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Development of atropine sulphate nasal drops and its pharmacokinetic and safety evaluation in healthy human volunteers

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ABSTRACT

Introduction: The increased use of organophosphate (OP) insecticides and the ever increasing possibility of terror groups using nerve agents underscore the need to develop effective and safe antidotes against OP poisoning. While intramuscular administration of nerve gas antidotes like atropine sulphate has certain lacunae, intravenous route is neither practical nor feasible in the field conditions for mass casualties. The objective was to develop a novel atropine sulphate nasal drop formulation, evaluate and characterize it using scintigraphy and to carry out safety–efficacy study in human volunteers with a view to obtain early pharmacological effects in comparison to the existing options, particularly the conventional intramuscular route.

Methods: Permeability studies were done using atropine sulphate solution containing variable amount of chitosan. Radiometric method was developed for scintigraphy studies while standard spectroscopy was used for the quantification of atropine sulphate in fluids. Concentration of atropine sulphate in nasal drops to produce therapeutic concentration in blood was calculated. Six volunteers (age range 18–53 years) were administered the formulation delivering 6 mg of atropine sulphate each. Bioavailability and atropinization were noted serially.

Results: Based on the results of in vitro, human scintigraphy and analytical data, 1% atropine sulphate–0.5% chitosan was chosen as the final nasal formulation. Human bioavailability curve was created which showed that the therapeutic concentration of the drug in blood was reached within 5 min with nasal drops suggesting that drug delivery through the nasal route is significantly better than the intramuscular route. Unpaired *t*-test between the means of baseline value of heart rate and that of each time interval showed that increase in heart rate of all the volunteers became significant at 15 min ($P < 0.01$) and extremely significant at 30 min ($P < 0.001$). Correlation was evident from 5 min ($c > 0.7$). Pupil diameter showed maximal increase at 30 min ($P < 0.01$).

Conclusions: This novel product, 1% atropine sulphate–0.5% chitosan nasal drops might be a safe and efficacious emergency treatment of organophosphorous poisoning with several advantages over the present management, including early atropinization and capability of mass treatment in least amount of time.

1. Introduction

Organophosphate (OP) poisoning is caused by exposure to insecticides like malathion, parathion, and more importantly by agents of chemical warfare like sarin, soman, tabun or VX (Ecobichon, 2001; Vesela et al., 2008). The exposure to nerve gas poisoning is toxic and leads to sudden death within minutes (Reutter, 1999). The cause of severe morbidity and death in these cases is severe and excessive systemic cholinergic response, the most important manifestation of which is paralysis of respiratory muscles (Bajgar, 2004; Pope et al., 2005). Moreover, in such a scenario the real challenge would be to manage the affected civilian population who would not be well protected and are unlikely to be pre-treated with drugs like pyridostigmine before nerve agent exposure like their army counterparts (Marrs et al., 2006). Mass causality in such a scenario is a reality, easily running into hundreds in confined environments. The increasing possibility of terrorist attacks with nerve agents and the escalating use of OP insecticides underscore the need to develop effective and safe antidotes against OP poisoning (Albuquerque et al., 2006).

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Physiological cholinergic antidotes like atropine sulphate and pralidoxime chloride are the mainstay of life saving therapy and need to be administered at the earliest and through the fastest route (Sidell, 1997). In the hospital setting, assuming the patient can be transported there, the intravenous bolus route is the fastest way to introduce and maintain a state of atropinization. The peak pharmacological concentration occurs within 10 min (Berghem et al., 1980). Unit dose is 2–4 mg atropine sulphate and the dose per day could be more than 50 mg. In the field conditions, the rescue workers (particularly during nerve gas attack) are provided with autoinjectors or spring-loaded syringes that deliver 1.67 mg of atropine (equivalent to 2 mg atropine sulphate) alone or along with pralidoxime chloride through the layers of clothing (Dunn et al., 1997; Bentur et al., 2006). The process is very painful. The peak plasma concentration of the drug occurs after 30 min of intramuscular injection (Berghem et al., 1980). Peak effects after oral dosing occur closer to 2 h (Gervais et al., 1997). Surprisingly there is no strategy in place for mass casualty in such a scenario or in case of accidental organophosphorous poisoning or suicide cases at the field level. Efforts are warranted to find methods to obtain therapeutic range and peak concentration of the drug in blood earlier than 30 min and as close to intravenous range as possible.

As intravenous or intramuscular routes are neither practical nor feasible in the field conditions for treating mass casualties, alternative routes need to be explored so as to combat such eventualities more efficiently and effectively. Nasal route is a painless, patient friendly route for self-administration and can be well employed in emergencies. Permeation enhancers and bioadhesive agents can be added for better efficacy (Illum, 1999). The main objectives of this study were (a) to make the nasal drops of atropine sulphate for systemic action using bioadhesion principles and (b) create its bioavailability data in humans and to evaluate its suitability for field use with a view to obtain early pharmacological effects in comparison to the existing options, particularly the conventional intramuscular route.

2. Materials and methods

2.1. Study design

Atropine sulphate and all other components of the formulation were of pharmaceutical grade. Atropine sulphate powder (monohydrate) was received from Cipla (Mumbai, India). All other chemicals and reagents including chitosan were purchased from Merck Ltd. (Mumbai, India). Technetium-99m (Tc-99m) was obtained from BRIT, BARC, India.

