Chloroquine treatment against naturally occurring *Giardia duodenalis* infection in dogs^{*)}

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Summary

The aim of the present study was to evaluate the efficacy of chloroquine on lessening or eliminating cyst shedding in dogs naturally infected with *Giardia duodenalis*. A total of 26 dogs naturally infected with *G. duodenalis*, from various breeds, ages and of both sexes were selected and randomly assigned into two groups based on treatment (group I, n = 20 dogs treated orally with 2.5 mg/kg chloroquine twice daily for 5 consecutive days) and control (group II, n = 6 untreated control dogs). Diagnosis was based on microscopic faecal examination and rapid test kits. Cyst excretion was determined on days 0, 3, 7 and 10, before and after treatment. Evaluation of the percentage reduction in cyst excretion revealed 99.8% efficacy on day 3 and 99.9% on days 7 and 10 in the treatment group. Geometric means of the number of excreted cysts did not change significantly in the control group. Chloroquine at the proposed dosage might be a practical in application, low priced and highly effective treatment option in dogs with giardiasis.

Keywords: *Giardia duodenalis*, dog, chloroquine, treatment

Giardia duodenalis (syn. *G. intestinalis*, *G. lamblia*), an endemic and enteric flagellated protozoan parasite, has commonly been encountered in humans and animals worldwide (30). The prevalence rates for *Giardia* infection in dogs and cats may change depending on the population, area, diagnostic method, and health status of the animal and is commonly distributed from 5% to 15% (31). Higher levels of infection, up to 100%, have been reported in younger dogs housed in kennels, breeding operations, and research facilities (2).

Giardia infections in dogs are not usually accompanied by clinical signs, although giardiasis may cause anorexia, weight loss, lethargy, vomiting and enteritis with acute or chronic diarrhea (31). Diarrhea is mainly caused by intestinal malabsorption and hypersecretion (8). Younger or immunosuppressed animals have been reported to be more susceptible to disease (12).

The role of dogs as a source of human giardiasis seems to be unclear: several reports are divergent about the zoonotic potential. Epidemiological and molecular studies revealed that *G. duodenalis* is composed by at least seven assemblages (A to G). Genotypes of assemblages A and B are mainly known to infect humans (2,

18). Assemblages C and D were determined as species-specific to dogs (30). However, DNA of assemblages A and B were also described in dogs (25).

The high prevalence rates, undesirable clinical findings, and potential zoonotic risk warrant suitable treatment of giardiasis in dogs. Although there is no approved veterinary drug on the market, several compounds such as drugs in the nitroimidazole (1, 16) and benzimidazole class (4, 5), febantel-praziquantel-pyrantel combinations (3), azithromycin (35), silymarin (10) have reported effectiveness in reducing the level of cyst shedding and clinical signs. Metronidazole seems to be the first line drug for the treatment of giardiasis by most of veterinary clinicians (31). Albeit it has been concluded that controlled studies are lacking and administration of metronidazole has been associated with central nervous system toxicity in dogs (11, 17). Moreover, inefficacy of metronidazole is commonly reported in the treatment of giardiasis, and reinfection has been compromised as the most common cause of treatment failure (29). Moreover, there is an increasing number of treatment failures reported in human medicine (14). Thus, both veterinary and human medicine are seeking new therapeutic options.

The aim of the present study was to determine the efficacy of chloroquine, a 4-aminoquinoline compound

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synthetic agent that has been recognized as an old drug with a new perspective against giardiasis (14), to those dogs naturally infected with *G. duodenalis*.

Material and methods

Animal population. The present study was performed among 26 dogs referred to the Adnan Menderes University, Faculty of Veterinary, Department of Internal Medicine located in Aydin city, Egean Region of Turkey. Animals from different breeds, aged 3 to 11 months, and of both sexes (14 female and 12 male) were selected to those of presenting clinical signs compatible with a susceptible *Giardia* infection such as diarrhea, abdominal pain and/or vomiting.

Prior to the study design, a total of 95 dogs suspected of *Giardia* infection were analyzed with rapid test kits (SNAP *Giardia* test, IDEXX Veterinary Diagnostics, United States). Out of those 95 analyzed cases, dogs with naturally occurring giardiasis ($n = 26$) were randomly assigned into two groups. Group I ($n = 20$) involved chloroquine (Kutlu 250 mg tablet[®], Keymen Ilac San. Tic. Ltd. Sti, Turkey) treatment orally at a dose of 2.5 mg/kg twice daily for 5 consecutive days; Group II ($n = 6$) were left as controls and received placebo. Chloroquine was applied directly into the mouth, followed by 5 ml water by the investigator; the placebo included an equivalent volume of water. General health control was carried out and faecal samples were collected on days 3, 7 and 10 after the first administration.

