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Optimized dexamethasone immunosuppression enables *Echinococcus multilocularis* liver establishment after oral egg inoculation in a rat model

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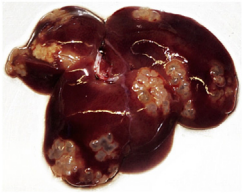
HIGHLIGHTS

- An animal model for alveolar echinococcosis in a naturally resistant host.
- Hepatic alveolar echinococcosis in dexamethasone treated rats after oral inoculation with parasite eggs.
- Immunocompetent RccHan™:WIST and F344/DuCrl rats are resistant to oral inoculation with parasite eggs.

GRAPHICAL ABSTRACT



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ABSTRACT

Comparable with immunocompetent humans, rats are considered highly resistant to *Echinococcus multilocularis* oncosphere invasion, both in nature and after experimental oral inoculation with eggs. Pharmacological immunosuppression with dexamethasone (DMX) was shown to abrogate the resistance of RccHan™:WIST rats, but due to weight losses >20%, many animals had to be excluded from previous experiments. The optimized DXM (Dexafort, MSD Animal Health, Germany) dosage regime presented in this study (each animal: 750 μg DXM at day -13 and 600 μg DXM at day -9 before inoculation) applied subcutaneously to RccHan™:WIST rats, resulted in weight losses ≤20%, but led to liver alveolar echinococcosis (AE) in all eight inoculated animals. Untreated control groups (each n = 8) including RccHan™:WIST (Wistar) and F344/DuCrl (Fischer-344) rats showed no parasite establishment. Antibodies against *E. multilocularis* metacestode vesicle fluid were present in 7/8 of the infected RccHan™:WIST rats 70 days after inoculation but in none of the control animals. Serology can therefore be used to diagnose AE. This optimized animal model enables a high infection rate in rats and may be applied in future immunological and experimental studies.

1. Introduction

Oral inoculation with *Echinococcus multilocularis* eggs, termed primary infection, is the natural route of infection in the intermediate or accidental host. After intestinal oncosphere invasion and venous migration, the parasite mainly establishes and develops in the liver, causing a disease referred to as alveolar echinococcosis (AE) (Stojkovic et al., 2014), which might be lethal if left untreated. Immunocompetent humans are considered rather resistant to infection after ingestion of eggs (Vuitton, 2003; Gottstein et al., 2015). However, in patients with impaired immunological status due to co-infections e.g., with HIV (Sailer et al., 1997) or due to drug-based immunosuppression (Chauchet et al., 2014), the progression of AE development may considerably be increased (Vuitton et al., 2015).

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Experimentally, secondary larval *E. multilocularis* infections comprising parenteral parasite inoculation have been used for decades. Thus, parasite isolates are maintained for research and diagnostics by serial propagation of metacestode material by intraperitoneal inoculation (Romig and Bilger, 1999). Although many AE animal models bypass the gastrointestinal exposure during oncosphere invasion including defensive local host immune regulations and responses, secondary AE infection models are widely used since parenteral application of metacestode material allows for the most rapid and potent development of AE (Asanuma et al., 2006; Küster et al., 2013; Song et al., 2014). Experiments with AE animal models extensively contributed to the current knowledge on host-parasite interaction such as host immune responses and parasite evasion strategies (reviewed by Gottstein et al., 2015, 2017), parasite development (Ohbayashi, 1960; Ohbayashi et al., 1971; Eckert et al., 1983; Mehlhorn et al., 1983) as well as treatment opportunities (Eckert, 1986; reviewed by Hemphill et al., 2014). However, since secondary echinococcosis in rodents considerably varies with regard to parasite growth and development characteristics, obtained results may be difficult to compare or even extrapolate to the natural situation after oral ingestion of parasite eggs. To overcome these limitations, primary infection models were propagated in several studies (Siles-Lucas et al., 2003; Deplazes et al., 2005; Matsumoto et al., 2010; Woolsey et al., 2016; Armua-Fernandez et al., 2016). Depending on host species and strains, metacestode growth and protoscolex formation (fertility) may be delayed, poor or absent (Matsumoto et al., 2010). A major obstacle of these experiments is the fact that parasite eggs are difficult to obtain, either after necropsy of wild foxes in endemic areas (most cost-effective option) or by experimental infections of foxes, dogs or raccoon dogs (Kapel et al., 2006; reviewed by Matsumoto and Yagi, 2008).

