Sildenafil improves clinical signs and radiographic features in dogs with congenital idiopathic megaoesophagus: a randomised controlled trial

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We evaluated the efficacy of oral sildenafil citrate in dogs with congenital idiopathic megaoesophagus (CIM). Twenty-one puppies were randomly assigned to two groups (treatment and control). The dogs were given sildenafil oral suspension 1 mg/kg every 12 hours for 14 days or placebo in a masked fashion. Clinical signs (frequency of regurgitation and weight gain) and oesophagrams (relative oesophageal diameter, ROD) were evaluated in order to assess the efficacy of drug treatment, by examiners who were unaware of the study protocol. In addition, a set of in vitro experiments on isolated samples of canine lower oesophageal sphincter (LOS) was performed, and the effects of increasing concentrations of sildenafil on basal tone and electrically-stimulated motility were assessed. Sildenafil administration significantly reduced the number of regurgitation episodes (0.88 ±1.40 v 2.65±1.56, P<0.0001) and significantly increased weight gain in the treated dogs compared to controls (79.76±28.30 per cent v 53.40±19.30 per cent, P=0.034). ROD values, at the end of the treatment period, were significantly decreased in the sildenafil group, compared to pre-treatment values (0.97±0.19 v 0.24±0.14, P<0.0001), in contrast to control subjects (0.98±0.17 v 1.10±0.25, P=0.480). In accordance with the in vivo findings, sildenafil dose-dependently reduced basal tone and increased electrically-induced relaxation of dog LOS samples. These results suggest that sildenafil citrate helps ameliorate clinical and radiographic signs in dogs with CIM by reducing LOS tone, and could represent a novel therapeutic tool for the treatment of this disease.

Introduction

The term megaoesophagus is used to describe a disease characterised by reduced or absent oesophageal motility which causes the accumulation of ingesta, dilatation of the oesophageal lumen, food regurgitation (which is often mistaken for vomit by the dog owner), and weight loss as the main clinical signs. Megaoesophagus may be idiopathic, congenital or acquired, or secondary to different aetiologies, such as myasthenia gravis, hypothyroidism or Addison’s disease. Congenital idiopathic megaoesophagus (CIM) is often observed at or before 10 weeks of life, and the condition frequently affects more than one animal in the same litter (Harvey and others 1974, Glidewell 1983). CIM causes poor weight gain (WG) in puppies shortly after weaning, and, even though most animals tend to show spontaneous improvement over time, they require long-lasting physical and nutritional support, and the risk of fatal complications such as aspiration pneumonia is high.

The pathogenesis of CIM is currently unclear. A predisposition for the disease has been reported in large and giant-breed dogs such as the German shepherd, Great Dane, Irish setter, Labrador retriever, Irish wolfhound and Newfoundland (Knowles and others 1990), and genetics might play a role in the aetiology of CIM because autosomal dominant inheritance has been demonstrated in miniature Schnauzers and fox terriers (Washabau 2003). A suspected hereditary form has also been reported in Bouvier des Flandres dogs (Peeters and others 1991).

It has been hypothesised that the congenital form of the disease is linked to a reduced or delayed development of the oesophageal neuromuscular system, in particular of the afferent vagal innervation, which fails to respond to the mechanical stimulus induced by food, thus resulting in ineffective peristalsis (Holland and others 1994, 1996, 2002). Manometric studies have found a normal tone and functioning of the lower oesophageal sphincter (LOS) in dogs with idiopathic megaoesophagus (Diamant and others 1975), unlike in other oesophageal motility
disorders in humans, such as achalasia or diffuse oesophageal spasm, where a hypertonicity of sphincter muscle is present (Pohl and Tutuian 2007, Roman and Kahrilas 2012). However, a failure by the LOS to relax in response to intraluminal balloon distension has been observed (Tian and Diamant 1987), further supporting the hypothesis of a functional defect of oesophageal sensitivity in man.

CIM treatment is frustrating, resulting in high mortality from directly related causes such as malnutrition and aspiration pneumonia or because euthanasia is required due to the continuing clinical signs (Harvey and others 1974, McBrearty and others 2011). In the majority of cases, drugs are not adequately effective, and the treatment is based mostly on nutritional support and alterations in body position (Chandra and others 1989). Several pharmacological approaches, especially with prokinetic drugs such as metoclopramide, domperidone or cisapride, have been proposed, with modest or varying results (Washabau 2003). However, recent studies with high-resolution manometry showed that cisapride significantly increased LOS pressure in healthy dogs, and this could represent a serious concern in dogs with megaesophagus (Kempf and others 2014, Ullal and others 2016).

