Full Length Research Paper

In vitro modulation of tracheal smooth muscle reactivity by extracts of some Senegalese medicinal plants

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Scientific validation of pharmacological actions of 7 Senegalese plants used in traditional medicine for the treatment of respiratory illness was carried out. Changes in contractility of isolated rat trachea were assessed in organ chambers. Rings were allowed to equilibrate for 60 min before experiments were carried out, during which time the resting tension was adjusted, as required. Rings were first exposed to methanolic plants extracts (10⁻² and 10⁻¹ mg/ml) or solvent. After a 30 min incubation period, they were contracted with acetylcholine in a cumulative manner (10⁻⁹ to 10⁻³ M). Extracts from leaves of Guiera senegalensis, Melaleuca leucodendron and Hymenocardia acida elicited a significant dose-dependant inhibition of the contractile agonist, suggesting further investigations on its chemical composition, the underlying mechanisms involved and its potential health value. However, in rings pre-incubated with extracts from Cymbopogon giganteus leaves and Salvadora persica roots, effect of the contractile agonist was not affected. Moreover, a hypereactivity was observed with extracts from Gossypium barbadense leaves and Cassia occidentalis seeds. These results provide evidence that Senegalese herbals may be of interest as valuable source of information for the selection of plants for focussed screening programmes and for therapeutically useful products.

Key words: Tracheal contractility; medicinal plants; respiratory illness

INTRODUCTION

Airway hyper-responsiveness and lung inflammation are reported to be the two majors factors involved in respiratory illness (del Giudice et al., 2004; Futrakul et al., 2005; Fahy 2006). According to the World Health Organization (WHO), they constitute a public health priority. In fact, according to recent estimates from WHO, 300 million people suffer from asthma, this number could reach 400 million in 2025 and is responsible for over 200,000 deaths per year (Meetoo, 2008). These respiratory diseases, in addition to inflammation (Geraldes et al., 2009; Yang et al., 2009), are often associated with bronchial hyperreactivity (Hamelmann, 1999). Bronchial responsiveness is the ability of airway response to bronchoconstrictor stimuli, be they physical, chemical or pharmacological. In response to these stimuli, the airways have the ability to reduce their diameter with bronchial responsiveness increased in many lung diseases, allergic or not. The bronchial hyperreactivity leads to abnormal sensitivity of the bronchi to contract excessively in response to stimuli that are ineffective in a normal subject. The prevention and treatment of bronchial hyperreactivity is a goal pursued intensively (Nie et al., 2005; Peters et al., 2006; Kim et al., 2009) and use of medicinal plants is a common practice in our traditional society.

Numerous in vitro studies have shown beneficial effects of phytotherapy (del Giudice et al., 2004; Govindan et al.,...
2004; Spasov et al., 2004; Schmiedel and Klein 2006; Boskabady et al., 2007) and reported Scientific validation of pharmacological actions of plants used in traditional medicine for the treatment of these pathologies have been reported (Borchers et al., 2000; Loi et al., 2004; Gazzaneo et al., 2005; Kufer et al., 2005; Leporatti and Impieri 2007; Redzic 2007; Nanyangi et al., 2008). However, none of such works cover plants used in Senegalese traditional medicine. Consequently, seven local plants (Guiera senegalensis, Melaleuca leucodendron, Hymenocardia acida, Cymbopogon giganteus, Salvadora persica, Gossypium barbadense and Cassia occidentalis) used by traditional health practitioners in Senegal for treatment of respiratory tract that have never been described were investigated.

This study also investigates the relationship between these plants extracts and the cholinergic system, after in vitro stimulation of rat trachea with a major neurotransmitter (acetylcholine) that activates smooth muscles in the respiratory tract.

MATERIALS AND METHODS

Materials

Acetylcholine was purchased from Sigma Chemical Co (Saint Quentin-Fallavier, France). Adrenaline was a generous gift from ‘Pharmacie Nationale d’Approvisionnement’, Dakar, Senegal. All reagent and extract were diluted in Krebs bicarbonate solution before use.

Preparation of methanol extract

Leaves of G. senegalensis, M. leucodendron, H. acida, C. giganteus and G. barbadense, roots of S. persica and seeds of C. occidentalis were shade dried and subjected to size reduction to get a coarse powder (Mortar grinder RM100). The powdered material (75 g) was subjected to extraction for 2 h using a 60% methanol/water solution (300 ml/200 ml, respectively) as solvent as previously described (Ghayur and Gilani, 2006). The extract was then filtered in vacuum conditions (Vacuum pump V-700, Büchler) by means of the phial of Kitassato and dried on a rotary evaporator (Buchler®). Methanolic extract evaporation was realized during 3 successive days until the obtaining of a dry crude extract. Evaporation conditions were the following ones: Temperature: +40°C; Cooling: +21°C; Rotation: 4000 tr. / min.

Tracheal reactivity studies

Male Wistar rats (12 weeks old), weighing 150-200 g were procured from local Institute, Dakar, Senegal. They were fed on standard rat feed and given free access to water. Tracheas were removed from rats after anaesthesia with pentobarbital (60 mg/kg, i.p.) and cleaned of connective tissue and cut into rings (3 - 4 mm in length).

