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CSF omeprazole concentration and albumin quotient following high dose intravenous omeprazole in dogs

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ABSTRACT

Clinical signs of syringomyelia and hydrocephalus occur secondary to cerebrospinal fluid (CSF) accumulation within the central nervous system. Omeprazole is recommended to treat these conditions despite little evidence of its capacity to decrease CSF production in the dog. Studies into new treatments are hampered by difficulties in measuring CSF production. The albumin quotient (QAlb), the ratio between CSF and serum albumin concentrations, may reflect CSF production and any decrease in CSF production should be associated with an increase in QAlb.

The primary objective of this study was to determine CSF omeprazole concentration after administration of a high intravenous dose of omeprazole and to evaluate its impact on QAlb in the dog. The second aim was to validate QAlb as a surrogate marker of CSF production.

Eighteen dogs were included in this prospective crossover placebo-controlled study. Each dog received omeprazole (10 mg/kg), acetazolamide (50 mg/kg) combined with furosemide (1 mg/kg) and saline. Blood and CSF samples were obtained on day 0 and then every 7 days, one hour after drug administration.

Omeprazole concentrations (2.0 ± 0.4 µmol/L) reached in CSF after high dose omeprazole were lower than the concentrations previously described as decreasing CSF production in dogs. There was no significant increase in QAlb following administration of acetazolamide/furosemide, prohibiting validation of QAlb as a surrogate marker for CSF production. Several dogs presented transient mild side effects after injection of acetazolamide/furosemide. High dose omeprazole was well tolerated in all dogs.

1. Introduction

Hydrocephalus and syringomyelia are two conditions in which disturbances in cerebrospinal fluid (CSF) flow may provoke clinical signs in dogs due to CSF accumulation in central nervous system (CNS) (Rusbridge et al., 2007; DeLahunta and Glass, 2015; Thomas, 2010). Medical treatment aims to decrease CSF production to reduce clinical signs and improve quality of life. Omeprazole, an inhibitor of the H^+-K^+ ATPase pump in gastric parietal cells, has been demonstrated to decrease CSF production under experimental conditions (Lindvall-Axelsson et al., 1992; Javaheri et al., 1997). Daily oral omeprazole is currently recommended in the long-term management of canine hydrocephalus and syringomyelia (Rusbridge et al., 2006; Thomas, 2010; Plessas et al., 2012). However, significant decreases in CSF production have only been demonstrated for a few hours following ventriculocisternal or intravenous administration of omeprazole in dogs and rabbits (Lindvall-Axelsson et al., 1992; Javaheri et al., 1997). Definitive evidence of clinical efficacy is lacking.

The albumin quotient (QAlb) is the ratio between CSF and serum albumin concentrations. This ratio is considered a reliable indicator of CSF flow in humans (Olsson and Pettersson, 1976; Reiber et al., 1993; Reiber, 1994; Reiber, 2003). Any decrease in CSF production should provoke an increase in QAlb (Reiber, 2003). Indeed, albumin in CSF originates only from blood and diffuses passively into CSF according to a controlled molecular size dependant process (Felgenhauer, 1974; Reiber, 2003). Thus, QAlb is independent of serum albumin and is a relevant parameter of CSF flow rate (r) with a non-linear relation $\Delta r \sim \Delta(QAlb)^{-0.5}$ (Reiber, 1994; Reiber, 2003). If CSF flow slowdown, there is more time for albumin to diffuse in CSF, resulting in an increase in QAlb. In a previous study, there was no change in QAlb in healthy dogs

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after 14 days of oral omeprazole administration (1 mg/kg/day) (Girod et al., 2016). Several hypotheses were offered to explain the absence of change in QAlb in that study: failure of omeprazole to affect CSF production, insufficient CSF omeprazole concentration to provoke a measurable difference, transient effect of omeprazole on CSF production or a lack of sensitivity of QAlb as a surrogate marker of CSF production.

Acetazolamide is a carbonic anhydrase inhibitor used in human medicine for the treatment of idiopathic intracranial hypertension, as it decreases CSF production (Sorensen et al., 1988; Schoeman, 1994; Kattah et al., 2015; Supuran, 2015; Markey et al., 2016). Furosemide is a loop diuretic that also decreases CSF production, partially by inhibiting carbonic anhydrase but also via another unexplained mechanism (McCarthy and Reed, 1974; Johanson et al., 1994). Acetazolamide and furosemide act synergistically to reduce CSF production in rabbits, cats and children (McCarthy and Reed, 1974; Melby et al., 1982; Shinnar et al., 1985; Schoeman, 1994) and the combination of both drugs is currently used to manage intracranial hypertension in people (Shinnar et al., 1985; Schoeman, 1994; Loo et al., 2016).

