Author's response to reviews

Title: Efficacy of Topical Cobalt Chelate CTC-96 Against Adenovirus Keratoconjunctivitis in a Rabbit Model.

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Author's response to reviews: see over
Dear Dr. Moylan,

Attached, please find our edited manuscripts: Manuscript 2126963171799982 “In Vitro Efficacy of Cobalt Chelate CTC-96 Against Adenovirus.” and Manuscript 541182537999725 “In Vitro Efficacy of Cobalt Chelate CTC-96 Against Adenovirus Keratoconjunctivitis in a Rabbit Model.”, representing individual original research done in our laboratory. In response to the reviewers comments, the above mentioned two documents have been edited and combined into one manuscript whose title has been slightly edited to reflect the addition of the *in vitro* material. We propose the title of the new document be: “Efficacy of Topical Cobalt Chelate CTC-96 Against Adenovirus in a Cell Culture Model and Against Adenovirus Keratoconjunctivitis in a Rabbit Model.”.

Our responses to the reviewer’s comments are as follows:

**Manuscript 2126963171799982 “In Vitro Efficacy of Cobalt Chelate CTC-96 Against Adenovirus.”**
1) The adenovirus forms plaques; plaque reduction assays are recognized as the gold-standard whereby the relative activity of different compounds may be compared. The ED50 and ED90 results by CTC-96 by plaque reduction should be included. Comparison between plaque reduction assays and virus inhibition assays carried out at higher multiplicity of infection may then be informative.

Both plaque reduction and virus inhibition (“viral inactivation”) assays were carried out and reported. Table 1 from this manuscript (Manuscript 2126963171799982) listing the viral titers has been added to the new combined manuscript. The ED50s and 90s have been estimated from the data and a new table (Table 2) added to the new combined manuscript demonstrating these values. Comparison between plaque reduction and virus inhibition (“viral inactivation”) assays carried out at various multiplicities of infection were informative with respect to both the mechanism of action and toxicity of CTC 96. Both of the original manuscripts, as well as the new combined one are efficacy studies of CTC 96. Neither of the original manuscripts, nor the new combined one are reporting on neither the toxicity of CTC 96 (which was previously studied and reported on in reference to the drug’s antiviral activities against *Herpes simplex*), nor the mechanism of action (which has likewise been previously studied and reported on both in relation to *Herpes simplex* and adenovirus).

2) Standard methods for establishing the mechanism of action should have been applied. These include time of drug addition studies (i.e. before, at the time of, and at various times after virus inoculation).

Both of the original manuscripts, as well as the new combined one, are efficacy studies of CTC 96. Neither of the original manuscripts, nor the new combined one, are reporting on the mechanism of action (which has been previously studied and reported on both in relation to *Herpes simplex* and adenovirus).

3) Drug resistant mutants should be selected. If none can be obtained, this would imply a cellular target for virus inhibition. If drug resistant mutants have been isolated and the resistance loci identified, sequence data should help to define the likely virus protein target(s) for drug action. These data should be included, or cited if published elsewhere.

Identification of the cellular target for virus inhibition is part of “standard methods for establishing the mechanism of action”. However, as previously stated, neither of the original manuscripts, nor the new combined one, are reporting on the mechanism of action of CTC 96 on neither *Herpes simplex* nor adenovirus. This has been previously studied and reported on both in relation to *Herpes simplex* and adenovirus.

4) Classical assays should have been carried out to determine cell toxicity and this should be defined preferably using several different tests including effects on cell viability and replication.

Both of the original manuscripts, as well as the new combined one are efficacy studies of CTC 96. Neither of the original manuscripts, nor the new combined one, are reporting on the toxicity of CTC 96 [which was previously studied and reported on in reference to the drug’s antiviral activities against *Herpes simplex* (Schwartz et al, J Virol]
2001, 75(9):4117-4128; Asbell et al, Cornea 1998, 17(5):550-557). Indeed, Doxovir™ is now in phase II clinical trials for Herpes simplex and FDA approval would not have been granted without toxicity data.

Manuscript 5411825337999725 “In Vitro Efficacy of Cobalt Chelate CTC-96 Against Adenovirus Keratoconjunctivitis in a Rabbit Model.”

1) The activity of the compound in cell culture and its lack of toxicity for cells at inhibitory concentrations (Paper 1, same authors) could be included as a single table of results ED50, ED90) and toxicity values.

Table 1 from Manuscript 2126963171799982 listing the viral titers has been added to this paper. In addition, the ED50s and 90s have been estimated from the data and a new table (Table 2) added demonstrating these values. However, this manuscript is not reporting on the toxicity of CTC 96, which was previously studied and reported on in reference to the drug’s antiviral activities against Herpes simplex (Schwartz et al, J Virol 2001, 75(9):4117-4128; Asbell et al, Cornea 1998, 17(5):550-557). Indeed, Doxovir™ is now in phase II clinical trials for Herpes simplex and FDA approval would not have been granted without this toxicity data.
2) I am slightly concerned that the frequency of sampling (9 times during the day 7:30 am – 7:30 pm) was followed by a 12h gap. If the compound has a very short half-life in the eye, it would appear that the 12 h gap may have an important influence. Preliminary experiments could be carried out with equal intervals between doses over 24h? A wider dose-response range at fixed intervals between doses may be more informative.

Animal treatment was performed during the day from 7:30am – 9:30 pm (thank you for catching this error), meant to mimic the treatment protocol of a human patient suffering from Adenovirus keratoconjunctivitis. Such patients do not typically wake up in the middle of the night to administer their topical antiviral agent. For this reason, efficacy of the drug was measured following this treatment protocol.

3) a) The figure legends should be more explicit so the treatment regimens are readily apparent and symbols such as “C”, “C+”, etc defined.

The figure legends have been accordingly revised.

b) “Ocular plaque assay viral titers” could be simply “infectious virus titers”.

Figure headings have been accordingly revised.

4) I do not see the need for including the data shown in Figures 2 and 3 since the pathogenesis of infection appears to be significantly different from that obtained in the antiviral experiment. eg In the developmental experiment, the virus excretion resolved from day 12-17 compared with day 31 in the second aeries (sic) (Figure 5) that was actually used for the antiviral experment (sic).

Figures 2 and 3 are meant to validate the method of infection which is different from that of some other authors (Gordon et al, Cornea 1996, 15(5):546; de Oliveira et al, Antiviral Res 1996, 31(3):165-172). We demonstrated that the corneal and conjunctival scarification method produced a conjunctival infection with occasional keratitis most similar to human disease, while the corneal injection method yielded primarily stromal involvement.

2) Adenovirus is the most common external ocular viral infection worldwide. Although not permanently blinding, ocular adenoviral infections are associated with significant patient morbidity, including symptomatic distress, and corneal changes causing visual disturbances that can last months to years. About one half of the over 50 serotypes of human adenovirus are known to cause ocular disease in patients. Currently there are no specific efficacious antiviral agents for topical or systemic treatment of Adenoviral infections.

As we did previously, we have carefully read and are familiar with the current “Instructions to Authors” and will comply with the instructions and stated conditions. We have all agreed to the revised draft of the paper, which has neither been published elsewhere nor simultaneously submitted to any other medical journal.

We thank you for your careful consideration of this paper for publication in BioMed Central Ophthalmology. Please contact us if we can be of help in the review process.

Sincerely,
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