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Toxic encephalopathy associated with high-dose metronidazole therapy in a dog: a case report

I. Hajek[1]*, V. Simerdova[1], M. Vavra[2], C.F. Agudelo[2]

[1]Small Animal Referral Centre Sibra, Bratislava, Slovak Republic
[2]Small Animal Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

*Corresponding author: hajeki101@gmail.com

ABSTRACT: This case report describes an episode of acute ataxia, tremor, vertical nystagmus and progressive weakness in a mixed breed dog treated with high doses of metronidazole. Complete blood cell count, serum biochemistry, coagulation profile, blood pressure measurement, urinalysis, computed tomography of the brain and cerebrospinal fluid examination were unremarkable. Metronidazole had been administered at a dose of 65 mg/kg/day and neurotoxicity was, therefore, suspected. Drug concentrations in the patient's serum and cerebrospinal fluid were measured using high-performance liquid chromatography and compared to control dogs. Metronidazole administration was immediately discontinued; supportive care consisted of fluid therapy and diazepam treatment. The neurological status of the patient improved rapidly within 72 h. The aim of this case report is to describe the clinical presentation of metronidazole intoxication in a mixed breed dog and to interpret the chromatographic analysis which can be a beneficial diagnostic and screening tool in dogs intoxicated with metronidazole.

Keywords: vestibular diseases; cerebellar dysfunction; canine diseases; gamma-aminobutyric acid; chromatography

Metronidazole (MTZ) is an injectable and oral synthetic, nitroimidazole antibacterial and antiprotozoal agent. It is commonly used in veterinary practice to treat a wide variety of conditions (Watson 1980; Tams 1984; Happonen et al. 2000; Olson et al. 2005; Cattin et al. 2008; Jergens et al. 2010; Senhorinho et al. 2012). The mode of action requires strict anaerobic conditions; susceptible infectious agents include *Bacteroides* sp., *Clostridium* sp., *Giardia* sp. etc. (Rossignol et al. 1984; Even et al. 1998; Plumb 1999; Marks and Kather 2003; Hausen et al. 2011). MTZ is primarily metabolised in the liver and crosses the blood-brain barrier rapidly; in mice, the unaltered drug accumulates in the cerebellum and hippocampal areas (Plumb 1999; Olson et al. 2005). The recommended dosage for long-term treatment in dogs is 10 mg/kg *p.o.* twice or three times a day (Plumb 1999). Adverse effects include neurological disorders, lethargy, weakness, neutropenia, hepatotoxicity, haematuria, nausea, vomiting and diarrhoea (Plumb 1999; Weiss 2005). MTZ toxicity can develop suddenly and if unrecognised, can lead to rapid deterioration and death (Wright and Tyler 2003). In cases of acute toxicity from a chronic overdose in dogs, the drug should be discontinued and the patients treated supportively and symptomatically (Wright and Tyler 2003). The aim of this case report is to describe a metronidazole-induced neurotoxicity with subsequent chromatographic analysis in a dog.

Case description

A six and a half-year-old 7.2-kg uncastrated male mixed breed dog was presented for neurological ex-

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amination with an acute generalised ataxia, tremor, nystagmus and progressive weakness of the hind limbs. During the preceding four weeks, MTZ had been administered to the patient in a private veterinary practice due to suspected colitis at a dose of 65 mg/kg a day. One day prior to the presentation, vomiting had been observed. Physical examination was unremarkable except for subfebrile rectal temperature (39.1 °C), polypnoea and right unilateral cryptorchidism. On neurological examination, the dog was disoriented with a wide-based stance and intention tremors. A severe generalised ataxia with a spastic tetraparesis and hypermetria of the forelimbs, spontaneous and positional bilateral vertical nystagmus and strong responsive mydriasis were detected. The dog's neurological signs suggested a cerebellar and central vestibular lesion. Differential diagnoses were vascular, inflammatory, metabolic, toxic or neoplastic disorders of the cerebellum and central vestibular system.

A diagnostic plan included laboratory examination with a coagulation profile, blood pressure measurement, diagnostic imaging of the brain and evaluation of the cerebrospinal fluid (CSF). A complete blood cell count and serum biochemistry revealed mild lymphopenia, neutrophilia without a left shift, hyperalbuminemia, mild hyperglycemia, hypophosphatemia and mild elevation of alanine aminotransferase (ALT; Table 1). The coagulation profile and urinalysis were unremarkable except for hypersthenuria (urine specific gravity 1.055). Oscillometrically measured mean arterial blood pressure of the *arteria mediana* was 92 mmHg (reference values 80–120 mmHg). A non-contrast and contrast computed tomography (CT) scan of the brain showed no evidence of an acute haemorrhage, mass effect or a midline shift. The CSF was collected from the cerebellomedullary cistern (spinal needle B. Braun Spinocan® 22 Ga. × 1½ in., 40 mm) and its examination was also unremarkable.