The objectives of the present study were to determine CSF omeprazole concentration after a single high dose of intravenous (IV) omeprazole and to assess the impact of the drug on QAlb. To evaluate the validity of QAlb as a surrogate marker of CSF production, a positive control (combined administration of acetazolamide and furosemide) and a negative control (saline solution) were also tested. We hypothesized that a high dose of IV omeprazole would reach therapeutic CSF concentration and lead to an increase in QAlb consistent with a decrease in CSF production.

2. Materials and methods

2.1. Animals

The study was conducted with approval from the Ethical Committee for Experimental Animals of the University of Liège (Record Number 14-1645). Eighteen entire beagle dogs, 9 of each sex, aged 2 to 13 years (mean 6.5 ± 3.7) and weighing 12 to 16 kg (mean 13.7 ± 1.4), were used. All dogs were clinically healthy at physical examination.

2.2. Experimental design

Dogs were divided into 6 groups of 3 dogs in a crossover, placebo-controlled design. Groups of dogs were balanced for age. Each dog received intravenously, in a randomized order and with a seven-day washout period between each injection, either high-dose omeprazole (10 mg/kg, Losec AstraZeneca), a combination of acetazolamide (50 mg/kg, Diamox MPI) and furosemide (1 mg/kg, Dimazon Intervet) or saline solution (placebo). To eliminate any effect of order of drug administration, groups received one of 6 possible injection sequences (3-period, 6-sequence, 3-treatment cross-over design; Table 1). Saline was added to all solutions to achieve a total volume of 20 mL and these were administered over 10 min. Dogs receiving acetazolamide/furosemide were hospitalized for 12 h on crystalloids fluids to prevent dehydration.

In each dog, blood and CSF samples were collected at day 0 (baseline values) and then one hour after IV administration of each preparation.

CSF was checked for erythrocyte (RBC) and leucocyte (WBC) counts. If significant blood contamination was present (i.e.: RBC > 13,200/μL (Di Terlizzi and Platt, 2009)), samples were excluded from the study. Subclinical inflammation was considered if the WBC count was > 5/μL ((Di Terlizzi and Platt, 2009). Serum and CSF albumin were measured to determine QAlb. Other parameters including pH, calcium and electrolyte concentrations were also assessed in blood and CSF. Dogs were physically examined daily throughout the study to monitor for adverse effects.

2.3. Samples

Blood and CSF sampling were performed under general anaesthesia, consisting of premedication with methadone (Comfortan Eurovet; 0.4 mg/kg), induction with propofol (Diprivan AstraZeneca; 5 mg/kg bolus) and maintenance with isoflurane (Isovet, Piramal healthcare UK: 1.5%; oxygen at 0.8 L/min). During the procedure, oxygen saturation was maintained over 96% and end tidal $C0_2$ between 35 and 45 mmHg.

Two +/− 0.5 mL samples of CSF were collected from the cerebellomedullary cistern and placed into two 1.8 mL microcentrifuge tubes (Cryotubes). CSF was immediately assayed for pH, erythrocyte and leucocyte count, total protein and electrolyte measurements. Two aliquots of CSF were frozen. One was kept at −80 °C for up to 2 weeks prior to measuring albumin concentrations by nephelometry. The second was frozen at −80 °C for up to 2 months prior to determining CSF omeprazole concentrations.

Five millilitres of blood were collected via jugular venipuncture immediately after obtaining CSF and divided into serum and heparin tubes. Blood pH was immediately measured on the heparinised sample. Serum total protein, albumin and electrolyte concentrations were also determined. An aliquot of plasma was frozen (−80 °C) for up to 2 months prior to determining omeprazole concentrations.

2.4. Analyses

Serum albumin concentration was measured using a colorimetric reaction with the bromocresol green technique (Response 920, Diasys). CSF albumin concentration was determined by nephelometry, as previously described (Girod et al., 2016).