Both groups of dogs were consuming commercially prepared dog food and were housed in individual pens having separate facilities for preventing cross-contamination during the study. The pens where the cases were kept were cleaned and disinfected with a product containing quaternary ammonium (Derdevice Plus Y, Deren Ilac, Turkey) for elimination of existing environmental parasitic contamination.

The study protocol was approved by the institutional laboratory animals ethics committee of Adnan Menderes University HADYEK (with no: 2012/088 and date 04.10.2012). Prior to enrolment in the present study, informed written consent was obtained from all of the owners/animal care takers. Taking into account ethical concerns, only a limited number of dogs served as controls. Albeit at the end of the trial, all positive control dogs were also treated with chloroquine at the same dosage to the previously treated animals.

Laboratory analysis. After initial physical examination, all dogs were screened on days 0, 3, 7, and 10 to confirm the presence/absence of *G. duodenalis* cysts (and for other possible intestinal parasites relevant to dogs, i.e. *Cryptosporidium* sp. oocysts in the faeces). On days 0 and 10 hematological (WBC, RBC, HCT, MCHC, PLT) and serum biochemical (ALT, AST, creatinine, triglyceride, urea) values were determined.

Faecal examination. Faecal samples were collected manually from the rectum of all dogs on days 0 (before treatment), 3, 7 and 10 (after treatment). Furthermore 1.5 g of faecal material was mixed with 33% ZnSO₄ solution (15 ml) and strained onto centrifuge tubes, which then was centrifuged at $880 \times g$ for 5 minutes, similarly to what has been described elsewhere (34). The latter procedure was followed by collection of a 50 μ l of the supernatant, which was then placed on a microscope slide with Lugol iodine,

covered by a slip. The slide was examined microscopically under $400 \times$ power for detection of *Giardia* cysts. Afterwards this step was repeated twice from different samples belonging to each dog by a single blind researcher. The number of cysts per gram of faeces (CPG) was calculated by $[(\text{number of cysts identified} \times 100)/1.5]$.

Assessment of treatment efficacy. Chloroquine treatment efficacy in the present study was assessed by microscopic examination of faecal samples collected on days 0, 3, 7 and 10, and percentage of reduction in CPG for the treatment group compared to the control group. The percentage of reduction in cyst excretion was calculated by use of the Henderson–Tilton formula (23), including geometric mean of CPG similar to those of Geurden et al. (19) which was also used by Ural et al. (34): $100 \times [1 - (Ta \times Cb)/(Tb \times Ca)]$.

Ta and Tb represented the geometric mean of CPG in the chloroquine treatment group before (Tb) and after (Ta) treatment, whereas Ca and Cb represented before (Cb) and after (Ca) placebo administration in the control animals.

Statistical analysis. Statistical analyses were performed using the SPSS statistical software package (version 22; SPSS Inc., Chicago, IL). The results for CPG in both control and treatment groups were tested for normality using the Kolmogorov-Smirnov test. The CPG was not normally distributed. Related samples: Friedman's two way-analysis of variance test was done before (day 0) and after the start of treatment (days 3, 7, 10) for each group. A Mann-Whitney-U test was used to compare differences between groups for each day. Probability (*P*) values: < 0.05 was considered to indicate a significant difference.

Results and discussion

Cyst excretion. The results of the cyst counts were presented in Table 1. Throughout the study period dogs in the control group remained positive; moreover 3 out of 6 dogs presented an increase in cyst counts on day 10 (ranged between 150 000-300 600 CPG) compared to the initial values (100 000-300 300 CPG), albeit there was no statistical significance. Treatment with chloroquine reduced cyst shedding in all cases on day 3 (99.98%), increase in the rate of reduction of cyst shedding (99.99%) and the absence of cyst excretion were detected in 11 patients on day 7, reduction of the cyst shedding was continuing (99.99%) and the absence of cyst excretion were determined in 16 cases on day 10.

Tab. 1. The geometric means of the number of the excreted CPG in the control and chloroquine treated groups at each sampling day (before treatment [day 0] and after treatment [days 3, 7, 10]). The percentage of reduction calculated based on geometric means is presented.

	Day 0	Day 3	Day 7	Day 10
Control ($n = 6$)	207 918 ^a	142 893 ^a	52 287 ^a	223 765 ^a
Treatment ($n = 20$)	165 293 ^a	315 ^b	125 ^b	7,7 ^b
p value	0.381	0.0001	0.002	0.0001
Reduction in cyst excretion	–	99.98%	99.99%	99.99%

Explanations: a, b – different letters indicate significant differences between rows and columns ($p < 0.05$).

