The gut-brain axis: effect of antibiotics on canine drug-resistant idiopathic epilepsy

De darm-hersenas: effect van antibiotica bij honden met medicatieresistente idiopathische epilepsie

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ABSTRACT

Approximately thirty percent of the dogs with idiopathic epilepsy develop multidrug resistance. Therefore, the search for new non-drug treatment alternatives is important. There is a growing interest in the gut-brain axis and its role in the pathogenesis of epilepsy. The gut microbiota can influence brain function by different neural, endocrine, immune and metabolic pathways, but this process is not yet fully understood. In this study, five client-owned dogs with drug-resistant, idiopathic epilepsy received amoxicillin-clavulanic acid as add-on orally (mean duration 32 days (range 21-64 days)). The mean isolated epileptic seizure frequency of these five dogs was 3.3/week (range 2.5-6.5) with a mean cluster seizure frequency of 0.9/week (range 0.4-1.5). During the amoxicillin-clavulanic acid treatment, three dogs showed complete seizure freedom, one dog showed an 80% decrease of both isolated epileptic seizure and cluster seizure frequency, and one dog showed an increase in isolated epileptic seizure and cluster seizure frequency of 54% and 38%, respectively. In the two-month follow-up period after cessation of the antibiotic administration, the mean isolated epileptic seizure and cluster seizure frequency increased again to 1.5 seizures/week (range 0.9-2.8) and 0.4 clusters/week (range 0.4-0.6), respectively. The preliminary results in this study highlight the need for future research into the role of the canine gut-brain axis in idiopathic epilepsy.

SAMENVATTING

Ongeveer dertig procent van de honden met idiopathische epilepsie ontwikkelen medicatieresistentie. Daarom is de zoektocht naar nieuwe niet-medicamenteuze behandelingsalternatieven belangrijk. De darmmicrobiota, een complexe verzameling van micro-organismen in de gastro-intestinale tractus, wordt steeds vaker vooropgesteld als een potentieel bepalende factor bij verscheidene neurologische aandoeningen. Ook in het huidige diergeneeskundig epilepsie-onderzoek is er een groeiende interesse voor de zogenaamde “microbiota-gut-brain axis”. De darmmicrobiota kunnen de hersenen beïnvloeden via verschillende neurale, endocriene, immuun- en metabole wegen, maar over dit proces is nog niet voldoende bekend. In deze studie kregen vijf eigenaarshonden met medicatieresistente, idiopathische epilepsie extra amoxicilline-clavulaanzuur oraal toegediend (gemiddelde duur 32 dagen (tijdspanne van 21-64 dagen)). De gemiddelde frequentie van de geïsoleerde epileptische aanvallen van deze vijf honden was 3,3/week (tijdspanne 2,5-6,5) met een gemiddelde frequentie van clusteraanvallen van 0,9/week (range 0,4-1,5). Tijdens deze toediening werden drie honden aanvalsvrij, één hond vertoonde een

afname van 80% van zowel geïsoleerde epileptische aanvallen als de frequentie van clusteraanvallen en bij één hond werd een toename van respectievelijk 54% en 38% van de geïsoleerde epileptische aanvallen en de frequentie van clusteraanvallen gezien. In de follow-up periode van twee maanden na het stopzetten van de antibiotica steeg de frequentie van de geïsoleerde epileptische aanvallen en van de clusteraanvallen opnieuw tot 1,5 aanvallen/week (tijdspanne 0,9-2,8) en 0,4 clusters/week (tijdspanne 0, 4-0,6), respectievelijk. De voorlopige resultaten van deze studie benadrukken de noodzaak van verder onderzoek naar de rol van de darm-hersenas bij de honden met idiopathische epilepsie.

INTRODUCTION

Epilepsy is the most common and challenging chronic neurological disorder in dogs characterized by spontaneous recurrent epileptic seizures (Berendt et al., 2015). Based on the International Veterinary Epilepsy Task Force (IVETF), epilepsy can be defined according to the cause into idiopathic or structural epilepsy (Berendt et al., 2015). Structural epilepsy is defined as epilepsy that is caused by an underlying structural brain pathology (Berendt et al., 2015; De Risio et al., 2015). Idiopathic epilepsy (IE) can be further divided into genetic epilepsy, suspected genetic epilepsy and epilepsy of unknown cause (Berendt et al., 2015).