Swallowing and oesophageal motility are complex processes involving a multifaceted interplay between excitatory innervation, mostly vagal cholinergic fibres, and inhibitory innervation, which releases nitric oxide (NO) as the main neurotransmitter. Endogenous NO induces smooth muscle relaxation through the synthesis of the second messenger cyclic guanosine monophosphate (cGMP). Sildenafil, a selective phosphodiesterase-type 5 (PDE-5) inhibitor, indirectly potentiates the action of endogenous NO by reducing cGMP degradation due to PDE-5 (Zhu and others 2007). Sildenafil is an effective vasodilator and is widely prescribed for the treatment of erectile disorders in man; however, it is also used to treat pulmonary hypertension, and it relaxes the smooth muscle of other organs such as the uterus (Méhats and others 2006) and the gallbladder (Degirmenci and others 2006). The vasorelaxant properties of sildenafil have also been observed in dogs (Souza-Silva and others 2005, Bach and others 2006), and this drug represents a valid option for the treatment of pulmonary hypertension in this species.

In humans and cats sildenafil has already been shown to induce the relaxation of the LOS (Zhang and others 2001, Fox and others 2007). Therefore, in the current study we evaluated the therapeutic efficacy of sildenafil in dogs affected by CIM, on the premise that a decreased LOS tone would facilitate the entry of the ingesta into the stomach, thus reducing the pressure at the premise that a decreased LOS tone would facilitate the entry of the ingesta into the stomach, thus reducing the pressure of the proximal stomach, and this could represent a serious concern in dogs with megaesophagus (Kempf and others 2014, Ullal and others 2016).

Radiographic evaluation
Lateral radiographs of each dog were taken, without any pharmacological restraint, before and immediately after the administration of 4 ml/kg of a barium suspension (Frontobario 60 per cent, Bracco Imaging Italia, Milan), mixed with 3–4 boluses of canned food, without keeping the dog in lateral recumbency. Radiographic evaluation was performed at D0, and the day after the last sildenafil or placebo administration (D15), (named W1 and W2, respectively) were used to calculate the WG for each dog, expressed as percentage (Fig 1). Dog owners were also asked to report immediately to the investigators any adverse event observed during or after the drug administration period. Apart from the regurgitation count, which took place in the breeding kennels, all the other evaluations were performed in the veterinary hospital facilities.
vented aspect of the vertebral column at the mid-point of the first rib, to the inner aspect of the manubrium at the point of the narrowest diameter of the TI. In order to minimise the differences in weight and size of the dogs in the two groups, the relative OD (ROD) was adopted instead of OD, using the function OD/TI, as proposed by Wray and Sparkes (2006) (Fig 2). All measures were performed with an image analysis software (Image J, version 1.49 NIH), by an examiner who was unaware of the study protocol.

In vitro experiments
Following laparotomy, the gastroesophageal junction was excised from six dogs of different breeds, euthanased at the Animal Hospital of the Department of Veterinary Science for reasons unrelated to pathologies of the digestive system. Each segment of oesophagus was put in cooled (4°C) modified Krebs-Henseleit Solution (KHS) of the following composition: sodium chloride (NaCl) 113.0 mM, potassium chloride (KCl) 4.7 mM, potassium phosphate (KH2PO4) 1.2 mM, sodium bicarbonate (NaHCO3) 25.0 mM and dextrose 11.2 mM, and immediately warmed to 37°C and continuously bubbled with 95 per cent oxygen (O2) and 5 per cent carbon dioxide (CO2). After a period of stabilisation (45–60 minutes), the mechanical activity was measured by means of an isotonic transducer with 95 per cent oxygen (O2) and 5 per cent carbon dioxide (CO2). After a period of stabilisation (45–60 minutes), the mechanical activity was measured by means of an isotonic transducer with 95 per cent oxygen (O2) and 5 per cent carbon dioxide (CO2).

