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Does sea-water made isotonic affect ciliary beat frequency?

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Abstract

Various nasal humidifying agents are being promoted for regular use. In this study we compared the effects of three formulations (medium 199, 0.9% saline and a commercially available isotonic sea-water formulation – Rhinomer) on ciliary beat frequency using rat tracheal cilia as a model. Surface response methodology was used to evaluate the effects of pH and osmolality of the formulation on ciliotoxicity. Our results showed that none of the formulations differed in their effects on cilia. Moreover, neither medium 199 nor Rhinomer protected the cilia from the effects of drugs (chlorbutol, xylometazoline, azelastine and lignocaine) known to be ciliotoxic.

Keywords: Ciliotoxicity; Seawater; Surface response; Chlorbutol; Xylometazoline; Lignocaine; Azelastine

1. Introduction

A number of intranasally administered drugs are ciliotoxic (Van de Donk et al., 1982; Jian and Li Wan Po, 1992). In addition to drugs, preservative agents, absorption enhancers and excipients may reduce ciliary beat frequency (Jian and Li Wan Po, 1993a,b). To date, we know of no study that has demonstrated that appropriate formulation reduces the ciliotoxicity of a given drug although work in our laboratory has shown that formulations can be optimised to minimise any such adverse effect contributed by excipients and other additives (Su and Li Wan Po, 1994).

Given the wide appeal of the so-called 'natural' approach to treatment (Eisenberg et al., 1993) and the fact that a formulation of sea-water made isotonic (Rhinomer®) was available for intranasal use as a humectant, we initiated this study to investigate whether such a formulation would modulate the ciliotoxicity agents known to have this effect. The rationale was that a multi-electrolyte medium might be more effective in reversing drug-induced damage to cilia than simple isotonic sodium chloride solution.

2. Methods

2.1. Preparation of incubation solutions

Medium 199 enriched with Hank's salts (Gibco, UK) was used as the control medium. Sterile sea-water made isotonic (300 mOsm; Rhinomer®) was obtained from Mattern and Partner

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(Germany). Dilutions of natural sea-water also obtained from the latter company were made with distilled water to provide a range of osmolalities for the surface-response study (see below). pH was adjusted using 1 M hydrochloric acid or sodium hydroxide.

Otrivine® and Rhinolast® sprays were purchased from local wholesalers. Isotonic sodium chloride (0.9% w/v) and chlorbutol solutions were prepared from the crystals and the appropriate medium. When concentrations of drug lower than those commercially available were required, dilutions were prepared with medium 199 unless otherwise indicated.

2.2. Preparation of ciliated tracheal rings

An adult male Wistar rat weighing between 350 and 450g was killed and the trachea immediately removed and incubated at 37° C in the enriched medium 199. Ring segments of about 1 mm were cut from the trachea and again stored in the enriched medium until required.

2.3. Recording of ciliary beat frequency

The appropriate test medium was placed in the microscope chamber which was set to maintain a 37° C environment through use of a plate thermostat. A tracheal ring was transferred to the medium and positioned to obtain a clear picture on the video monitor. Continuous video recordings were made at three different sites and the experiment repeated using three different tracheal rings. Different rats were used if necessary. Site-to-site, ring-to-ring and rat-to-rat variability were considered within the random error term in any subsequent statistical analyses.

The video recorded ciliary movements were reprocessed as previously described (Jian and Li Wan Po, 1992, 1993a,b; Su and Li Wan Po, 1994) to convert the movements into a frequency spectrum using fast Fourier transform.

2.4. Defining the decay curve and response variable

The beat frequencies was expressed as a percentage of that observed at time zero. When exposed to a ciliotoxic solution the beat frequency decays to produce a decay curve (see section 3). For statistical analyses, the area under the curve (AUC) between the axis $y = 100\%$ and time was used as the response variable. This can be viewed also as the complement of the more traditional AUC between the axis $y = 0\%$ and time (Jian and Li Wan Po, 1993a).