MTZ intoxication was suspected due to the patient's history, neurological signs and unremarkable clinical investigations. After the immediate discontinuation of the MTZ treatment, the patient improved greatly during the first three days with supportive care (acetated Ringer's solution intravenously through a cephalic catheter, assisted feeding, monitoring of vital signs) and administration of diazepam at a dose of 0.5 mg/kg twice a day *p.o.* for five days. For the evaluation of MTZ concentrations, venous blood was taken on the first and the third day of the hospitalisation, and two and four weeks after the treatment withdrawal. The serum and CSF MTZ concentrations were measured using high-performance liquid chromatography (HPLC) in the pharmaceutical laboratory at the University of Veterinary and Pharmaceutical Sciences Brno, and compared to the control individuals (Table 2). HPLC is a technique in analytical chemistry used to separate and quantify every single component of a mixture based on their differing interactions with an adsorbent material. A mixture of methanol and water (85 : 15) was used as a mobile phase. An Eclipse Plus C18 column (Agilent Technologies, USA) was used. Dichlormethane was used to extract MTZ from the samples. The samples were processed on the day of collection without any storage delay. These screenings are not easily available (in the Czech Republic) but to our knowledge, they are accessible in human faculty hospitals.

Table 1. Haematology and serum biochemistry of the patient

Parameter	Values	Range
Leukocytes (10^9/l)	14.8	6.0–17.0
Erythrocytes (10^{12}/l)	7.37	5.5–8.5
Haemoglobin (g/l)	164	120–180
Haematocrit (l/l)	0.52	0.37–0.55
Platelets (10^9/l)	352	200–500
Neu bands (10^9/l)	0.296	0.0–0.45
Neu segments (10^9/l)	13.024	3.3–10.5
Lymphocytes (10^9/l)	0.888	1.0–3.6
Monocytes (10^9/l)	0.444	0.0–0.5
Eosinophils (10^9/l)	0.148	0.0–0.6
Total protein (g/l)	77.6	55–75
Albumin (g/l)	38.1	23–34
Bilirubin (μmol/l)	3.8	0–7
Creatinine (μmol/l)	70.6	35–110
Glucose (mmol/l)	7.7	3.1–6.7
Urea (mmol/l)	4.2	3.3–8.3
ALP (μkat/l)	1.41	0.1–4.0
ALT (μkat/l)	1.92	0.1–1.0
Calcium (mmol/l)	2.59	2.3–3.0
Phosphorus (mmol/l)	0.93	1.0–2.1
Sodium (mmol/l)	154	140–155
Potassium (mmol/l)	4.1	4.0–5.5
Chloride (mmol/l)	118.4	100–120

ALP = alkaline phosphatase, ALT = alanine aminotransferase, Neu = Neutrophils

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Table 2. Metronidazole concentrations measured using high-performance liquid chromatography. The regular patient was treated with a long-term metronidazole dose of 12 mg/kg twice a day

Individuals	Weight (kg)	Sample	Concentration (μg/ml)
Intoxicated patient	7.3	serum A	1.3551
		CSF	1.388
		serum B	0.209
		serum C	0.1551
		serum D	0.1416
Regular patient	18.6	serum	0.5033
Healthy dog	11.2	serum	0.0

A = day of presentation, B = two days after discontinuation (AD), C = two weeks AD, CSF = cerebrospinal fluid, D = four weeks AD

DISCUSSION AND CONCLUSIONS

The presented case report depicts the risks of a long-term (more than one week) high-dose MTZ therapy in dogs. The symptoms detected in our patient revealed a vestibulocerebellar disorder which corresponds to the supposed mechanism of MTZ toxicity. These signs are commonly described in the literature but some dogs are presented with a history of seizures or only spastic tetraparesis (Dow et al. 1989; Fitch et al. 1991). Due to the history and symptomatology of our patient, MTZ toxicity was the first differential diagnosis that was considered. Because of peracute progressive signs, the owner accepted a complete neurodiagnostic procedure to exclude every potential cause of the patient's condition.

