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A new approach for targeting to *Cryptosporidium parvum* using mucoadhesive nanosuspensions: research and applications☆

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Abstract

A new strategy to deliver antibiotics to the *Cryptosporidium*-infected gastrointestinal tract is presented. In an effort to augment the anticryptosporidial effect of clinically used drugs, mucoadhesive nanosuspensions were prepared. They have the ability to reside in the gastrointestinal tract for an extended period. The hydrogel contained bupravaquone nanosuspensions and an adhesive polymer (chitosan) powder dispersed in water. By the development of mucoadhesive nanosuspensions, a potential drug delivery system for poorly soluble drugs has been investigated to overcome bioavailability problems caused by the pathophysiological diarrhoeic situation in patients suffering from cryptosporidiosis. Adapting drug delivery systems to the situation of *Cryptosporidium parvum* infections in man allows increased retention times with a prolonged action at reduced elimination in the gastrointestinal tract. In this communication, in vivo data are presented to document the efficiency of bupravaquone formulated as mucoadhesive polymers to improve its activity against *C. parvum*.

Keywords: Cryptosporidium; Mucoadhesion; Bioadhesion; Bupravaquone; In vivo; In vitro; HIV; Drug delivery; Chitosan; Nanosuspension

Challenges in developing new therapeutic strategies include not only identifying novel active agents, but also improving the delivery of a drug at the biologic level. The development of a new drug delivery strategy is important; even for the therapy of cryptosporidiosis, where no strategy to deliver the drug efficiently to the infected gastrointestinal tract is known. *Cryptosporidium parvum* is a protozoan parasite associated with municipal water supplies that causes diarrhea. In patients with a normal immune system, the disease manifests itself with watery diarrhea, cramps, nausea and anorexia. In immunocompromised people, such as those receiving immunosuppressant drugs or those infected with HIV-1, symptoms are more severe (10–15% of patients with

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AIDS in the United States may acquire *Cryptosporidium* infection). The disease is prolonged, and diarrhea can persist for months, even years. Today, there is no efficient chemotherapy to prevent or treat cryptosporidiosis (Hoepelman, 1996; Laing, 1999).

Many compounds have been tested for their anticryptosporidal potential; most of them failed, but few have shown promise for further development like paromomycin (Nelson et al., 1999), nitazoxanide (Theodos et al., 1998), azithromycin in combination with paromomycin (Smith et al., 1998), roxithromycin (Uip et al., 1998), or by protease inhibitors used in 'highly active antiretroviral therapy' (Nelson, et al., 1999). The importance of bringing drug delivery strategies at an early stage to drug development should not be underestimated. Regarding the pathophysiological situation of *Cryptosporidium*, the localisation of the pathogen in the epithelial membrane of the gastrointestinal tract will be of advantage for developing a mucoadhesive drug delivery system. Mucoadhesive drug delivery systems appear attractive because, as a general feature, nanosuspensions tend to stick to the intestinal wall. Major advantages are that the drug can directly interact with the pathogen coating of the entire infected gastrointestinal tract and avoid non-adequately coated infected segments. Considering the situation of serve diarrhea characterised by rapid excretion of a given dose, integration of an antibiotic in a mucoadhesive hydrogel will prolong retention time and contact time to the pathogen, and therefore improve its bioavailability.

To discuss the potential of mucoadhesive nanosuspensions, bupravaquone was integrated in a hydrogel using 0.5% (M/M) chitosan as polymer. Bupravaquone was identified in vitro and in vivo as a new potent anticryptosporidial drug, reducing parasite growth with no evidence of drug-associated toxicity (Jacobs et al., 2000).