2.5. Surface-response study

To investigate the effect of tonicity and pH of the sea-water on ciliary beat frequencies, a central composite design was used (Box and Wilson, 1951; Su and Li Wan Po, 1994). Nine replicates of the 13 point designs were carried out so that in the statistical modelling of the surface response, 117 observations were analysed. The full surface-response model can be represented by Eq. 1:

$$\mathrm{AUC} = \beta_0 + \beta_1(\mathrm{pH}) + \beta_2(\mathrm{Os}) + \beta_3(\mathrm{pH})^2 + \beta_4(\mathrm{Os})^2 + \beta_5(\mathrm{Os})(\mathrm{pH}) \quad (1)$$

where Os refers to the osmolality and β_0–β_5 are coefficients of the model.

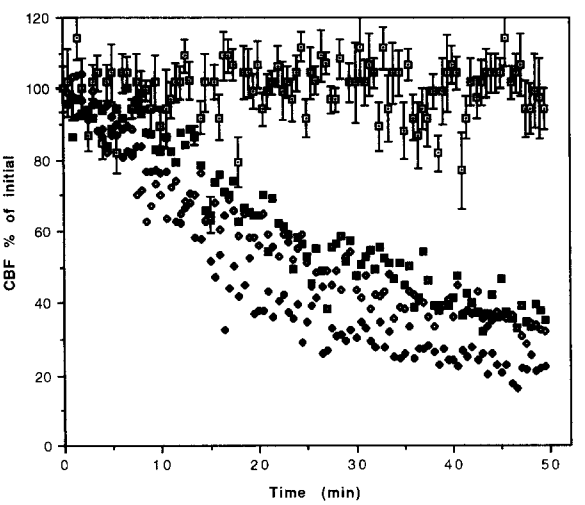

Fig. 1. Beat frequency of cilia exposed to various solutions of xylometazoline (Otrivine® spray) and isotonic sea-water. (■) Isotonic sea-water; (■) 0.01% xylometazoline + 0.9% NaCl (50% v/v); (♦) 0.01% xylometazoline; (◇) 0.01% xylometazoline + isotonic sea-water (50% v/v).

For comparing the ciliotoxicities of various media and for fitting the model, analysis of variance was used.

3. Results and discussion

Fig. 1 shows the effect of exposure of the cilia to 0.01% xylometazoline with and without dilution with an equal volume of 0.9% sodium chloride or sea-water made isotonic. In line with earlier studies (Jian and Li Wan Po, 1992), xylometazoline causes decay in ciliary beat frequency and dilution with either medium led to a reduction in toxicity but there was no significant difference between the results obtained with 0.9% sodium chloride and the isotonic sea-water. For clarity only the standard deviations associated with the results obtained with the control medium are shown.

We then investigated whether 0.9% sodium chloride or the isotonic sea-water could reverse the effects of a 15 min prior exposure to 0.02% xylometazoline (Otrivine® spray) or a 10 min exposure to 0.03% azelastine (Rhinolast® spray). The results showed that the ciliotoxic effects of neither the xylometazoline (Fig. 2) nor the azelastine (Fig. 3) could be reversed.

Lignocaine is an anaesthetic agent which also inhibits ciliary beat frequency. Again, neither 0.9% sodium chloride solution nor isotonic sea-water could reverse this ciliotoxic effect (Fig. 4).

Chlorbutol is an interesting compound with respect to its ciliotoxic effects in that cilia exposed to it show a decay in ciliary beat frequency which is fully reversible when the preservative agent is removed. We were therefore interested in evaluating whether either 0.9% sodium chloride solution or isotonic sea-water could speed up this recovery process. Cilia were exposed to 0.5% chlorbutol in 0.9% NaCl for 15 min and the solution removed to be replaced with fresh 0.9% sodium chloride solution or isotonic sea-water. Fig. 5 shows that there were no differences between the effects of various solutions on the recovery process.