Data are expressed as mean±sd. Unpaired t tests were used for the comparison of data between the treatment and control groups, while paired t tests were employed to compare pre- and post-treatment data in the same group. All analyses were performed using a commercial statistical software (GraphPad Prism for Mac V.6.0f, GraphPad Software Inc, USA).

Results
The trial was conducted between November 2013 and January 2016. The treatment group (n=12) consisted of seven Great Danes (four of which were littermates), three German shepherd dogs (four of which were littermates) and three English Springer Spaniels (two of which were littermates). The control group (n=12) consisted of 12 English Springer Spaniels (two of which were littermates).

In order to monitor the regurgitation frequency developing a passive load of 2 g to the preparation throughout the entire experiment. In a separate set of experiments, electrical field stimulation (EFS) was applied with a pair of coaxial platinum electrodes positioned 10 mm from the longitudinal axis of the preparation and used to deliver trains of square wave pulses (0.4 ms duration, 50 V amplitude) every 120 seconds to the tissue for Mac V.6.0f, GraphPad Software Inc, USA).

### TABLE 1: Weight values of dogs in control and treatment group at day 0 (W1), day 15 (W2), and WG. Regurgitation frequency (D=day number)*

<table>
<thead>
<tr>
<th>Weight assessment</th>
<th>Number of regurgitation episodes in 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D0</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
</tr>
<tr>
<td>W1 (kg)</td>
<td>3.63</td>
</tr>
<tr>
<td>W2 (kg)</td>
<td>±0.92</td>
</tr>
<tr>
<td>WG (%)</td>
<td>±0.39</td>
</tr>
<tr>
<td>Treatment group</td>
<td>3.23</td>
</tr>
<tr>
<td>W1 (kg)</td>
<td>±0.92</td>
</tr>
<tr>
<td>W2 (kg)</td>
<td>±0.39</td>
</tr>
<tr>
<td>WG (%)</td>
<td>±0.39</td>
</tr>
</tbody>
</table>

*All values are expressed as mean±sd. p=0.034 treatment v control group. WG, weight gain.

Drugs
Sildenafil citrate pharmacological forms (Revatio oral suspension, Revatio intravenous solution) were purchased from Pfizer Italia; atropine, guanethidine, indomethacin, tetrodotoxin (TTX), L-NG-nitroarginine methyl ester (L-NAME), and 1H-[1,2,4]oxadiazolo[4,3,-

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### FIG 1: Study design scheme indicating: the duration of the study (from day 0 to day 45, D0–D45); sildenafil (1 mg/kg twice daily) or placebo administration protocol; times of regurgitation frequency evaluation; weight recordings (W1 and W2), and radiographic evaluation times (RX)
The control group it was 3.63±0.92 kg (range 2.8–35 days) in the treatment and control groups, respectively (P=0.389). Mean weight at the start of the study (W1) for the control group (Co) and treatment group (Sil). **P<0.0001 Sil D15 v Sil D0

The number of regurgitation episodes were notably significantly lower (0.24±0.14) (range 0.02–0.44) compared to the control group dogs, in which the mean ROD value was 1.10±0.25 (range 0.82–1.47) (P<0.0001). Sildenafil administration was also able to reduce mean OD in a significant fashion, as observed by comparing ROD values at D0 v D15 (P<0.0001). By contrast, no significant difference was recorded in the control group between the ROD values at D0, with respect to D15 (P=0.480).

The thoracic inlet (TI) was also measured in the same radiograph, from the ventral aspect of the vertebral column at the mid-point of the first rib, to the inner aspect of the manubrium at the point of narrowest diameter of the TI. The relative OD (ROD) was calculated, using the OD/TI ratio

dogs and two Labrador retrievers. The control group (n=9) consisted of five Great Danes (two of which were littermates) and four German shepherd dogs. The mean ages of the dogs were 28.17±6.07 days (range 22–45 days) and 28.44±3.00 days (range 25–35 days) in the treatment and control groups, respectively (P=0.389). Mean weight at the start of the study (W1) for the treatment group was 3.23±0.92 kg (range 2.8–35 days) in the treatment group dogs were significantly lower (0.24±0.14) (range 0.02–0.44) compared to the control group dogs, in which the mean ROD value was 1.10±0.25 (range 0.82–1.47) (P<0.0001). Sildenafil administration was also able to reduce mean OD in a significant fashion, as observed by comparing ROD values at D0 v D15 (P<0.0001). By contrast, no significant difference was recorded in the control group between the ROD values at D0, with respect to D15 (P=0.480).

