A novel CDPK1 inhibitor—a potential treatment for cryptosporidiosis in calves?

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Abstract Cryptosporidium parvum is a zoonotic agent that infects humans and animals occasionally causing severe, watery diarrhoea. In immunocompetent hosts, cryptosporidiosis is self-limiting but can have a fatal outcome in immunocompromised individuals. Cryptosporidium is one of the most common causes of waterborne diseases (recreational water and drinking water) in humans, a leading cause of moderate to severe childhood diarrhoea, and a major agent of diarrhoea in calves leading to high economic losses and up to 10 % lethality. So far, available treatment options are insufficient for both veterinary and human clinical disease cases. Here, we report for the first time that the novel bumped kinase inhibitor (BKI) 1294 targeting the calcium-dependent protein kinase 1 (CDPK1) of Cryptosporidium is able to reduce the oocyst shedding of C. parvum by calves—its natural host—without obvious side effects.

Keywords Cryptosporidium parvum · Cryptosporidiosis · Treatment · CDPK1 · Bumped kinase inhibitor

Cryptosporidium parvum is a unicellular, zoonotic agent that infects a wide range of hosts. In humans, cattle and goats, it causes severe watery diarrhoea which is often self-limiting but can also have a fatal outcome. In young calves, C. parvum is the most common cause of diarrhoea affecting nearly every animal (Göhring et al. 2014). Consequently, calves are supposed to be a major source of oocysts infecting humans especially in rural areas with poor sanitation and close contact between humans and livestock. A recent global enteric multicenter study (GEMS) identified Cryptosporidium sp. as the second most common pathogen in children with moderate to severe diarrhoea, and Cryptosporidium was significantly associated with an increased risk of death of 12- to 23-month-old children (Kotloff et al. 2013).

Unfortunately, the treatment options for Cryptosporidium infections are limited. There are only two approved products on the market which are moderately effective against Cryptosporidium. Nitazoxanide is approved in the USA to treat humans, and halofuginone is approved in Europe for the treatment of calves (Lendner et al. 2011).

Calcium-dependent protein kinases (CDPKs) are plant-derived kinases which differ from mammalian kinases in having an enlarged adenosine triphosphate (ATP) binding pocket that is selectively inhibited by bumped kinase inhibitors (BKIs), such as 1294 (Castellanos-Gonzalez et al. 2013). CpCDPK1 has an orthologue in Toxoplasma gondii which was shown to play a crucial role in invasion (Lourido et al. 2010). Consequently, BKIs have been developed to prevent invasion by inhibiting CDPK function (Lourido et al. 2010; Ojo et al. 2010; Johnson et al. 2012). In T. gondii, it has been shown that BKI 1294 selectively targets through TgCDPK1 by overexpressing a mutant form of TgCDPK1. Mutant T. gondii displayed decreased sensitivity to BKI 1294 (Ojo et al. 2010). It was shown that BKI 1294 is a potent inhibitor of CpCDPK1 and prevents the invasion of host cells by C. parvum in vitro and the establishment of the disease in mice (Castellanos-Gonzalez et al. 2013). However, C. parvum does not infect immunocompetent mice making the transfer of results from experiments using immunosuppressed mice to the natural host difficult. Here, we demonstrate for the first time that BKI 1294 reduces C. parvum oocyst shedding in its natural host.
Table 1  Oocyst shedding and reduction in shedding of the BKI 1294 group compared to the other groups; shown is the sum of oocysts shed by each group and per animal

| Group      | No. of animals | Σ Oo per field of vision and group | Σ Oo per field of vision and animal | Percent reduction | Mean no. of Oo per field of vision |
|------------|----------------|-----------------------------------|------------------------------------|-------------------|-----------------------------------|
| BKI1294    | 6              | 38.13                             | 6.40                               |                   | 0.6348                            |
| Mock       | 6              | 155.75                            | 25.96                              |                   | 2.584 (p=0.0008)*                 |
| Untreated  | 5              | 98.18                             | 19.64                              |                   | 2.039 (p=0.0232)*                 |

Oo oocyst

*ANOVA (p≤0.05) and Mann-Whitney test, p values indicate significance to the BKI1294 group

Three groups (n=6) of newborn calves were infected with a single dose (2×10⁷) of C. parvum and were orally treated five times every 2 days with 400 mg/animal BKI 1294, mock control (solvent; 70 % ethanol, 30 % DMSO) or milk replacer starting 1 h after infection. Oocyst output (semiquantitative analysis according to Keidel and Daugschies 2013) and health status were assessed daily in this double-blinded study. The average oocyst output per animal was calculated by summing up the oocyst output of each group for study days 4 to 14 and dividing it by the number of animals per group.

The single dose infection with C. parvum led to mild symptoms and only a few days with watery diarrhea in all groups. In the untreated group, one animal (excluded) died on study day 6 due to a bacterial infection and one animal of the treatment group died on study day 17 due to an omphalogenic systemic infection associated with a patent urachus. BKI 1294-treated animals only showed a single peak of oocyst excretion at day 5 p.i., whereas the control groups showed a second much higher peak at day 8 p.i. The overall reduction in oocyst shedding in the BKI 1294 group was 76 % compared to the mock control group and 68 % compared to the untreated group (see Table 1).

This study shows that BKI 1294 might have a potential to treat cryptosporidiosis in calves and potentially in humans as well. However, only minor differences in faecal consistency and dehydration could be observed between the treatment group and the control groups. This is due to the fact that the infection with C. parvum in general was moderate in terms of diarrhoea and dehydration even in the controls. Seven of the animals, in one of the two cohorts of this experiment, had bloody faeces, which is unusual for C. parvum monoinfection, making it likely that the animals were infected with undetected enteropathogens. Since severe cryptosporidiosis in calves may reflect a multifactorial aetiology (Göhring et al. 2014), we analysed the faecal samples for other common enteropathogens such as rotavirus, coronavirus, Escherichia coli K99 and Giardia with commercially available fast tests (rotavirus, coronavirus, E. coli K99 with strip tests (Bio X diagnostics), Giardia with an ELISA (ProSpecTM)).

Further studies are needed to evaluate if BKI 1294 would reduce symptoms in a setting where animals develop long lasting (>5 days) moderate to severe diarrhoea without co-infections. Since cryptosporidiosis is a multifactorial disease in which severe symptoms occur predominantly in co-infected animals (Göhring et al. 2014), it would also be necessary to test if BKI 1294 would lead to an improvement with co-infections.

Altogether, the study shows that BKI 1294 could have the potential to treat cryptosporidiosis in calves and might also be a candidate to treat humans. The specificity of BKI 1294 for targeting CpCDPK1, and not human protein kinases (Castellanos-Gonzalez et al. 2013), makes it highly probable that no relevant side effects would occur in humans, as it was the case for calves in this experiment.

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