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Original article

Drug targeting of airway surface liquid: A pharmacological MRI approach

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Abstract

Pharmacological MRI at 4.7 T was used to investigate the secretory response to Sylvestris pine oil stimuli in the rat airways, with the aim of developing an *in vivo* model in a small laboratory animal. The availability of such a model would greatly facilitate the drug discovery process using compounds active on airway surface liquid (ASL) production, and would make it possible to obtain information on chemoreceptoral mechanisms and to test the effects of environmental substances on the airways. T1- and T2-weighted images were acquired in the trachea and larynx before and at various times after exposure to pine oil. Several post-processing procedures were tested in order to improve the visibility of the secretory response and to measure the enhancement of the signal intensity of ASL. A semiautomatic application software was written to localize and to measure the volume involved in the secretory response to a compound administration. A significant effect of the pine oil administration on the secretory response was founded in trachea ($p < 0.01$) and in the salivary glands ($p < 0.01$). 3D reconstructions of MRI data and virtual endoscopy permitted a quick visualization of tracheal morphology and localization of the greatest response to stimulus. The study demonstrated that, despite technical problems due to the air/tissue interface and to the small dimensions of the experimental animals, the secretory response can be evaluated and the pharmacological MRI (phMRI) of the rat airways is feasible. The potential and the limitations of phMRI investigation in drug targeting of ASL are discussed.

Keywords: trachea; magnetic resonance imaging; airway surface liquid

1. Introduction

The secretory mechanisms of the airways play an important role in many pathologies. Abnormal ASL secretion or absorption is a feature of several airway diseases such as chronic rhinosinusitis, asthma and cystic fibrosis. In general, secretions are modified in chronic airway pathologies, and in particular the viscoelasticity of ASL is greater than the optimal values for mucociliary clearance [1]. ASL is composed of mucus, which is a superficial gel layer, with periciliary fluid interposed between the mucous layer and the epithelium. A thin layer of surfactant separates the mucus from the periciliary fluid. The mucous layer extends from the intermediate airway to the upper airway and is approximately 2–10 μm thick in the trachea. ASL is the secretory product of goblet cells and submucosal glands. It is a non-homogeneous, adhesive, viscoelastic gel composed of water, carbohydrates, proteins, and lipids [2].

Mucus acts as a physical and chemical barrier to which particles and organisms adhere. Cilia lining the respiratory tract propel the overlying mucus to the oropharynx, where it is either swallowed or expectorated. Mucus plays a fundamental role in the defense of the lung, which is continually at risk of exposure to noxious environmental agents and respiratory pathogens [3]. Chemical analysis of mucus shows that it

Abbreviations: ANOVA, analysis of variance; ASL, airway surface liquid; FOV, field of view; FWHM, full width at half maximum; MRI, magnetic resonance imaging; PAS, periodic acid-Schiff; phMRI, pharmacological magnetic resonance imaging; RARE, rapid acquisition with refocused echoes; ROI, region of interest; SI, signal intensity; T1W, T1-weighted sequence; T2W, T2-weighted sequence; TE, echo time; TEM, transmission electron microscopy; TR, repetition time.

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contains several different groups of glycoproteins [4]. Tracheal glycoproteins from different epithelial-cell sources have distinctive chemical compositions, and their secretions may be independently regulated [5]. Failure of the defense mechanisms of the airway, or of their co-operation, results in chronic airway disease. The pharmacological approach to these pathologies involves a great variety of specific drugs: expectorants (which increase the volume or hydration of airway secretions), mucolytics (which degrade polymers in secretions), mucokinetics (which increase mucociliary efficiency), adhesives (such as surfactants, which decrease mucus attachment to the cilia and epithelium, augmenting both coughing and mucociliary clearance), mucoregulatory agents (including anti-inflammatory drugs, which reduce the volume of airway mucus secretion) [6].

The epidemiological data on diseases of the airways prompts investigation of new mucoactive drugs and *in vivo* assessment of their effects through analysis of mucus properties before and after treatment.

Considerable interest in plant extracts with mucoactive properties has been expressed in the course of time. Essential oils are interesting compounds and they are chemically diverse in their effect. Family Pinaceae is one of the important objects for investigation due to its medical properties. Pine was first investigated by Hippocrate, the father of Western medicine, due to its benefits to the respiratory system [7] for its decongestant, disinfectant, expectorant and tonic properties.

