Veterinary Parasitology 198 (2013) 39–44

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Anticoccidial effects of a novel triazine nitromezuril in broiler chickens

Chenzhong Fei[a], Chao Fan[a], Qiping Zhao[a], Yang Lin[b], Xiaoyang Wang[a], Wenli Zheng[a], Mi Wang[a], Keyu Zhang[a], Lifang Zhang[a], Tao Li[a], Feiqun Xue[a,*]

[a] Key Laboratory of Veterinary Drug Safety Evaluation and Residues Research, Chinese Academy of Agricultural Sciences, Shanghai Veterinary Research Institute, CAAS, No. 518 Ziyue Road, Minhang District, Shanghai 200241, PR China
[b] Institute of Veterinary Feed Control of Zhengzhou, Henan 450003, PR China

ARTICLE INFO

Article history:
Received 13 May 2013
Received in revised form 27 August 2013
Accepted 27 August 2013

Keywords:
Avian coccidiosis
Anticoccidial
Triazine compounds
Cross-resistance
Nitromezuril
Anticoccidial index

ABSTRACT

The anticoccidial efficacy of 2-(3-methyl-4-(4-nitrophenoxy)phenyl)-1,2,4-triazine-3,5(2H,4H)-dione (nitromezuril, NZL), a novel triazine compound, was evaluated in three different studies under experimental conditions. The anticoccidial efficacy was chiefly evaluated using the anticoccidial index (ACI). The resistance level was determined by calculating ACI, percentage optimum anticoccidial activity (POAA), reduction in lesion scores (RLS) and relative oocyst production (ROP). In the dose determination study (study A), NZL was added to the diet at doses of 1, 2, 3, 4, 5 and 6 mg/kg to test its efficacy against coccidiosis caused by *Eimeria tenella*. Groups treated with NZL 1 mg/kg feed could observe the faecal dropping scores and caecal lesions. ACIs of NZL-treated groups reached 179–199. In the study on the anticoccidial efficacy of 3 mg/kg NZL in the diet (study B), only a few faecal oocysts and slight lesions were observed. NZL significantly promoted weight gain (WG) and reduced lesion scores (LS) compared to controls receiving diclazuril (DZL) ($P < 0.05$). ACIs of NZL-treated groups were 193, 192, 191 and 163 for *E. tenella*, *Eimeria necatrix*, *Eimeria acervulina* and *Eimeria maxima*, respectively, whereas those of DZL-treated groups were 185, 176, 176 and 148. In the cross-drug resistance study (study C), ACIs of NZL and toltrazuril (TZL)-treated groups ranged from 188 to 204, which were significantly higher than those of DZL-treated groups ($P < 0.05$). NZL- and TZL-treated groups were sensitive to experimentally induced DZL-resistant *E. tenella*, whereas DZL-treated groups showed complete resistance. No cross-resistance was observed between DZL and NZL or TZL. Based on the abovementioned studies, it was concluded that diets containing 3 mg/kg NZL had an excellent efficacy in preventing coccidiosis in broiler chickens. The activity of 3 mg/kg NZL in the diet was equal or superior to that of 1 mg/kg DZL. These results are of great significance for the future applications of NZL; however, its actual mechanism of action remains unknown. NZL is a potential novel anticoccidial agent suitable for further development.

1. Introduction

Coccidiosis is one of the most important diseases affecting the poultry industry. The estimated total annual cost derived directly or indirectly from diseases caused by *Eimeria* species in raising fowl reaches up to US $800 millions (Williams, 1998). The control of coccidiosis chiefly depends

* Corresponding author. Tel.: +86 02134293460.
E-mail addresses: fqxue@shvri.ac.cn, feiqun@gmail.com (F. Xue).

0304-4017/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetpar.2013.08.024

on prophylactic chemotherapy with anticoccidial drugs (McDougald and Reid, 1994; Peek and Landman, 2011). However, certain *Eimeria* strains, the causative agent of coccidiosis, exhibit or develop resistance to most drugs, thereby rendering the anticoccidials inefficient and leading to clinical failure (Abbas et al., 2011; Allen and Fetterer, 2002; Harfoush et al., 2010; Zhang et al., 2013). This development of resistance has prompted a constant search for products that could replace drugs against which organisms have acquired resistance. Many natural plant extracts and chemical compounds have been reported to have anticoccidial activities in vivo and in vitro (De Pablos et al., 2010; Dkhil et al., 2011; Giannenas et al., 2012; Khalafalla et al., 2011; Kobayashi et al., 1996; Zaman et al., 2012). However, the systemic anticoccidial activity of a novel chemical compound has seldom been reported in recent years.