QAlb was calculated as the ratio between CSF and serum albumin concentrations:

$$\text{QAlb} = (\text{CSF albumin [g/L]})/(\text{serum albumin [g/L]})$$

CSF erythrocytes and leucocytes were counted using a Fuchs-Rosenthal counting chamber CSF and blood pH were measured using a pH meter (Mettler Toledo MP225) directly after collection. Each value was corrected with the Rosenthal factor to allow for the difference between the temperature of the patient and that of the measuring device:

$$\text{Blood pHt1} = \text{blood pHt2} - 0.0147(t1 - t2)$$

where t1, t2, blood pHt1 and blood pHt2 represent the rectal temperature, the temperature of the meter's electrode (37 °C), the blood pH and the pH read by the electrode at 37 °C, respectively.

Plasma and CSF omeprazole/omeprazole-D3 concentrations were determined by High-Performance Liquid Chromatography (HPLC) in Tandem Mass Spectrometry Method as previously described (Wojnicz et al., 2015). A CSF and plasma calibration curve in concentrations ranging from 1.5 to 2000 ng/mL was used. Because of the high concentration in some samples (over 2000 ng/mL), dilution by a factor of 10 was necessary. For that purpose, intra- and inter-day precision and accuracy assays were used as a partial validation of that dilution factor (Table 2).

2.5. Statistical analyses

Sample size was determined on the a priori assumption of a two-tailed alpha error of 0.05 with 80% power. All data were analysed with statistical software (SAS software, GLM procedure). Normality testing was performed using a Shapiro–Wilk test. Baselines values, saline solution, acetazolamide/furosemide and omeprazole were considered as four independent treatments. Data were analysed using a two-way repeated-measures analysis of variance (ANOVA 2) according to the equation: $y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$ where μ, α_i, β_j and ε_{ij} represent the overall mean, effect of the dog, effect of drug and effect of any other interaction, respectively. The model allowed for correction of

Table 1
Eighteen healthy dogs were included in a prospective, cross-over, placebo-controlled study (3-period, 6-sequence, 3-treatment cross-over design). Each dog received intravenously sequentially one of the following 3 injections: high dose omeprazole (10 mg/kg), acetazolamide (50 mg/kg) in combination with furosemide (1 mg/kg) (A + F) or saline solution (placebo). Dogs were separated into 6 groups of 3 dogs in a way that each group of dogs received one of the 6 sequences possible.

Dog	Age	Sex	Weight (kg)	Day 0	Day 7	Day 14	Day 21
1	13	Male	17.2	Baseline values	A + F	Omeprazole	Saline
2	8	Male	12.5	Baseline values	A + F	Omeprazole	Saline
3	2	Female	10.5	Baseline values	A + F	Omeprazole	Saline
4	11	Female	14.8	Baseline values	Omeprazole	Saline	A + F
5	8	Male	15.5	Baseline values	Omeprazole	Saline	A + F
6	2	Male	14	Baseline values	Omeprazole	Saline	A + F
7	8	Female	14.5	Baseline values	Saline	A + F	Omeprazole
8	3.5	Female	14	Baseline values	Saline	A + F	Omeprazole
9	2	Female	13	Baseline values	Saline	A + F	Omeprazole
10	8	Female	15.2	Baseline values	A + F	Saline	Omeprazole
11	11	Male	17.8	Baseline values	A + F	Saline	Omeprazole
12	2	Male	17.4	Baseline values	A + F	Saline	Omeprazole
13	8	Male	17.3	Baseline values	Omeprazole	A + F	Saline
14	2	Female	12.8	Baseline values	Omeprazole	A + F	Saline
15	8	Male	12	Baseline values	Omeprazole	A + F	Saline
16	8	Male	16	Baseline values	Saline	Omeprazole	A + F
17	8	Female	13,2	Baseline values	Saline	Omeprazole	A + F
18	2	Female	10.8	Baseline values	Saline	Omeprazole	A + F

Dog 14 was excluded from statistical analysis for drug effect due to repeated CSF blood contamination.

differences between dogs prior to comparing treatment effects on QAlb, CSF and blood parameters. All results were expressed as mean +/− SD. Differences were considered significant when $P < .05$.

3. Results

Data from dog 14 were excluded from statistical analyses regarding treatment effect due to repeated CSF blood contamination. However, data from this dog were included in omeprazole concentration measurements as blood contamination does not interfere with HPLC measurement (Mano et al., 2015).