Pharmacological magnetic resonance imaging (phMRI) could be capable of investigating the secretory response, and in some conditions could make it possible to qualitatively analyze ASL. phMRI is not invasive, and with high spatial and temporal resolution allows functional studies of several organs. It has in the past been applied to the study of secretions in saliva, the stomach, and the intestine [8–10]. The aim of this study was to develop an *in vivo* model to evaluate the airway secretory response using phMRI on a small laboratory animal. The availability of such a model would greatly facilitate the drug discovery process using compounds active on mucus production, and would make it possible to obtain information on the chemoreceptoral mechanisms recently discovered in the airways [11–13] and to test the effects of environmental substances on the airways.

2. Materials and methods

The study was carried out on 4-month-old female Wistar rats ($n = 6$) kept under controlled environmental conditions and veterinary monitoring. The animals were accustomed to the experimenters prior to the procedures employed in the present study. The experiments were conducted following the principles of the NIH Guide for the Use and Care of Laboratory Animals and the European Community Council (86/609/EEC) directive. Before experimental session rats were anaesthetized by intraperitoneal injection of pentobarbital (50 mg/kg) and placed in the prone position. To evaluate spontaneous tracheal secretion due to the anesthesia, $n = 4$ animals in the same basal conditions were examined.

All MRI experiments were carried out using a Biospec Tomograph System (Bruker, Karlsruhe, Germany) equipped with a 4.7 T, 33-cm bore horizontal magnet (Oxford Ltd, UK) with a gradient power of 20 G/cm. A 7.2 cm i.d. birdcage resonator was used for transmission, while detection of the MRI signal was achieved using a 1.5 cm surface coil that was positioned at the ventral surface of the neck; the animals were placed in prone position. Acquisitions were made with ParaVision 3.0.2 software running on a Linux-based workstation. Sagittal and coronal spin-echo images were acquired for trachea localization and afterwards, axial multislice T1-weighted (T1W) and T2W (RARE) images were acquired using, respectively, TR = 800 ms, TE = 15 ms and TR = 5000 ms, TE = 65 ms. Other parameters were: number of slices = 15; slice thickness = 2 mm; field of view (FOV) = 40 × 40 mm; matrix size 256 × 128 pixels for T1W images and 256 × 256 pixels for RARE images.

Preliminary experiments showed that T2W sequences, and specifically RARE sequences, were able to show an ASL layer in the trachea as a thin hyperintense ring in the axial images. Best results were obtained using TR = 5000 ms and TE = 65 ms. In T1W sequences, however, it was not possible to distinguish the ASL layer from the tracheal ring.

Sylvestris pine oil stimuli were examined using the following protocol: at the end of the T1W and T2W series of images, the rats were administrated for 5 min continuously with a mixture of air and Sylvestris pine essential oil. Five and ten minutes after the beginning of the stimulation, two further RARE acquisitions (5 min each) were performed.

MRI images were acquired in the tracheal region using 15 contiguous slices, 2 mm in thickness, which covered the region between the trachea and epiglottis. In our experimental conditions the first ten slices covered the trachea, and the remaining five the larynx (respectively at the distal subglottis, proximal subglottis, glottis, distal epiglottis and proximal epiglottis). In this anatomical region the secretory response was analyzed and the corresponding volume measured.

Quantitative analysis of images was performed on the same squared ROI (40 × 40 pixels, corresponding to 6.24 × 6.24 mm^2) around the tracheal lumen.

Extensive analysis of the images (post processing) was carried out in order to extract the greatest possible amount of information. All the routines were written in Matlab7.1.

2.1. Compound tested

The chemical composition of the Sylvestris pine essential oil can varies with relation to the extraction procedures (steam, maceration, cold pressing and solvent extraction). Sylvestris pine oil is rich in monoterpene hydrocarbons (α-β-pinene, limonene, β-caryophylene, germacrene D, Δ-3-carene [7]. The expectorant and fluidificant properties of the Sylvestris pine oil are well-known from ancient times and useful in case of cold, bronchitis and rhinitis. Sylvestris pine oil (Omit, Dolisos Italia S.r.l., Italy) was administered using a two-way purpose-built apparatus.