The study was designed with the following sequential components:

(a) *Chitosan was chosen as excipient*. Chitosan is a bioadhesive in nature and is able to interact strongly with the nasal mucus layer and with nasal epithelial cells. The clearance of chitosan formulations from the nasal cavity of sheep and humans has been shown to be significantly slower than that of simple aqueous solutions (Soane et al., 1999, 2001). Hence, nasal chitosan drug formulations provide longer time for drug transport across the nasal membrane before the formulation is cleared by the mucociliary clearance mechanism. Furthermore, chitosan has also been shown in Caco-2 cell culture studies to open the tight junctions between cells transiently, which enable hydrophilic drugs to pass through the membrane by paracellular route (Dodane et al., 1999).

(b) *Formulation development of various proportions of the excipient and drug.*
   (1) The amount of chitosan was varied from 0.5 to 1%. Tests were carried out to choose the best formulation on the basis of best release kinetics.
   (2) The pH of the formulations containing chitosan was taken as 6.0 as chitosan is insoluble at pH above 6 (Sogias et al., 2008).
   (3) 1% Atropine sulphate was taken as the active drug.

(c) *In vitro experiments to estimate permeability rate constants*. The permeability of the formulations was determined through intestinal mucosa of mouse using Franz diffusion cell. Animal experiments were conducted after obtaining permission from the animal ethical committee of Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi.

(d) *Human experiment to assess residency time of the formulation in the nasal cavity*. Pharmacoscintigraphy is a useful technique in the development and evaluation of drugs and their formulations (Singh et al., 2004). It is considered the gold standard for imaging and quantifying the in vivo distribution and bioabsorption pattern of the formulations (Davis et al., 1992; Wilding et al., 2001) and was used in the formulation development process of atropine sulphate nasal drops. Technetium-99m (Tc-99m) was used as the radionuclide of choice in the scintigraphy study for assessing the release rate of atropine sulphate nasal drops because of its short half-life of 6 h, easy availability, and being a pure gamma emitter. Two drops of Tc-99m labeled diethylene triamine pentaacetic acid (DTPA) (mol. wt. 393.34 g) were added (100–200 μCi) to atropine sulphate formulation for determining the clearance rate of the formulation from the nasal cavity.

(e) *Phase-1 safety–efficacy study with atropine sulphate–chitosan nasal drops*. The permission for the conduct of clinical study was obtained from duly constituted Institutional Human Ethical Committee (INM/TS/IEC/006-017/07) as well as from the regulatory agency. Written informed consent was obtained from all participants.

2.2. In vitro experiments

Various concentrations of atropine sulphate in phosphate buffer saline, pH 7.4 were prepared and the absorbances were determined with UV–vis spectrophotometer (ECIL, India) at 262 nm.

The standard curve was prepared in phosphate buffer saline, pH 7.4 in the range of 0.005–0.03 mg/ml and was used for in vitro permeability studies for the formulations through intestinal mucosa of mouse using Franz diffusion cell.

Five formulations of atropine sulphate were made:

- Formulation-I: chitosan 0.5 %, pH 6,
- Formulation-II: chitosan 0.75 %, pH 6,
- Formulation-III: chitosan 1.0%, pH 6,
- Formulation-IV: chitosan 0 %, pH 6 and
- Formulation-V: chitosan 0 %, pH 9.

The large intestine (caecum) of strain 'A' mouse was put on Franz diffusion cell containing physiological saline, facing lumen side upwards within 15 min of dissection. Saline was replaced with phosphate buffer saline pH 7.4 as receptor media.100 μl of the nasal drops was placed on the membrane. 1 ml of samples was collected at intervals of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80 and 90 min. These samples were analyzed at 262 nm in UV–vis spectrophotometer (ECIL, India). Blank formulation was prepared in the same way excluding the drug. Amount of atropine sulphate in the samples was calculated from the standard curve. Cumulative drug released per unit surface area was plotted against time. The experiment was repeated for each of the five test formulations.

The cumulative amount of drug released per unit surface area is given by the formula:

$$Q = \left\{ C_n V + \sum_{i=1}^{n-1} C_i S \right\} / A$$

Q: cumulative amount of drug released per unit surface area of membrane (μg/cm^2); C_n: concentration of drug determined at nth sampling interval; V: volume of individual Franz diffusion cell; $\sum_{n-1}^{i=1} C_i$: sum of concentration of drug (μg/ml) determined at sampling intervals 1 to $n-1$; S: volume of sampling aliquot; A: area of sampling well.

The formulation showing maximal cumulative drug released per cm^2 was further used for human studies.

2.3. Evaluation of the formulation for clearance rate from the nasal cavity using scintigraphy

Method. Three drops of 99mTc-DTPA (500 μCi) was added to 3 ml of normal saline containing 1% atropine sulphate–0.5% chitosan. 0.6 ml each was instilled in the larger of the nasal cavity in three human volunteers in supine position. An appropriate nasal catheter was used to deliver the fluid with the tip placed deep enough to avoid the nasal hair so that the fluid slid down to its resting position in the posterior nasal cavity from where the bulk of absorption of the drug was expected. The volunteers were made to lie down under the gamma camera and clearance rates of the formulation from the site of administration were recorded by taking dynamic views (1 frame per minute) for 30 min. This was done to determine the residency time/clearance rate of the formulation in nasal cavity of human beings so that the fraction available for bio-transfer to the blood from nasal mucosa could be estimated. As a control, 2 subjects were nasally administered Tc-99m DTPA mixed in 0.6 ml normal saline (without chitosan).

2.4. Assessments performed before start and during the therapy

Demographic data, age, and weight of the volunteers were recorded in the case report form at the time of enrolment and history of any disease was noted.