Plasma omeprazole concentrations ranged from 4.3 µmol/L to 14.3 µmol/L (mean 10.1; SD 2.8) and CSF omeprazole concentrations ranged from 1.1 µmol/L to 2.6 µmol/L (mean 2.0; SD 0.4) (Table 3).

There was no significant change in QAlb following administration of acetazolamide/ furosemide. QAlb mildly increased following omeprazole ($P = .02$) and saline ($P = .03$) but there was no statistical difference in QAlb values between the three treatment regimens (Table 4, Fig. 1).

Changes in CSF composition are reported in Table 5. CSF pH decreased after administration of acetazolamide/furosemide compared to baseline value ($P = .02$) and this decrease was significant compared to saline solution and to omeprazole ($P = .01$). A mild significant increase in CSF potassium concentration was observed following omeprazole ($P = .04$), acetazolamide/furosemide ($P < .001$) or saline solution administration ($P < .001$) but without significant difference between the 3 treatments. A significant decrease in CSF chloride concentration was also observed after each treatment ($P < .001$) but without difference between the 3 treatments.

Four dogs (dogs 2, 8, 11 and 18) had a mild increase in CSF WBC count (9, 6, 11, and 7 cells/µL respectively) on day 14 (dogs 2, 11, 18) or on day 21 (dog 8). CSF WBC count normalized one week later in 3 out of the 4 dogs. Dog 8 had no more CSF analysis as day 21 was the last day of the experiment, but this dog did not develop any clinical signs of CNS inflammation over the following weeks.

Table 3
CSF and plasma omeprazole concentrations in 18 dogs 1 h after the intravenous administration of 10 mg/kg of omeprazole.

Dogs	Plasma omeprazole concentrations (µmol/L)	CSF omeprazole concentrations (µmol/L)
1	13.4	2.1
2	5.9	1.9
3	9.6	1.7
4	11	2.6
5	8.6	2.6
6	11.7	1.9
7	11.2	2.2
8	10.7	2
9	14.3	2
10	6	1.9
11	11	2.6
12	14	1.8
13	9	2.1
14	11.1	1.7
15	4.3	1.1
16	12	2.3
17	9.7	1.9
18	7.6	1.5
Mean +/− SD	10.1 +/− 2.8	2.0 +/− 0.4

Findings regarding changes in blood composition are summarized in Table 6

Blood pH decreased significantly after administration of acetazolamide/furosemide compared to baseline value ($P = .03$) and to other treatments ($P < .001$). Blood pH increased after administration of omeprazole compared to baseline ($P = .04$) but this increase was not

Table 2
Precision and accuracy intra- and inter-day with dilution factor 10 (DF10). Data were obtained from 4 quality controls of omeprazole (400 ng/mL after the DF 10) repeated 5 times on the same day for precision and accuracy intra-day and on 3 consecutive days for inter-day assays. The mean ± SD of the number of total experiments is shown.

	Concentration(ng/mL)	Mean ± SD(ng/mL)	CV[a](%)	Accuracy(%)
Intra-day run ($n = 5$)	400 (DF10)	351.95 ± 3.91	1.11	−12.01
Inter-day run ($n = 15$)	400 (DF10)	371.09 ± 30.52	8.22	−7.23

[a] CV, coefficient of variation.

Table 4
CSF albumin concentrations, serum albumin concentrations and albumin quotient (QAlb) (expressed as means +/− standard deviation) before (basal values) and 1 h after injection of saline solution (negative control), acetazolamide with furosemide (positive control) and omeprazole in 17 healthy dogs.

	Basal values	Saline(negative controls)	Acetazolamide + furosemide (positive controls)	Omeprazole
CSF albumin (g/L)	0.14 +/− 0.06	0.14 +/− 0.07	0.16 +/− 0.06	0.15 +/− 0.07
Serum albumin (g/L)	30.2 +/− 2.4	28.6 +/− 3.0	31.7 +/− 2.7	28.7 +/− 2.2
QAlb ($\times 10^{-3}$)	4.61 +/− 2.10	5.14 +/− 2.70[a]	5.00 +/− 1.92	5.17 +/− 2.52[a]

[a] Indicates significant difference in QAlb compared to basal values.

significant compared to saline solution.