Rings were suspended in organ baths (Panlab-TRI 202P) containing oxygenated (95% O2; 5% CO2) Krebs bicarbonate solution (mM: NaCl 119, KCl 4.7, KH2PO4 1.18, MgSO4 1.18, CaCl2 1.25, NaHCO3 25 and D-glucose 11, pH 7.4, 37°C) for determination of changes in isometric tension as previously described and adapted (Boskabady and Shaikh, 2000). Following equilibration for 60 minutes under a resting tension of 2 g, rings were contracted with acetylcholine (1 µM) and relaxation to phenylephrine (1 µM) was determined. After washout and a 30 min equilibration period, rings were incubated with methanolic plants extracts (10⁻² and 10⁻¹ mg/ml) or solvent for 30 min before a concentration-contraction curve to acetylcholine (10⁻³ to 10⁻³ M) was constructed.

Statistical analysis

Values are expressed as mean ± SEM. Statistical evaluation was performed with Student’s t test for paired data or ANOVA. Values of p < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Several studies have reported the effects of traditional medicine plants in an attempt to rationalize some of its medical uses (Ko et al., 2003; Li et al., 2006; Castro et al., 2006; Ghayur et al., 2007). This present study provides pharmacological evidence for the medicinal use of Senegalese plants in respiratory disorders.

One major finding of this study is that extracts from G. senegalensis, M. leucodendron and H. acida inhibited the contractions of rat tracheal smooth muscles induced by acetylcholine. To investigate the effects of these medicinal plants, rat trachea were incubated with hydroalcoholic extracts of G. senegalensis leaves, M. leucodendron leaves and H. acida leaves. As shown in Figure 1, after a 30 min incubation period, the contractions induced by cumulative concentrations of acetylcholine were significantly inhibited by these extracts when administered at 10⁻¹ mg/ml, in comparison to control rings not exposed (Emax: 2.142g ± 0.131). These hyporesponsiveness to the contractile agonist was not observed at 10⁻² mg/ml. Moreover, the inhibitory effect evoked by H. acida (Emax: 0.675 g ± 0.191; Figure 1F) was more potent than those with either M. leucodendron (Emax: 0.972g ± 0.085; Figure 1D) or G. senegalensis (Emax: 1.677 g ± 0.408; Figure 1B) which appeared to be the least potent among these 3 extracts. With regards to G. senegalensis, an anti-tissue action has been reported. The leaf and roots which contain total alkaloids (Fiott et al., 2006) appears to be the most frequently used plant parts (Silva et al., 2008). No organic toxicity in relation with renal and hepatic functions, heart, lungs, brain, cerebellum, spleen and haematological features in relation with erythropoiesis, haemoglobinogenesis and leucopoiesis was found (Diouf et al., 2000). H. acida also contains alkaloids and phenolic contents (Sofidiya et al., 2009). This plants is known to have in vitro anti-trypanosomal (De Vooght et al., 2009), antisickling (Farag et al., 2004; Mpiana et al., 2007), antioxidant and antibacterial activities (Sofidiya et al., 2009), but no data is available for its bronchodilatory effect. Melaleuca tree appears not to be a significant source of aeroallergen and the Melaleuca odour is not a respiratory irritant (Stablein et al., 2002).

The results with C. giganteus and S. persica are not surprising. No data is available about their efficacy against respiratory disorders. As shown in Figure 2, no
Figure 1. Effect of in vitro exposure of the leaves of Guiera senegalensis, GS (A and B), Melaleuca leucodendron, ML (C and D) and Hymenocardia acida, HA (E and F) at 0.01 mg/ml (left panel) or 0.1 mg/ml for 30 min (right panel) on acetylcholine-induced contraction of isolated rat trachea. Each values represents the mean ± S.E.M, (n = 5). ns = not significant ; *p< 0.05, ** p < 0.01, one-way ANOVA).

significant differences were seen between rings incubated with extracts from C. Giganteus leaves (Figures 2A and B) and S. persica roots (Figures 2C and D) regarding tracheal reactivity in comparison to control rings. However, these plants have some efficacy in other biological systems. Indeed, C. giganteus was used against chloro-
Figure 2. Effect of in vitro exposure of Cymbopogon giganteus, CG leaves (A and B) and Salvadora persica, SP roots (C and D) at 0.01 mg/ml (left panel) or 0.1 mg/ml for 30 min (right panel) on acetylcholine-induced contraction of isolated rat trachea. Each values represents the mean ± S.E.M, (n = 5). ns = not significant ; *p < 0.05, one-way ANOVA.

CONCLUSION

Altogether, these results justified the use of some selected plants in the management of respiratory disorders by
Figure 3. Effect of in vitro exposure of *Gossypium barbadense*, GB leaves (A and B) and *Cassia occidentalis*, CO seeds (C and D) at 0.01 mg/ml (left panel) or 0.1 mg/ml for 30 min (right panel) on acetylcholine-induced contraction of isolated rat trachea. Each values represents the mean ± S.E.M, (n = 5). ns = not significant ; *p < 0.05, one-way ANOVA).

traditional oral health practices which constitute an important part of lifestyle in Senegalese population. The mechanisms by which these plants interact with SMCs contractions remains to be elucidated. However, we can not exclude the involvement of the cholinergic system (stimulated by acetylcholin) and the interaction with calcium signalling.

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