In the experiments performed in vitro, sildenafil (10−7 to 10−5 M) induced a concentration-dependent decrease of basal tone of LOS preparations, as shown by the fall of the baseline with respect to pre-drug level (Fig 4). In the presence of atropine, guanethidine and indomethacin, EFS evoked non-cholinergic non-adrenergic phasic relaxations of the LOS muscle (Fig 4), which were abolished by neuronal sodium channel blocker (TTX), by NO-synthase inhibitor (L-NAME), and by guanylyl cyclase inhibitor (ODQ). Sildenafil (10−10 to 5x10−7 M) enhanced the amplitude of these relaxations in a concentration-dependent fashion (Fig 4).

**Discussion**

To date, there is no specific and effective pharmacological treatment for idiopathic megaoesophagus. In dogs, the oesophagus possesses a striated muscle layer throughout its entire length, excluding the LOS; therefore prokinetic agents which act on smooth muscle, such as metoclopramide and cisapride, are ineffective and could be contraindicated (Washabau 2005). In accordance with this, 5-HT3 serotonin receptors were not detected in the oesophageal muscle of dogs (Cohen and others 1994). Moreover, metoclopramide and cisapride tend to increase LOS tone, further hindering the emptying of oesophageal content, and thus worsening the clinical signs (Washabau and Hall 1997). On the other hand, bethanechol, a muscarinic agonist, was instead shown to increase the amplitude of contractions in dogs with idiopathic megaoesophagus (Diamant and others 1974).

Due to the scarce results obtained with drugs aiming to enhance the contractions of the oesophageal body, a possible therapeutic strategy could be to relax LOS smooth muscle, in order to promote the emptying of the oesophagus. Indeed, calcium channel blockers were shown to be able to decrease LOS pressure in humans with oesophageal motor dysfunctions (Bauack and others 1991), and nifedipine administration resulted in a temporary clinical improvement in dogs with idiopathic megaoesophagus (Chanda and others 1989). A possible detrimental effect exerted by calcium antagonists on overall oesophagus peristalsis cannot be excluded, though, and it may represent a serious concern.

The importance of NO in basal and swallowing-induced LOS relaxation, as well as the ability of sildenafil to modify LOS tone, have been demonstrated several times in different species over the past two decades. For example, seminal work in the opossum demonstrated that the inhibition of NO synthesis antagonised swallowing-induced LOS relaxation, and caused an increase in basal LOS pressure (Tetttrup and others 1991, Yamato and others 1992). Indeed, sildenafil was shown to decrease LOS tone in healthy humans or in patients with achalasia or other oesophageal motility disorders (Bortolotti and others 2000, 2002, Rhee and others 2001, Eherer and others 2002, Lee and others 2005, Fox and others 2007). An average basal LOS relaxation of 

**FIG 2:** Radiographic measurement technique as proposed by Wray and Sparkes (2006). The oesophageal diameter (OD) was measured in each radiograph at its widest point, perpendicularly to the oesophageal longitudinal axis, at the level of its luminal surface. The thoracic inlet (TI) was also measured in the same radiograph, from the ventral aspect of the vertebral column at the mid-point of the first rib, to the inner aspect of the manubrium at the point of narrowest diameter of the TI. The relative OD (ROD) was calculated, using the OD/TI ratio.

**FIG 3:** Mean±sd of relative oesophageal diameter (ROD) (OD/ thoracic inlet (TI)) values measured at day 0 (D0) and day 15 (D15) for control group (Co) and treatment group (Sil). **P<0.0001 Sil D15 v Co D15;##P<0.0001 Sil D15 v Sil D0**
50 per cent was also observed in sildenafil-treated cats (Zhang and others 2004).