Triazine coccidiostats, including diclazuril (DZL) and toltrazuril (TZL), are widely used in chickens and turkeys for their remarkable clinical effects on the members of the genus *Eimeria*. TZL is administered via drinking water at 25 mg/L, continuously over 48 h. DZL is registered as an anticoccidial feed additive for broilers at a dose of 1 mg/kg in the diet. The structure of 2-(3-methyl-4-(4-nitrophenoxy)phenyl)-1,2,4-triazine-3,5(2H,4H)-dione, also known as nitromezuril (NZL; CAS:1352755-63-5), is similar to that of TZL and DZL. It is a novel anticoccidial triazine compound developed using systematic structure–activity relationship studies of these compounds in our laboratories (Rhyu and Hopfinger, 1995). The chemical synthesis of this compound has been published in China Patent No. CN 102285930. Early trials have shown that NZL has activity against chicken coccidiosis caused by *Eimeria tenella* (Fei et al., 2010), but its suitable dosage has not been determined. To further confirm the efficacy of NZL against coccidiosis, three batch-controlled studies in battery cages were conducted to determine the dose–effect relationship; the anticoccidial efficacy of NZL against *E. tenella*, *Eimeria necatrix*, *Eimeria acervulina* and *Eimeria maxima* and the cross-resistance of NZL and DZL in DZL-resistant *E. tenella*.

2. Materials and methods

2.1. Birds and feed

One-day-old Pudong yellow broiler chicks were purchased from a local hatchery. The birds were reared on wire-floored batteries under hygienic conditions with ad libitum access to water and a standard diet without drug supplements. The size of the cages was 600 mm × 500 mm × 430 mm; sufficiently large for the growth of 10 birds. Electric radiators and ventilation fans were used to maintain the recommended temperature, and a 24 h light was maintained. The protocol conformed to the guidelines of the Institutional Animal Care and Use Committee of China and was approved by the Ethics Committee of the Faculty of Veterinary Medicine.

2.2. Drugs and parasite

DZL (>99%) was synthesized by the Shanghai Veterinary Research Institute, Chinese Academy of Agriculture Sciences (CAAS). Toltrazuril (Baycox®, 2.5% toltrazuril solution, Bayer HealthCare) and NZL (>98%) were synthesized by the Shanghai Veterinary Research Institute, CAAS. Oocysts of *E. tenella*, *E. necatrix*, *E. acervulina*, *E. maxima* and experimentally induced DZL-resistant *E. tenella* were maintained at the Key Laboratory of Animal Parasitology of the Ministry of Agriculture. *Eimeria* species were identified based on oocyst morphology, predominant caecal lesions, clinical signs and the presence of characteristic schizonts and gametocytes in fresh caecal mucosal smears (Reid et al., 1984). Sporulated oocysts were stored for less than 2 months at 4 °C in 2.5% potassium dichromate. These cells were washed three times with phosphate-buffered saline (PBS; pH 7.2) before use and diluted to the required concentrations in 1-mL solutions. Infected chicks were fed with the respective 1-mL solutions of sporulated oocysts, whereas uninfected chicks were sham-inoculated with PBS alone.

2.3. Experimental design

Our experiments followed the recommendations of the World Association for the Advancement of Veterinary Parasitology (Holdsworth et al., 2004). At approximately 2 weeks of age, the chickens were weighed and allocated to cages using a restricted randomization procedure that approximately equalized their initial weights.

2.3.1. Experiment A: dose determination test

At 14 days of age, 270 chickens were divided into nine groups, with 30 chickens in each group. Each group was further subdivided into three cages of 10 chickens each as biological replicates. The chickens in Groups A1–A8 were using 1-mL oral gavages containing 8×10^4 sporulated oocysts of *E. tenella*. Chickens in Groups A1–A6 were administered NZL at doses of 1, 2, 3, 4, 5 and 6 mg/kg feed, respectively. Chickens in Group A7 were administered 1 mg/kg DZL in the diet. Chickens in Group A8 served as infected non-medicated controls (INC) and those in Group A9 served as the non-infected non-medicated controls (NNC).

2.3.2. Experiment B: anticoccidial efficacy test

At 12 days of age, 260 chickens were divided into four groups with 60 chickens in each group, and the remaining 20 chickens served as shared NNCs. The chickens in the four groups were infected using 1-mL oral gavages containing 8×10^4 sporulated *E. tenella* oocysts, 10×10^4 sporulated *E. necatrix* oocysts, 15×10^4 sporulated *E. acervulina* oocysts and 10×10^4 sporulated *E. maxima* oocysts, respectively. The 60 chickens in each group were further subdivided into three subgroups of 20 chickens each. Chickens in Subgroup B1 were continuously administered a regular diet containing 3 mg/kg NZL, those in Subgroup B2 were administered a regular diet containing 1 mg/kg DZL and those in Subgroup B3 served as INC.