Comparable to the situation in humans, the genus *Rattus* is considered highly resistant to oral *E. multilocularis* egg exposure and does not contribute to the maintenance of the general life cycle in nature. In a highly endemic region such as Hokkaido, Japan, only two natural AE cases in *Rattus norvegicus* were described (Okamoto et al., 1992; Iwaki et al., 1993). Under experimental conditions, no hepatic AE could be induced in Wistar or T-cell deficient nude rats after oral egg inoculation (Webster and Cameron, 1961; Iwaki et al., 1995; Armua-Fernandez et al., 2016). In contrast, intrahepatic or intraperitoneal inoculations of metacestode or oncosphere material led to local AE development in rats (Yamashita et al., 2013; Armua-Fernandez et al., 2016). These observations suggest that resistance to oncosphere invasion or AE development most probably involves different mechanisms.

Glucocorticoids such as dexamethasone (DXM) are potent immunosuppressive and anti-inflammatory agents which induce multiple effects on immune cell counts and functions (reviewed by Coutinho and Chapman, 2011). After immunosuppression of Wistar rats with DXM, the barrier-mediating resistance was broken, leading to the assumption that immune-derived mechanisms or the intestinal endothelial or villous structure are responsible for the resistance of rats against *E. multilocularis* oncosphere invasion. Since recent DXM dosage schemes of Wistar rats led to the exclusion of several animals from the experiment due to weight losses >20% (Armua-Fernandez et al., 2016), the aim of this study was to improve the rat model by an optimized treatment regime, causing less animal weight losses but allowing hepatic AE development in the liver after oral egg inoculation. As different strains of mice are well known to vary in susceptibility to oral egg inoculation, as documented by differences in the number of hepatic AE lesions (Matsumoto et al., 2010), a second rat strain named F344/DuCrl was orally inoculated with eggs without immunosuppressive treatment.

2. Materials and methods

2.1. Parasite eggs

Echinococcus multilocularis eggs were obtained from naturally infected foxes during the regular Swiss hunting season in spring 2016. After harvesting adult parasites from the small intestines of foxes, *Echinococcus* eggs were collected after squashing and filtering the worms through 41 μm followed by 21 μm meshes (Lanz-Anliker AG, Switzerland) before being kept at 4 °C in PBS with 100 IU penicillin, 100 μg streptomycin (Life Technologies, Switzerland) for 11 weeks until use. Eggs resistant to sodium hypochlorite (SH) were considered as viable (Deplazes and Eckert, 1988). Briefly, 0.3 ml of a SH solution (2% active chlorine, pH 12) was added to 0.4 ml egg suspension (500–1000 eggs/ml). Within 1 min (i.e. before destruction of embryophores occurred), the total number of eggs was determined in a McMaster-chamber. Five minutes later, oncospheres with intact membranes were counted again. SH resistance was calculated from triplicate counts as percentage of intact oncospheres. Animals were inoculated by oral gavage with eggs suspended in 0.2 ml of PBS.

2.2. Experimental animals

The animal experiment described in this paper was authorized by the Cantonal Veterinary Office of Zurich, Switzerland (permission no. 294/2014). RccHan™:WIST (Wistar) rats (2–3 month of age, female weight range 215–254 g, male weight range 356–402 g) and F344/DuCrl (Fischer-344) rats (2–3 month of age, female weight range 162–170 g, male weight range 255–272 g) were purchased from Envigo (Switzerland) and Charles River Laboratories (United Kingdom), respectively. C57BL/6 mice (2 month of age) were obtained from Charles River Laboratories (Germany). They represented in vivo controls for infectivity of the utilized eggs and were inoculated along with the rats in each experiment. Each experimental and control group consisted of 4 female and 4 male rats.

2.3. Experimental design

The design of the experiment is summarized in Table 1. Rats were divided into five groups (R1-R5) of eight animals each. Immunocompetent control groups consisted of C57BL/6 mice (group M), RccHan™:WIST rats (group R1) and F344/DuCrl rats (group R2) without immunosuppressive treatment. Each rat of group R3 received 750 μg DXM (Dexafort, MSD Animal Health, Germany) at study day (SD) -13 and 600 μg DXM at SD -9, group R4 received 600 μg DXM at SD -9 and group R5 received 600 μg DXM at SD -4. All animals were orally inoculated with 1000 viable *E. multilocularis* eggs at SD 0. Animals were weighted weekly and blood was taken by sublingual venous puncture at SD 0, 21, 35, 49 and 70. Necropsy was carried out 10 weeks post inoculation (p.i.) with all the animals.

2.4. Macroscopical examinations

At necropsy, developed metacestode samples were collected and 1–2 metacestodes per animal were investigated for fertility in a smear sample by direct visualization of protoscoleces under a light microscope.

2.5. PCR for the identification of E. multilocularis

Liver samples of the rats with no macroscopically-clear metacestode establishment were collected. DNA was extracted from the