The present study provides the first evidence documenting the benefits of sildenafil citrate in relieving the clinical signs associated with CIM in dogs. Although a decrease in the number of regurgitation episodes from D0 to D14 was observed in both groups, it was notably higher in the sildenafil group. Moreover, in the puppies treated with sildenafil, the mean frequency of regurgitation episodes in 24 hours was significantly lower, compared to non-treated subjects, and the clinical improvement was supported by a significant increase of WG in the treatment group with respect to controls. The beneficial effects of the drug were also observed radiographically: in the treatment group dogs, a marked reduction of the OD was measured at the end of the treatment period, as indicated by the significantly lower mean ROD values, compared to control group. By contrast, in all the dogs enrolled in the control group the OD was wider at D15, with respect to the beginning of the study. In placebo-treated dogs a gradual decrease of regurgitation episodes was observed despite a worsening of oesophageal enlargement; although this discrepancy might seem surprising, there is usually a poor correlation between the severity of clinical signs and the degree of oesophageal distension in dogs with megaesophagus (Guifford 1990), and spontaneous improvement with time may be due to feeding from the upright position (Sokolovsky 1972).

NO is the principal inhibitory neurotransmitter released from myenteric neurons which induces relaxation of the LOS, through activation of cGMP synthesis (Mittal and Bhalla 2004).

The importance of the NO/cGMP pathway for the relaxation of LOS muscle in dogs was corroborated by the results of in vitro experiments, which indicated that NO-synthase inhibitor L-NAME inhibited EFS-evoked relaxation spikes of LOS preparations, in accordance with what was observed previously (Yamato and others 1992). Moreover, ODQ, a guanylyl cyclase inhibitor, abolished such relaxations, confirming that they were mediated by cGMP, and thus could be susceptible to sildenafil action. In fact, sildenafil enhanced EFS-evoked relaxation spikes and reduced basal tone in a concentration-dependent manner, showing that this PDE-5 inhibitor is able to induce the relaxation of isolated LOS in the dog. These results strongly support the hypothesis that the clinical and radiographic improvement observed in dogs treated with sildenafil are indeed due to a reduced LOS tone, with subsequent easier transit of food from the oesophagus into the stomach.

The ability of sildenafil to relax smooth muscle could also represent a concern, though, as it might hinder oesophageal peristalsis further. As a matter of fact, in previous studies in humans and in cats, sildenafil significantly reduced oesophageal contractile pressures (Bortolotti and others 2000, 2002, Eherer and others 2002, Zhang and others 2004). Unlike in humans and cats, however, oesophageal muscle in dogs is almost entirely of the striated type, and thus is not affected by sildenafil. Indeed, the work by Zhang and others showed that the contractile amplitude in oesophageal portions with striated muscle was unaffected. Another concern of the reduced tone of LOS induced by sildenafil could be represented by a potential increased risk of gastro-oesophageal reflux (GOR); a previous study, though, found that sildenafil altered LOS function without causing GOR in human patients (Kim and others 2006).

Since peristalsis of the oesophagus is unchanged, the dogs affected by CIM treated with sildenafil would still require to be fed from an elevated position; however, they could benefit greatly from the easier oesophageal emptying and the decrease in oesophageal dilatation, resulting in an improvement in clinical signs and general health status. Moreover, serious complications such as aspiration pneumonia are less likely to occur. Interestingly, sildenafil seemed to achieve results that go beyond mere symptomatic treatment, since puppies in the sildenafil group had only occasional episodes of regurgitation up to 30 days after the drug administration was discontinued, whereas the clinical signs, though improved, were considerably worse in the control subjects. CIM is a chronic disease, so it would be very important in future studies to expand the knowledge about sildenafil effects over time. Further experiments with different doses of sildenafil and with similar drugs, like tadalafil, will be necessary for a better understanding of the efficacy of PDE-5 inhibitors against idiopathic megaesophagus in dogs. Moreover, gastro-oesophageal manometric studies should be performed to determine the effect of sildenafil activity on oesophageal and LOS tone and contractility.

The current dosage was well tolerated in all treated puppies. Apart from the possible decrease in blood pressure, several adverse reactions following sildenafil administration have been reported in the literature. Abbott and others (2004), for example, described species-specific effects in dogs (Beagle pain syndrome), mice and rats. For this reason, additional clinical studies in dogs would benefit from arterial pressure measurement, urinalysis, haematological and serum biochemical analyses in sildenafil-treated patients.

In conclusion, this preliminary study suggests, for the first time, that sildenafil citrate, by reducing LOS tone and facilitating the emptying of the oesophagus, could represent a useful drug for the clinical management of CIM in dogs.

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