Similar results were obtained after a shorter 10 min exposure of the cilia to the chlorbutol solution (Fig. 6).

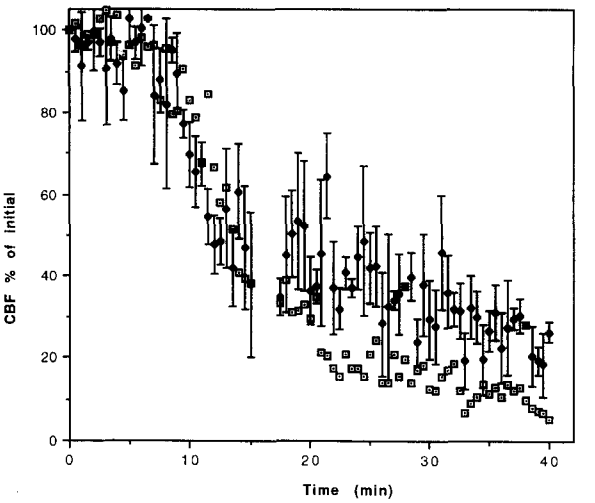

Fig. 2. Comparison of isotonic sea-water (■) and medium 199 (◆) on beat frequency of cilia exposed to 0.02% xylometazoline (Otrivine® spray) for 15 min. Error bars refer to SD ($n = 3$).

If sea-water is to be formulated as a humectant formulation what would be the best pH and tonicity? Fig. 7 shows that clearly both variables are important as low pH and high osmolality are both highly toxic. The surface response study provided the range of acceptable limits and the

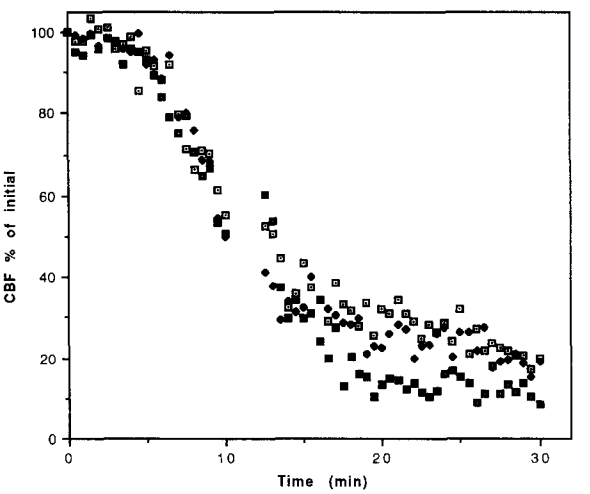

Fig. 3. Comparison of isotonic sea-water (■), medium 199 (■) and 0.9% NaCl (◆) on beat frequency of cilia exposed to 0.03% azelastine (Rhinolast® spray) for 10 min (SD are not shown, $n = 6$).

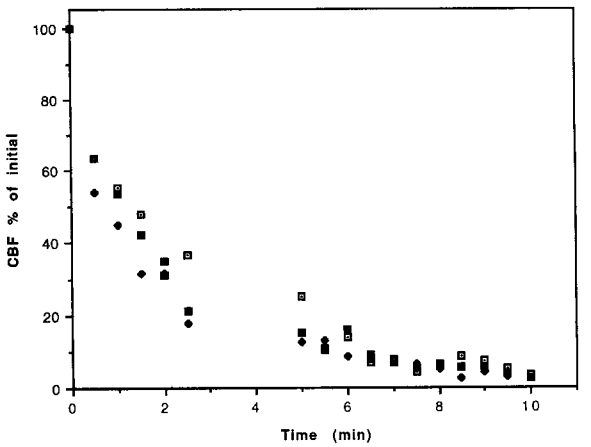

Fig. 4. Comparison of isotonic sea-water (♦), medium 199 (▣) and 0.9% NaCl (■) on beat frequency of cilia exposed to 2% lignocaine hydrochloride for 3 min (SD are not shown, $n = 3$).