While serum sodium concentration increased significantly after administration of acetazolamide/furosemide compared to baseline ($P < .001$) and to other treatments ($P < .001$), potassium concentration decreased significantly compared to baseline ($P < .001$) and to other treatments ($P < .001$). Chloride concentration decreased significantly after acetazolamide/furosemide compared to baseline ($P < .001$) and this decrease was significant compared to saline solution ($P < .04$) and to omeprazole ($P < .02$). Chloride concentration significantly decreased after administration of omeprazole compared to baseline ($P = .02$) but this was not significantly different to chloride concentration after saline solution.

Following administration of acetazolamide/furosemide, three dogs (dogs 9, 11 and 15) presented mild tremors, one dog (dog 18) had a short episode of pruritus, one dog (dog 1) had polypnoea and lip licking and one dog (dog 5) demonstrated responsive mydriasis, tremor and ataxia (hypermetria). All these signs appeared within 15 min after administration, were transient and completely resolved within an hour. None of the dogs displayed side effects after administration of omeprazole or placebo.

4. Discussion

The present study failed to identify any significant difference in QAlb values following administration of acetazolamide combined with furosemide in healthy dogs. Although QAlb increased after omeprazole, a similar magnitude change was seen with saline suggesting that the effect was not attributable to the omeprazole. QAlb was proposed as a surrogate marker of CSF flow and, therefore, would be expected to differ from baseline following the administration of acetazolamide/furosemide.

Acetazolamide has been demonstrated to decrease CSF production in animals and humans (Holloway Jr. and Cassin, 1972; McCarthy and Reed, 1974; Watanabe et al., 1976; Knuckey et al., 1991). In rats, a significant attenuation of hydrocephalus was demonstrated following administration of 30 mg/kg of acetazolamide intraperitoneally (Gao et al., 2016). In cats, the IV administration of 50 mg/kg of acetazolamide led to a 50% decrease in CSF production from 30 min to > 5 h after administration (Vogh, 1980). The association of acetazolamide and furosemide has shown even greater efficacy to decrease CSF production in humans (Shinnar et al., 1985; Schoeman, 1994; Loo et al., 2016). In sheep, a 50% decrease in CSF production caused an increase in QAlb by approximately 2×10^{-3} (Chen et al., 2010). As the combination of acetazolamide and furosemide was selected as a positive control, the absence of a significant increase in QAlb in the present study prohibits the validation of QAlb as a surrogate marker for CSF flow in dogs.

Jafarzadeh et al. (2014) demonstrated an absence of a decrease in CSF production in some humans after administration of acetazolamide ("non responders"). This phenomenon could contribute to the absence of significant change in QAlb after acetazolamide/furosemide in dogs in the present study and a larger number of dogs may be needed to overcome this effect. It is also possible that the administration of acetazolamide/furosemide has no impact on CSF production in healthy dogs. However, this seems less likely as a significant decrease in CSF production after acetazolamide administration has been reported in healthy cats (Kister, 1956; Vogh, 1980).

In the present study, the mean concentration of omeprazole measured in CSF (2×10^{-6} mol/L) one hour after high dose IV omeprazole was lower than the concentration reported to decrease CSF production in dogs. Javaheri et al. (1997) demonstrated that a CSF concentration of omeprazole of 10^{-5} mol/L decreased CSF production by 26% in the dog. The effect of lower concentrations was not evaluated. In rabbits, a concentration of 10^{-6} mol/L decreased CSF production by 36% and a