Despite the administration of anti-seizure drugs (ASDs), approximately 30% of dogs with IE are drug-resistant, which is very similar to the situation in human medicine where up to 36% of the patients do not respond to the available ASDs (Kwan et al., 2010). In drug-resistant dogs with IE, there is no reduction of at least 50% of the epileptic seizure frequency, even when a minimum of two ASDs are administered at a maximal dose or at optimal serum concentrations (De Risio et al., 2015). Furthermore, the side effects of ASDs and the epileptic seizure frequency do not only contribute to a reduced quality of life of dogs and owners, but may lead to the owner's decision for euthanasia (Potschka et al., 2015). The importance to search for new non-ASD therapeutic options for epilepsy management in dogs is therefore increasing.

Currently, there are a few non-drug treatment alternatives to manage IE in dogs. Both dietary changes and neurostimulation have regained attention lately (Berk et al., 2020; Charalambous et al., 2020; Law et al., 2015; Molina et al., 2020; Pilla et al., 2020). Dogs with drug-resistant IE receiving a diet high in medium chain triglycerides (MCT-diet) show a reduced epileptic seizure frequency compared to a standardized placebo diet (Law et al., 2015). It is assumed that the mechanism of action involves the alteration of gut microbiota (Pilla et al, 2020). The connection between the gut microbiota and the brain, which is bidirectional, is called the gut-brain axis (GBA). In multiple human and canine studies, it has been hypothesized that the GBA could play a role in the pathogenesis of epilepsy (De Caro et al., 2019; Olson et al., 2018; Pilla et al., 2020).

Manipulation of the GBA by probiotics, antibiotics, diets high in MCT and fecal microbiota transplantation (FMT), may offer a benefit in the epileptic seizure control of dogs with IE (Gomez-Eguilaz et al., 2018; He et al., 2017; Law et al., 2015). Antibiotics have been administered in humans with drug-resistant epilepsy with promising results (Braakman and van Ingen, 2018).

At the Small Animal Hospital at the Faculty of Veterinary Medicine (Ghent University), a client-owned one-year-old male Presa Canario was observed with drug-resistant IE. The dog received an antibiotic treatment during one month for a presumed infectious mono-arthritis. The dog became seizure-free during antibiotic treatment. Based on the findings in this particular dog, the aim of this clinical trial was to evaluate the effect of amoxicillin-clavulanic acid on the isolated epileptic seizure and cluster seizure frequency in four other dogs with drug-resistant IE. Cluster seizures can be defined as the occurrence of two or more epileptic seizures within 24 hours (Berendt et al., 2015). To the best of the authors' knowledge, there are currently no reports on the effect of antibiotic treatment on canine epileptic seizure frequency.

Material and methods

A neutered male Presa Canario (dog 1) was diagnosed with IE at the age of 6.5 months based on a normal complete blood examination (hematology, biochemistry, electrolytes, ammonia, calcium) and computer tomography (CT) and magnetic resonance imaging (MRI)-scan of the brain. The dog developed a drug-resistance at the age of one year and one month. At that time point, the oral maintenance treatment of the dog consisted of phenobarbital (3.3 mg/kg BID, Phenoleptil, Kela, Belgium) and potassium bromide (16 mg/kg BID, Libromide, Dechra, UK) with optimal serum concentrations of 30 mg/l and 2036 mg/l, respectively. Additionally, levetiracetam was administered as an oral pulse therapy when cluster seizures occurred, and intranasal midazolam was given in case of a status epilepticus. At the age of one year and two months, the dog was admitted to the Small Animal Hospital (Ghent University) with fever, lethargy and left pelvic limb lameness. A suspicion of an infectious mono-arthritis was made. At that moment, the dog's mean isolated epileptic seizure frequency was 2.5/week and mean cluster frequency was 0.9/week in the last two months.

After initiation of amoxicillin-clavulanic acid (20 mg/kg twice per day; Kesium, Ceva, Belgium), the clinical signs disappeared quickly and the treatment was continued for 28 days. During antibiotic treat-

ment, the owner noted an absence of isolated epileptic seizures and epileptic cluster events. Twenty-nine days after cessation of the antibiotic treatment, the epileptic seizures re-occurred and a mean isolated epileptic seizure frequency of 0.9/week and a mean cluster frequency of 0.4/week were noted till two months after the antibiotic treatment.

Based on this observation, another four dogs with drug-resistant IE were given amoxicillin-clavulanic acid during a mean time period of 33 days (range 21-64 days). During the amoxicillin-clavulanic acid treatment, the dose of the ASDs was not altered in any dog, but the diet was altered in one dog (dog 3). In dog 3, the owner changed the diet from commercially available MCT-diet (Purina Pro Plan NC NeuroCare, Nestlé Purina PetCare, USA) to raw food for ten days during the antibiotic treatment of 57 days, because the dog lost his appetite.