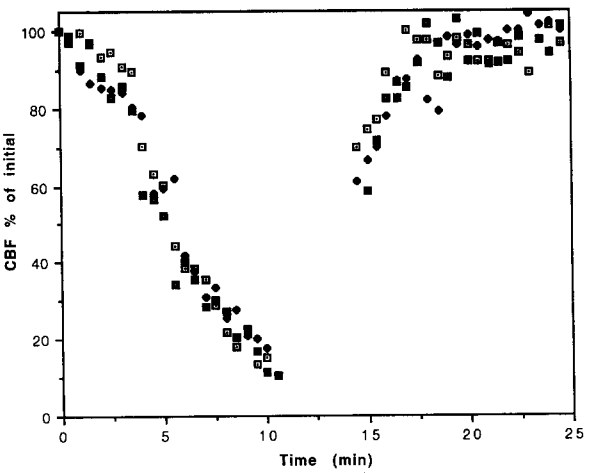

Fig. 6. Comparison of isotonic sea-water (♦), medium 199 (▣) and 0.9% NaCl (■) on beat frequency of cilia exposed to 0.5% chlorbutol solution in 0.9% NaCl for 10 min.

optimum formulation within the range of pH and osmolalities studied (Fig. 8).

The response surface equation is given by:

$$\mathrm{AUC} = 12106.4 + 1936.8\mathrm{pH} + 29.5\mathrm{Os} + 154.6(\mathrm{pH})^2 + 0.056(\mathrm{Os})^2 + 1.17(\mathrm{pH})(\mathrm{Os}) \quad (2)$$

All the coefficients were significant, indicating interaction between pH and osmolality. The optimum formulation with minimum ciliotoxic effect was one with a pH of 6.25 and an osmolality of 332 mOs. If the AUC bounds are restricted to -100 and 500% min^{-1}, then the region of acceptance for pH and osmolality is shown in Fig. 9.

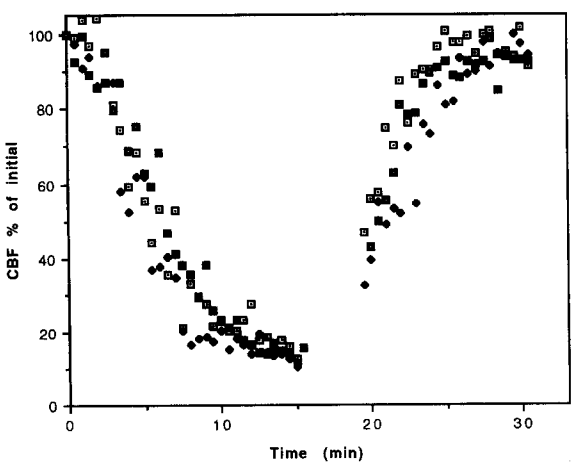

Fig. 5. Comparison of isotonic sea-water (♦), medium 199 (▣) and 0.9% NaCl (■) on beat frequency of cilia exposed to 0.5% chlorbutol solution in 0.9% NaCl for 15 min.

4. Conclusion

The use of a saline solution as a humectant has been recommended in the clinical rhinological literature for the management of rhinitis sicca, allergic rhinitis and when frequent nasal administrations of drugs with antiallergic and/or vasoactive properties are required. For example, Nuutinen et al. (1986) reported beneficial effects of application of a saline solution in a study involving 93 patients with chronic rhinitis.

The present study has demonstrated, that the administration of sea-water made isotonic has no ciliotoxic effect. The surface response study showed, that within the range of pH and osmolalities studied the minimum ciliotoxic effect was observed at pH 6.25 and an osmolality of 332 mOsm. For these reasons the use of sea-water made isotonic (Rhinomer®, Zyma GmbH, München) would be suitable for humidifying the nasal mucosa.

