqueous solution) in two groups of sensitized guinea pigs (6 per group) that were pretreated intraperitoneally with either 2.9% alcoholic solution (AST-1 solvent) or 0.9% saline (diphenhydramine solvent). Half an hour after the injections, each site was encircled by a red flare with a clear central area. The size of the red flare reached a maximum two hours after the injections and the diameter of the encircled area increased from 0.4 ± 0.1 cm (at time zero) to a maximum of 1.8 ± 0.1 cm for histamine and 1.2 ± 0.1 cm for ovalbumin. These responses subsided four hours after the injections were given.

One week after the control experiments, each animal in the two groups of sensitized guinea pigs was pretreated intraperitoneally with either AST-1 (0.3 ml of 8 mg/ml in 2.9% alcoholic solution) or diphenhydramine (0.3 ml of 300 mg/ml in 0.9% saline) 15 minutes before injection of either histamine or ovalbumin at separate demarcated sites on the right shaven flanks. At five minutes, half an hour and two hours after the injections, the demarcated sites were

examined and it was noted that the diameters were not increased beyond the zero time value of 0.5 ± 0.1 cm which was due to the volume of the injection fluid. In fact, there was no evidence of development of the red spot or the surrounding red flare. Even the small diameter produced by the volume of the injection fluid was not evident two hours after the injections were given. But although these results indicated that AST-1 and diphenhydramine prevented the development of the cutaneous responses, it was necessary to confirm that the animals were still sensitized and responsive to intradermal injection of histamine and ovalbumin. In the confirmatory test which was performed one week after termination of the treatment with AST-1 and diphenhydramine, both histamine and ovalbumin produced the initial red spot followed by the central wheal and surrounding red flare with mean maximum diameters which measured respectively 1.5 ± 0.3 cm and 1.3 ± 0.1 cm in one group, and 1.4 ± 0.4 cm and 1.1 ± 0.2 cm in the other group of guinea pigs.

DISCUSSION

An alcoholic extract (AST-1) was prepared from parched and ground whole slugs for investigation in this study. Preliminary chemical analysis of AST-1 ruled out the presence of alkaloids. Thin Layer Chromatography separation showed the presence of two compounds but the structural compositions were not determined.

From pharmacological investigation of AST-1 in this study, it was shown that the extract inhibited muscle tension produced by histamine-induced contraction of the *in vitro* tracheal muscle preparation of the guinea pig. The magnitude of the inhibition was dependent on the concentration of AST-1; however the inhibition was not totally surmounted by increasing concentration of histamine. This result is an indication of non-competitive inhibitory interaction (4) of AST-1 with the histamine receptors, but this pharmacodynamic property could not be accurately confirmed with the semi-pure AST-1.

There was, however, supporting evidence for histamine-H_1 receptor mediation of the AST-1 effect as indicated by concentration-dependent inhibition of contraction produced by HTMT dimaleate, a selective H_1 receptor agonist (7). Histamine-H_2 receptors were not involved in the AST-1 effect, since selective stimulation of H_2 receptor with dimaprit hydrochloride (7) did not increase tension in the tracheal muscle preparation. The absence of H_2 mediated contraction of the tracheal smooth muscle is consistent with findings reported in the literature in which a relaxant effect was attributed to H_2 receptor stimulation of the respiratory smooth muscle while contraction of this muscle was linked to H_1 receptor stimulation (8) as demonstrated in the present study.

Other H_1 mediated effects inhibited by AST-1 in this study were the cutaneous triple response produced by intradermal injection of ovalbumin and histamine in ovalbumin sensitized guinea pigs. The phases of the response due to intradermal injection of histamine were first described by Lewis (9) and they were characterized by an initial red spot due to vasodilation (4) at the site of injection followed by the development of a wheal (indicated by a central clear area) due to localized oedema (4) at this site and a surrounding red flare due to vasodilation produced by axon reflex (4). All of these phases of the triple response were attributed to stimulation of H_1 receptors by histamine released from cutaneous mast cells during allergy (4). In the present study, intradermal injection of ovalbumin and histamine produced all phases of the response in ovalbumin sensitized guinea pigs, and they were inhibited by AST-1 and diphenhydramine, a selective H_1 receptor antagonist (8).

The results with diphenhydramine confirmed that H_1 receptors mediated the cutaneous triple response produced in the sensitized guinea pigs in this study. But AST-1 also inhibited the response and this similarity of the effect with diphenhydramine indicates that H_1 receptors mediated the AST-1 effect in this study.

When these results are considered together, they represent supportive evidence of H_1 antihistamine properties for AST-1. These properties were associated with inhibition of tracheal smooth muscle contraction and cutaneous responses produced by allergen sensitization in this study. In this regard, they provide a possible explanation for the Jamaican folklore claim attributed to the use of parched and ground slug material to control respiratory difficulties that are most likely associated with allergy in asthmatic attacks. This potential of AST-1 should be investigated further by isolating the pure active compound and characterizing the antihistamine properties that may be of therapeutic value.

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