RESULTS

During the amoxicillin-clavulanic acid treatment, three dogs (dog 1, 2 and 5) showed epileptic seizure freedom within one day after initiation of the antibiotic administration for a mean time of 51 days (range 41-57 days). Dog 4 showed an 80% decrease in both isolated epileptic seizure frequency and epileptic clusters. Dog 3 showed an increase in isolated epileptic seizure frequency and epileptic clusters of 54% and 38%, respectively. The baseline characteristics and the epileptic seizure control before, during and after the antibiotic treatment of all five dogs are presented in Tables 1 and 2, respectively. After cessation of the antibiotic administration, three dogs (dog 1, 2 and 5) exhibited an increase in isolated epileptic seizure frequency and epileptic cluster seizures after a mean time of 25 days (range 13-34 days) (Table 2).

DISCUSSION

To the authors' knowledge, the effect of amoxicillin–clavulanic acid administration on the isolated epileptic seizure frequency and epileptic cluster events in dogs with drug-resistant IE has never been described before. Three of the five dogs reported in this study showed complete seizure freedom and one dog showed a decrease of 80% of both isolated epileptic seizure frequency and epileptic cluster seizures during antibiotic administration. These preliminary results are interesting, certainly given the high number of epileptic seizures prior to antibiotic administration.

An area of research which is gaining more attention lately in canine and human epilepsy research, is the one that examines the connection between the brain (epilepsy) and the gut microbiome. The gut microbiome is the collection of genes of all micro-organisms, whereas gut microbiota refers to the specific micro-organisms themselves. The gut microbiota can influence brain function by different pathways (De Caro et al., 2019). There is increasing evidence that not only the gut microbiota can produce signals to the brain, but also the brain can influence the gut microbiota. The connection is bidirectional and is globally named the GBA. However, the specific mechanism of both signals is not yet fully elucidated, but neural, endocrine, immune and metabolic influences are suspected (Peng et al., 2018; Wanleenuwat et al., 2020). Interestingly, the canine gut microbiome resembles more the human gut microbiome than the gut microbiome of mice and pigs. This means there is a big similarity in gut microbiome of dogs and humans. This offers opportunities to extrapolate from human microbiome studies to canines and to use canine models for future human applications in which microbiome manipulations may be interesting (Wanleenuwat et al., 2020).

Based on the study by Peng et al. (2018), there is evidence that the gut microbiota composition in patients with drug-resistant IE is considerably altered in comparison to drug-sensitive patients. Especially the microbial community richness is higher in drug-resistant humans than in drug-sensitive humans. This bacterial enrichment is a result of an increase in several rare bacteria mainly belonging to the *phylum Firmicutes* and *phylum Verrucomicrobia*. On the contrary, the patients with drug-sensitive IE show a higher abundance of *Bifidobacteria species* and *Lac-*

Table 1. An overview of the baseline characteristics of all five dogs.

Dog	Age of epileptic seizure onset	Gender	Breed	TIER-level	Diet (during antibiotic-treatment)	ASDs
1	10m	Mc	Presa Canario	2	Raw food	▪ Phenobarbital ▪ Potassium bromide ▪ If clusters: levetiracetam ▪ If status epilepticus: intranasal midazolam
2	1y 7m	Vc	Australian Shepherd dog	1	MCT-diet	
3	3m	Vc	French bulldog	2	First: MCT-diet Later: MCT-diet + raw food End: MCT-diet	
4	3y 5m	Mc	Mixed breed	2	MCT-diet	▪ Phenobarbital ▪ Levetiracetam
5	2y 6m	M	Samoyed	1	MCT-diet	

Abbreviations: ASDs, anti-seizure drugs.

Table 2. Mean isolated epileptic seizure frequency/week (IS/week) and mean cluster seizures/week (CS/week) two months before, during and two months after antibiotic treatment of all five dogs.