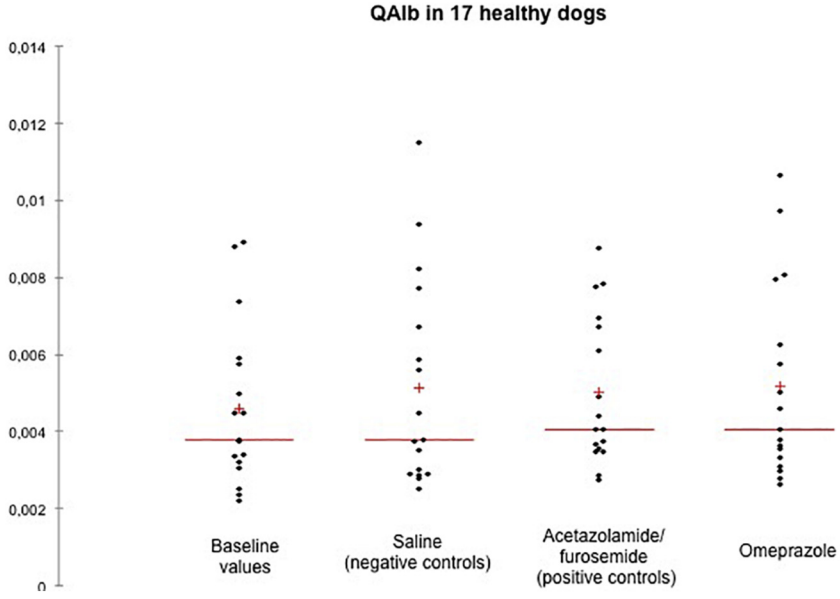

Fig. 1. Scatterplots showing the albumin quotient (QAlb) in 17 healthy dogs before (baseline values) and 1 h after injection of either a saline solution (negative controls), a combination of acetazolamide and furosemide (positive controls) or high dose omeprazole. The cross (+) represents the mean value and the horizontal line represents the median. There was no significant difference in QAlb value between saline, acetazolamide/furosemide and omeprazole ($P > .05$).

Table 5
CSF values before (basal values) and 1 h after injection of saline (negative control), acetazolamide with furosemide (positive control) and omeprazole (expressed as means +/− standard deviation) in 17 healthy dogs.

	Basal values	Saline solution (negative controls)	Acetazolamide + furosemide (positive controls)	Omeprazole
WBC count (cells/μL)	1.18 +/− 1.24	2.12 +/− 3.37	1.41 +/− 1.73	1.59 +/− 1.87
pH	7.41 +/− 0.09	7.46 +/− 0.08	7.35 +/− 0.08[b]	7.42 +/− 0.05
Ca^{2+} (mmol/L)	1.37 +/− 0.10	1.37 +/− 0.16	1.4 +/− 0.13	1.38 +/− 0.15
Na^{+} (mmol/L)	145.0 +/− 1.9	144.9 +/− 1.4	145.0 +/− 1.1	145.1 +/− 1.6
K^{+} (mmol/L)	2.76 +/− 0.09	2.83 +/− 0.07[a]	2.83 +/− 0.07[a]	2.80 ± 0.07[a]
Cl^{+} (mmol/L)	139.3 +/− 10.1	126.9 +/− 6.7[a]	125.8 +/− 4.9[a]	122.9 +/− 2.6[a]

[a] Indicates values that are significantly different to basal values.
[b] Indicates values that are significantly different to basal values and to other treatments.

Table 6
Blood values before (basal values) and 1 h after injection of saline solution (negative control), acetazolamide with furosemide (positive control) and omeprazole (expressed as means +/− standard deviation) in 17 healthy dogs.

	Basal values	Saline solution (negative controls)	Acetazolamide + furosemide (positive controls)	Omeprazole
pH	7.34 +/− 0.05	7.39 +/− 0.06	7.30 +/− 0.06[b]	7.38 +/− 0.06[a]
Ca^{2+} (mmol/L)	2.51 +/− 0.12	2.45 +/− 0.24	2.45 +/− 0.16	2.41 +/− 0.14
Na^{+} (mmol/L)	144.8 +/− 1.1	144.9 +/− 1.8	148.1 +/− 1.0[b]	144.8 +/− 1.4
K^{+} (mmol/L)	3.93 +/− 0.39	3.96 +/− 0.34	3.51 +/− 0.36[b]	3.92 ± 0.41
Cl^{+} (mmol/L)	112.0 +/− 3.3	108.9 +/− 2.5[a]	106.8 +/− 3.6[b]	109.4 +/− 3.2[a]

[a] Indicates values that are significantly different to basal values.
[b] Indicates values that are significantly different to basal values and to other treatments.

minimum concentration of 10^{-8} mol/L was required to decrease CSF production (Lindvall-Axelsson et al., 1992). Based on these observations, 10 mg/kg of omeprazole was well tolerated but seems insufficient to reach a CSF concentration that could decrease CSF production in dogs. Higher doses could be tested but the clinical safety remains undetermined. Moreover, the use of a standard dose of omeprazole (1 mg/kg/day) to treat hydrocephalus or syringomyelia may be insufficient to have a therapeutic effect and merits further investigation.