Bupravaquone (Fig. 1) was kindly provided by Dr S.L. Croft, London School of Hygiene and Tropical Medicine, London, UK. The mucoadhesive nanosuspension was prepared according to Jacobs et al. (2001). The in vivo testing for anticryptosporidial activity of mucoadhesive drug formulations is well described by Waters and Harp (1996) and Harp et al. (1992). Briefly, TCR-α-deficient mice are incapable of clearing *C. parvum*; thus, they are useful for screening compounds with potential as a treatment for an ongoing infection. In this study, neonatal mice were challenged with *C. parvum* at 7 days of age, treated with either phosphate-buffered saline (PBS) (controls) or compounds beginning at 10 days of age (treatments were administered twice daily for 6 or 7 days), and euthanised at 21 days of age. Intestinal sections were obtained and examined for *C. parvum* and histopathological changes. Purified oocysts were isolated from faeces collected from calves experimentally inoculated with *C. parvum* oocysts by a method described previously. Oral challenge of mice consisted of 10^3 oocysts in 100 μl of 0.15 M PBS. Mice were challenged with *C. parvum* oocysts at 1 week of age by gavage using a 24-gauge animal feeding needle. Mice were euthanised, and intestinal sections from the distal ileum and cecum fixed in 10% formalin and embedded in paraffin. Histologic sections were cut at a thickness of 4 μm, stained with hematoxylin and eosin, and examined microscopically for *C. parvum* and intestinal lesions. Infectivity scores were determined as described previously. Briefly, a score of 0 represents no *C. parvum* detected, 1 represents few *C. parvum* detected, and 2 represents many *C. parvum* detected. Scores were determined upon examination of individual tissue sections, means calculated for each treatment group, and data presented as group means ± S.E.M.

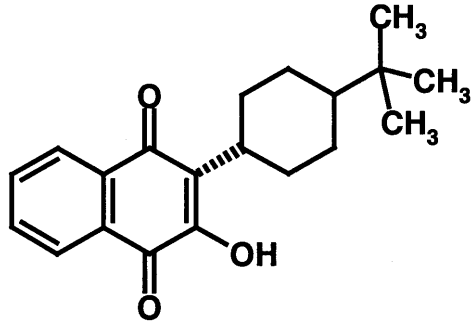

Fig. 1. Structure of bupravaquone.

Table 1
Anticryptosporidial activity of bupravaquone and its formulation as a nanosuspension[a]

Compound formulation	Infectivity score	
	Ileum	Cecum
Bupravaquone in DMSO	1.56 ± 0.18	0.46 ± 0.17
Bupravaquone nanosuspension	1.17 ± 0.15	0.67 ± 0.12
Bupravaquone mucoadhesive nanosuspension[b]	0.17 ± 0.12	0.17 ± 0.14
PBS (control)	2.0 ± 0.2	2.0 ± 0.2

[a] With permission of the Controlled Release Society (Jacobs et al., 2000).
[b] Containing 0.5% (M/M) chitosan

Anticryptosporidial activity of the tested bupravaquone formulations is displayed in Table 1. The present work is the first evidence demonstrating the in vivo usefulness of produced mucoadhesive bupravaquone nanosuspensions for the eradication of *C. parvum*. In comparison with bupravaquone dissolved in DMSO, the infectivity score (IS) for the upper gastrointestinal part (ileum) was reduced from 1.56 to 0.17 by formulating the drug as a mucoadhesive nanosuspension using chitosan as polymer. Formulation of bupravaquone to nanosuspensions without chitosan reduced the IS only moderately (IS = 1.17). Different IS values in the upper and lower part of the gastrointestinal tract (GIT) for the non-modified nanosuspension (IS = 1.17 and 0.17, respectively) indicate that most of the administered bupravaquone will be transported rapidly in the lower gastrointestinal part to accumulate there. This will lead to an insufficient effective dose in the ileum to kill *C. parvum* and may result in a relapse of resistant strains. In contrast, using mucoadhesive hydrogels, the entire GIT is coated and will allow increased retention time also in the upper parts, expressed by a significant reduced IS value (IS(ileum) = 0.17).

In conclusion, the mucoadhesive nanosuspensions more effectively cleared *C. parvum* from the gastrointestinal tract than unmodified nanosuspension due to the prolonged gastrointestinal residence time resulting from mucoadhesion. A dosage form consisting of mucoadhesive nanosuspensions containing an appropriate antimicrobial agent should be useful for the eradication of *C. parvum*. There is also a possibility that the use of mucoadhesive bupravaquone nanosuspensions would allow one to reduce the dose of bupravaquone, which is important from the viewpoint of reducing adverse effects and application frequency for the patient. Chitosan is an acceptable polymer that permits a stable hydrogel without incompatibilities with the nanosupension. The combination of both drug formulation strategies had synergistic effects and provides suitable information to develop a rational treatment for intestinal *Cryptosporidium* infections

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