Distribution of cyst number in the treatment group before treatment ranged from 105 000-310 000 CPG; cyst number reduced gradually following chloroquine treatment on days 3 (0-20 000 CPG), 7 (0-15 000 CPG) and 10 (0-7200 CPG).

Hematological and serum biochemical analysis.

There was no statistically significant difference in hematological and serum biochemical variables on days 0 and 10 between the groups studied (data was not shown).

Treatment applications. Dogs in both groups had clinical signs compatible with naturally occurring giardiasis, involving diarrhea on day 0. No observable and significant side effects to chloroquine application were detected in the treatment group during the study. Neither coccidiosis nor cryptosporidiosis infection were found in any of the animals enrolled throughout the study period.

G. duodenalis has been noticed as a significant and common intestinal pathogen. Relatively few choices are commercially available for anti-giardial treatment and there is no approved veterinary drug on the market (31). 5-nitroimidazole compounds such as metronidazole are first line anti-giardial treatment choice in human medicine (9). However, cases refractory to treatment applications within the latter compounds are becoming frequent (14). Several drugs have been tested against giardia infections in dogs (3-5, 10, 16, 35) but metronidazole is used routinely for treatment of giardiasis (16, 31). However, controlled studies are lacking in veterinary medicine and administration of metronidazole has been associated with toxicities (11, 17). Therefore, it is prudent to suggest that there is a need for searching for new treatment choices both in human beings and in the veterinary field.

Chloroquine, an old but promising agent, has now been recognized as an old drug with a new perspective against giardiasis (13). Chloroquine, a 4-aminoquinoline compound, is a synthetic agent. The latter compound was first synthesized at the Bayer laboratories in 1934, modified from quinacrine via replacing its acridine ring by a quinoline ring (13). For a good number of years, chloroquine was used as a first line option for the vast majority of treatments of malaria. Currently it has widely been used in many locations of the world as a reliable treatment against uncomplicated malaria (13). Furthermore the usage of this drug has not been limited to malaria, since it is recommended as a second-line treatment option for other infections, such as HIV (7), sarcoidosis (15), amoebiasis (28) and noninfectious diseases in humans (14).

In a prior study, *in vitro* activity of chloroquine against 25 isolates of *G. duodenalis* trophozoites demonstrated that more than half of the isolates were extremely susceptible (21). Furthermore, chloroquine was shown to be more effective than metronidazole on *G. duodenalis* trophozoites (26). Although the mechanism of action of chloroquine is not entirely under-

stood, as the latter compound's efficacy was attributed to a reduced ability of *Giardia* trophozoite *in vitro* for attaching to surfaces. Hence it was also claimed that *in vivo* conditions possessed the existence of a similar effect (6). Chloroquine has also been recognized as a lysosomotropic agent. Lysosomes prevent endosomal acidification that cause the inhibition of endocytosis, degradation, recycling, and secretion of protein (32). *Giardia* exhibits peripheral vacuoles rather than a defined endosomal/lysosomal system. It may be suggested that the latter compound might inhibit peripheral vacuolar functions (33). These mechanisms might be related to the high efficacy against *G. duodenalis* in the present study.

The efficacy of chloroquine in dogs is comparable to what has been described in lambs and calves naturally infected with giardiasis, in which an oral dose of 2.5 mg/kg during 5 consecutive days resulted in 100% efficacy, as no cysts were found in faeces after treatment (22, 24). In the present study chloroquine treatment significantly reduced the cyst excretion by 99.8% on day 3, afterwards 99.9% on days 7 and 10.

Cleaning and disinfection of the environment are recommended to reduce the re-infection risk (19, 20). Studies showed that dogs re-excreted cysts shortly after the end of treatment (20). In addition, giardia cysts may survive more than a month in soil (27). Therefore, treatment protocols should be combined with disinfection of the environment (20). In our study, individual boxes of dogs were cleaned and disinfected every day with a quaternary ammonium product. No re-infection was determined on days 7 and 10 in the treatment group. However, it may be suggested that a long term follow-up is necessary to evaluate re-infection.

The high cyst reducing activity of chloroquine against giardiasis may provide an important benefit in private veterinary clinics or other veterinary service facilities (34). The easily availability of this cheap drug on the market, its cost (approximately 0.05 dollars per dog for 5 days treatment), have been significant impediments for usage of this antimalarial drug against giardiasis in dogs.

In conclusion chloroquine might be a practically applicable, reasonably priced and highly effective treatment option in dogs with giardiasis. Moreover, side effects in dogs receiving higher doses should be evaluated both in healthy and diseased subjects. Further studies with different doses and durations of treatment in a larger and longer study might be beneficial.

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