The administration of acetazolamide/furosemide was associated with a significant decrease in blood pH and serum potassium. These results were expected as acetazolamide inhibits carbonic anhydrase in the kidney and shifts the equilibrium of the Henderson-Hasselbach equation ($CO_2 + H_2O < -> H_2CO_3 < -> HCO_3^- + H^+$) to the right. K^+ and HCO_3^- are excreted in the urine and H^+ is reabsorbed, resulting in a metabolic acidosis with hypokalaemia (Leaf and Goldfarb, 2007; Jafarzadeh et al., 2014). Furosemide increases H^+ and K^+ secretion in the renal tubule contributing further to the hypokalaemia but possibly moderating metabolic acidosis (McCarthy and Reed, 1974). The metabolic acidosis and hypokalaemia were mild and of no clinical consequence.

The significant increase in sodium following administration of acetazolamide and furosemide was probably secondary to a mild degree of dehydration induced by the diuretics. The mild decrease in serum chloride following acetazolamide/furosemide was surprising as acetazolamide has been associated with systemic hyperchloraemic metabolic acidosis (Leaf and Goldfarb, 2007; Jafarzadeh et al., 2014). Nevertheless, as furosemide increases Cl^- excretion by inhibiting renal Na^+-K^+-$2Cl^-$ cotransporter of the ascending limb of the loop of Henle, it could be responsible for the mild hypochloraemia (Harada et al., 2015).

The decrease in CSF pH following administration of acetazolamide/furosemide may be explained by the diffusion gradient for HCO_3^- between CSF and plasma induced by acetazolamide. The tendency for HCO_3^- ions to move out of CSF along a concentration gradient lowers CSF pH (Leaf and Goldfarb, 2007).

Finally, the administration of acetazolamide/furosemide provoked only minor and transient side effects. Neurological side effects such as paresthesia, fasciculation and ataxia have previously been reported following acetazolamide administration in humans (Vahedi et al., 2002; Kim et al., 2007). These are assumed to be secondary to metabolic acidosis and hyperammonaemia induced by acetazolamide (Vahedi et al., 2002; Kim et al., 2007). Administration of furosemide has uncommonly been associated with headache or polydipsia in humans and animals (Verma et al., 2004; Harada et al., 2015). The administration of a high dose of omeprazole was well-tolerated by all dogs.

This study had several limitations. The number of dogs could have been insufficient to demonstrate an effect of drugs on QAlb (type II error). The power calculation assumed a minimum change in QAlb of 2×10^{-3}. A larger number of dogs might be required to highlight a smaller treatment effect on QAlb. In this study dogs of different ages were used. As CSF production has been shown to decrease with age, an additional decrease of CSF production could be more difficult to show in an old dog (with reduced CSF production), than in a young dog (Reiber, 1994, 2003; Silverberg et al., 2003).

Furthermore, healthy beagle dogs may not be representative of the broader canine population and absence of findings here may not be applicable to clinical cases.

Validated techniques for direct measurement of CSF production such as volumetric assays were not performed concurrently (Kolecka et al., 2015). Any reduction in CSF production provoked by the positive control could be too brief or too small to elicit a detectable change in QAlb.

A further limitation of the study is the cross-over design. A 3-period, 6-sequence, 3-treatment cross-over design was chosen as it is uniform in sequence and period, meaning that period and sequence effects are removed by the design. It is also a balanced design with respect to first-order carryover effects. Thus, treatment difference is not affected by carryover effects if a sufficient washout period is respected. A 7-days washout period was chosen as this exceeds > 5 times the half-life of all relevant drugs in the blood (1 h in dogs for omeprazole and furosemide; 8 h in humans for acetazolamide). Nevertheless, sustained clinical effects in CNS of previous medication could mean that a change in QAlb was overlooked.

Finally, CSF omeprazole concentrations were only measured at a single time point. It cannot be excluded that the concentration of omeprazole continued to rise to reach levels in excess of previously established thresholds for efficacy. Further studies would be required to establish a clearer pharmacokinetic model of omeprazole within canine

CSF

5. Conclusion

Administration of a high dose of omeprazole produced CSF omeprazole concentrations lower than those previously reported to be effective at reducing CSF production in dogs.

The administration of acetazolamide in combination with furosemide was not associated with an increase in QAlb one hour after administration when compared to placebo administration. This suggests a lack of sensitivity of QAlb as a surrogate marker of CSF production in dogs.

Declaration of Competing Interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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