	Two months before antibiotics		During antibiotics		Two months after antibiotics		Antibiotic intake (days)
	IS/week	CS/week	IS/week	CS/week	IS/week	CS/week	
Dog 1	2.5	0.9	0	0	0.9	0.4	28
Dog 2	6.5	1.5	0	0	1.6	0.4	21
Dog 3	2.6	0.4	4.9	1.0	2.8	0.6	64
Dog 4	2.5	0.6	0.5	0.1	1.0	0.4	21
Dog 5	2.6	1.0	0	0	1.4	0.3	28

Abbrevations: IS: mean isolated epileptic seizure frequency; CS: mean cluster seizures.

tobacillus species, which is like the microbiota composition in healthy humans. Patients that have been treated with probiotic mixture (*Lactobacillus, Bifidobacterium* and *Streptococcus* strains) show a decrease in epileptic seizure frequency (Gomez-Eguilaz et al., 2018). It has been shown that probiotics alter the gut microbiota composition and thereby potentially influence epileptic seizure control in humans (Dahlin and Prast-nielsen, 2019). Based on the results of this study, it is assumed that *Lactobacillus species* would play a protective role in the development and progression of neurological disease. Muñana et al. (2020) evaluated the gut microbiome in dogs and found a similar abundance of *Lactobacillus species* in dogs with drug-sensitive IE and in healthy dogs. A trial in dogs with IE receiving an MCT-diet showed seizure freedom or a reduction in seizure frequency in 71% of the dogs in comparison with a placebo diet (Law et al., 2015). Pilla et al. (2020) investigated the changes in the microbiota composition in dogs with IE and found significant changes in microbiota composition before and after an MCT-diet trial. The MCT-diet did not affect the abundance of the gut microbial communities, but increased bacterial richness. A specific bacterial *genus X57N15* was identified as a potential biomarker for MCT-diet consumption. This genus was correlated with *Akkermansia species,* indicating they occupy a similar niche. In two dogs *Parabacteroides species* were found (Pilla et al., 2020). Those two bacterial genera have been associated with seizure protection in mice, that were treated with a ketogenic diet (Olson et al., 2018). In humans, 83% of the drug-resistant IE patients show seizure freedom until two weeks after the antibiotic treatment (Braakman and van Ingen, 2018). The patient described by He et al. (2017) received FMT and became seizure-free during a twenty-month follow-up. In the previous studies with antibiotics by Braakman and van Ingen (2018) and with FMT by He et al. (2017), no microbiota analyses were performed; however, because of the positive impact on the epileptic seizure frequency, these results suggest a remodeling of the gut-microbiota. The results of these aforementioned studies suggest that gut-microbiota alterations are the driving force behind those non-drug approaches (i.e. antibiotics, probiotics, MCT-diet and FMT).

In the present study, one dog showed an increase in epileptic seizures and clusters during antibiotic administration. However, during this administration, the owner changed the dog's diet because of a loss of appetite. Multiple human and canine studies have shown that the diet influences the fecal microbiome and metabolome. The fecal metabolome is the collection of small molecules, metabolites, within the feces, such as bile acids, cholesterol, GABA, isomaltose, etc (Gomez-Eguilaz et al., 2018; Pilla et al., 2021; Schmidt et al., 2018).

Antibiotic treatment in canine epilepsy will not become common practice and can be associated with some important adverse effects such as antibiotic resistance and dysbiosis. Furthermore, certain antibiotics (β-lactams, cephalosporins, fluoroquinolones and carbapenems) can induce epileptic seizures. The effect was presumed due to direct and indirect gamma-aminobutyric acid (GABA) antagonism inhibition of GABA-synthesis by β-lactams or glutaminergic N-methyl-D-Aspartate (NMDA) receptor agonistic activity by cephalosporins, fluoroquinolones and carbapenems (Wanleenuwat et al., 2020). Also, administration of antibiotics, together with antiseizure drugs, may lead to enhanced seizure risk due to drug interactions, which predisposes to alterations in drug metabolism and therapeutic efficacy (Wanleenuwat et al., 2020). Amoxicillin-clavulanic acid had no significant effect on the pharmacokinetic profile of valproic acid (Wanleenuwat et al., 2020).

The main limitations of the present study include the absence of fecal microbiota analysis before and after antibiotic administration, the low number of cases and the lack of a placebo group. The dogs in this study had a severe epilepsy phenotype, the owners were desperate and considered euthanasia. For that reason, antibiotics were immediately started as a last resort. In addition, not all the dogs were fed the same diet on the start and during the antibiotic treatment, so the fecal microbiome analysis would not have been valuable and standardized.

CONCLUSION

In conclusion, the observed positive impact of antibiotic administration in reducing epileptic seizure frequency points to a possible contribution of the gut microbiota in the management of epilepsy. The present study provides a step towards further research investigating the gut-brain axis as a target for new management modalities in canine epilepsy. Microbiota manipulations, such as an MCT-diet and FMT, may provide a better epileptic seizure control and moreover improve future insights in drug-resistant canine IE.

CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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