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Subacute toxicity and pharmacokinetics of colistin in rats following two intravenous doses of colistin sulfate and dextrin-colistin conjugates

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Introduction

The recent emergence of plasmid-mediated polymyxin resistance highlights an urgent need for new antibiotic compounds or reformulation of promising antibiotic drugs that are currently restricted due to high toxicity or poor pharmacokinetics. We have developed dextrin-colistin conjugates[1], whereby colistin is passively targeted to sites of infection/inflammation by the 'Enhanced Permeability and Retention (EPR) effect'[2]. Here, colistin is then released from the accumulated conjugate by amylase-triggered degradation of dextrin (using Polymer masked-UnMasked Protein Therapy)[3], thereby reducing colistin's clinical toxicity and improving targeting to sites of infection.

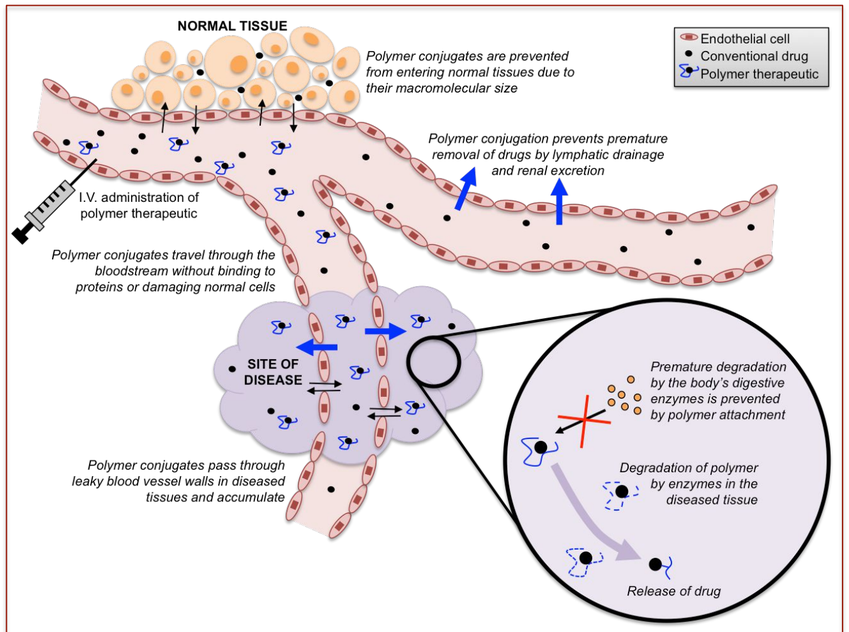

Figure 1. Proposed mechanism of action of dextrin-colistin conjugates.

These conjugates demonstrated sustained colistin release in infected wound exudate[4] and had comparable antibacterial activity to Colomycin®, but with reduced *in vivo* toxicity and prolonged plasma half-life in comparison to colistin sulfate[5].

AIM

This Study compared the pharmacokinetics and toxicity of 8-hourly intravenous doses of colistin sulfate and dextrin-colistin conjugate in rats.

Methods

Characterization of dextrin-colistin conjugates. Colistin was bound to 1.1 mol% succinoylated 8,000 g/mol dextrin using EDC and sulfo-NHS as coupling agents[1,4] and purified by FPLC. The conjugates used in the *in vivo* studies had a colistin content of 6.9% w/w.

***In vivo* study procedure.** Colistin sulfate (20 mg/kg) or dextrin-colistin conjugate (0.5, 5, 20 mg/kg colistin equiv.) were dissolved in 0.9% w/v sterile saline, filtered (0.22 μm) and administered intravenously to 3 Sprague-Dawley rats with a sham intrabronchial respiratory tract infection (agar plug). One hour post-infection, each treatment group were administered two doses intravenously, 8 hours apart. Blood samples were collected 5 min after each dose and 12 and 16 h after the first dose. Plasma concentrations of colistin and TNFα were quantified by ELISA. Cage-side observations of general well-being and behavior were made throughout the study.

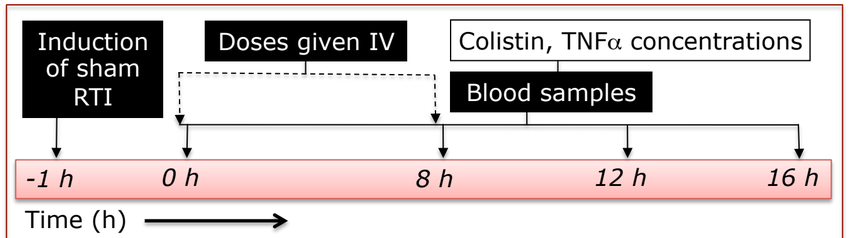

Figure 2. Overview of the experimental design.

Results

Repeat doses of dextrin-colistin conjugate were well tolerated at all dose levels, however animals administered colistin sulfate at 20 mg/kg appeared agitated then lethargic and cold to the touch after the first dose and one animal died 10 min after the second dose.

Throughout all treatment timecourses, circulating TNFα levels were below detectable limits of the ELISA used (<16 pg/mL), showing that dextrin-colistin conjugates did not induce an immune reaction.

Analysis of plasma colistin concentration showed higher levels and extended retention of colistin in rats treated with an equivalent dose of dextrin-colistin conjugate compared to colistin sulfate (plasma colistin concentration 12 h post-dose = 1.5 and 0.8 μg/mL, respectively).

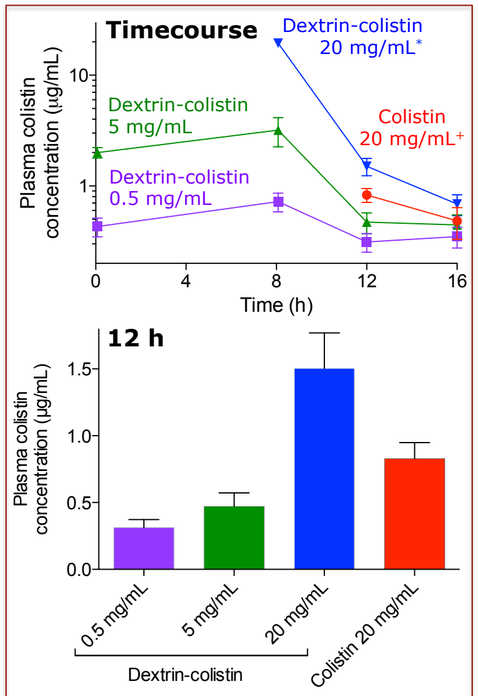

Figure 3. Mean plasma concentration versus time profile for colistin sulfate and dextrin-colistin conjugates (n=3). *0.08 h blood sample not taken, +0.08 and 8.08h blood samples not taken, n=2 (1 rat died).

Antibiotic resistance often occurs when bacteria are exposed to suboptimal concentrations of antibiotic, therefore it is encouraging that dextrin-colistin conjugates were better tolerated at higher concentrations in this study.

In addition, tolerance to repeat dosing of conjugates combined with prolonged plasma retention seen in previous studies indicate that therapeutic colistin concentrations can be achieved and maintained by polymer conjugation. Accumulation of dextrin-colistin conjugates at sites of infection is also expected to increase their efficacy and reduce the emergence of resistance.

CONCLUSIONS

This study demonstrates the safety of repeat dosing with dextrin-colistin conjugates, and defines safe dosing levels for subsequent *in vivo* efficacy models of infection. Ongoing studies are testing the effect of polymer conjugation on the mechanisms and extent of colistin's nephrotoxicity.

References

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