This study has also shown that sea-water made isotonic and 0.9% aqueous sodium chloride solu-

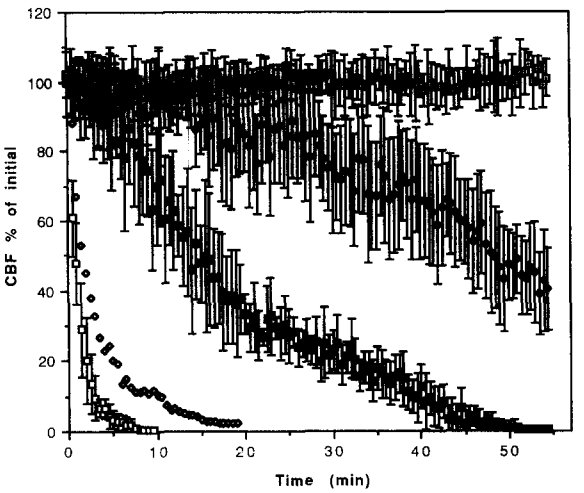

Fig. 7. Effect of sea-water of different pH and osmolality on ciliary beat frequency (error bars refer to SD, $n = 9$). (■) Control (medium 199); (♦) 220 mOsm + pH 9.05; (■) 100 mOsm + pH 8.00; (♦) 100 mOsm + pH 3.00; (□) 220 mOsm + pH 1.97.

tion did not influence the ciliotoxicity of xylometazoline or azelastine. The decrease in ciliary beat frequency following continuous or transient exposure to those two agents was not stopped or slowed by either of those two isotonic solutions or enriched medium 199. Following exposure to chlorbutol the decrease in ciliary beat

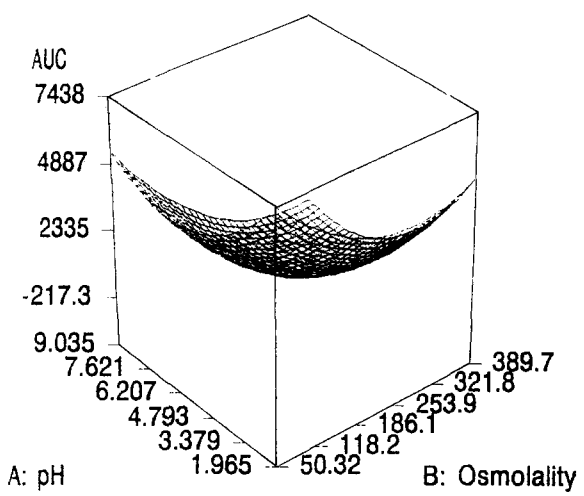

Fig. 8. Surface response for the effect of osmolality and pH of sea-water on ciliary beat frequency (CBF). AUC: area under the CBF curve (see text).

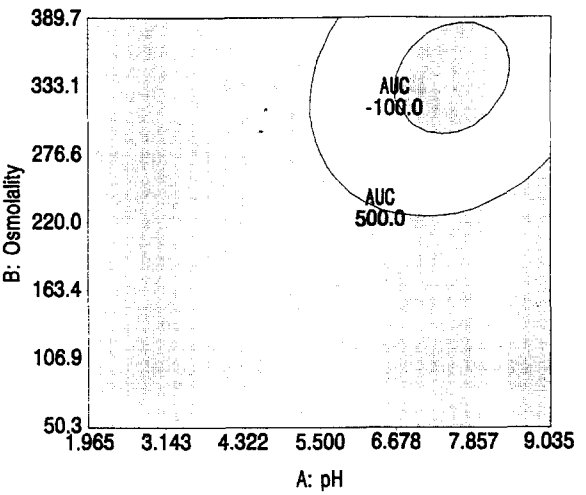

Fig. 9. Region of acceptance for pH and osmolality if area under the curve for ciliotoxicity is restricted to −100 to 500% min.

frequency was fully reversible in the presence of all three solutions (enriched medium 199, isotonic sodium chloride and isotonic sea-water).

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