In human patients, cerebellar toxicity can infrequently result from MTZ treatment and is related to high cumulative doses ranging from 25 to 1080 g (Patel et al. 2008). Most commonly, neurotoxicity in dogs is manifested after a chronic-moderate to high-dose therapy. Signs of intoxication include anorexia, depression, mydriasis, nystagmus, ataxia, and head-tilt, as well as deficits of proprioception, joint knuckling, disorientation, tremors, seizures, bradycardia, rigidity and stiffness (Dow et al. 1989). At high doses, MTZ has been reported to induce severe central nervous system (CNS) dysfunction in dogs – in the past, a dosage of 66 mg/kg/day was recommended for the MTZ treatment of giardiasis (Dow et al. 1989). After a number of reports of intoxication, the dosage rate of 30 mg/kg/day, which is closer to those prescribed in humans, was suggested (Dow et al. 1989). MTZ intoxication has also been described in cats and the forebrain signs are more common than the vestibulocerebellar ones in this species (Saxon and Magne 1993; Olson et al. 2005). In three cats with MTZ neurotoxicosis, nystagmus, which is a typical feature of the MTZ intoxication in dogs, was missing (Saxon and Magne 1993). The dose administered in our patient can lead to neurotoxicity within two weeks – susceptibility to MTZ seems to vary depending on the individual and the risk of treatment lies in the extent of depot with cumulative doses. In five dogs, MTZ administered for 3–14 days (dosage rate 67.3–129.0 mg/kg/day) led to the acute development of CNS dysfunction. Clinical signs began with anorexia and intermittent vomiting and progressed to generalised ataxia and positional vertical nystagmus (Dow et al. 1989). Moreover, extreme doses (more than 100 mg/kg/day) can produce clinical toxicity in a few days (Dow et al. 1989; Wright and Tyler 2003). The duration of common MTZ treatment should not generally exceed 10 days (according to the instructions in the manufacturer's leaflet). For longer therapy, there should be a strict indication or cultivation results – neither of these were available for our patient, who later suffered from neurological complications.

Post-mortem histopathological examination of the CNS of intoxicated dogs revealed lesions and deaths of Purkinje cells, axonal damage as a consequence of vasogenic oedema, as well as an inhibition of RNA synthesis (Scharer 1972; Fitch et al. 1991; Agarwal et al. 2016). In our patient, who survived the intoxication and improved greatly upon the discontinuation of therapy, *ante-* or *post-mortem* biopsies were considered unnecessary and were not taken. In cases of MTZ toxicosis, magnetic resonance imaging (MRI) can be useful and may detect affected predilection areas. In humans, abnormal bilateral symmetric T2 hyperintense lesions in the cerebellar dentate nucleus, midbrain, dorsal pons, medulla, *corpus callosum* and cerebral white matter were described (Ahmed et al. 1995; Kim et al. 2007). In our case, we conducted only a CT as a time-saving step which was necessary to rule out other pathological conditions (mainly vascular or neoplastic diseases which may resemble metronidazole poisoning) with respect to the

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acute patient's clinical course (Fitzgerald 2013). We therefore did not have any brain MRI scans which may be a limitation of our diagnostic procedure.

The exact mechanism of MTZ toxicity is unknown, but is hypothesised to be modulated by the affinity of MTZ for gamma-aminobutyric acid (GABA) receptor sites in the vestibulocerebellum; it is interesting that both the chemical structure as well as clinical signs of the MTZ toxicity are similar to the benzodiazepine antagonist flumazenil, which also binds GABA receptors (Evans et al. 2003). The treatment of intoxicated patients consists of immediate MTZ discontinuation and supportive care in combination with diazepam administration. A return to normal clinical condition takes 1–2 weeks; diazepam significantly shortens the recovery time. In 21 dogs intoxicated with MTZ, the recovery time was 38 h in patients treated with diazepam and 11 days in untreated ones (Evans et al. 2003). Diazepam is believed to exert its anti-vertiginous effect by promoting the effects of GABA and reducing subsequent vestibular system excitement (Ryu and McCabe 1974; Matsuoka et al. 1975; Steiner and Felix 1976; Pettorossi et al. 1982; Evans et al. 2003). The excitatory signals are transmitted through the NMDA and AMPA glutamate receptors in the vestibular system; strategies aimed at controlling these pathways are still in the experimental stages (Li et al. 2012). A sufficient dose of diazepam in these cases is 0.5 mg/kg orally or intravenously twice or three times a day for at least three days. In our patient, we observed a great improvement within 72 h at a dose of 0.5 mg/kg orally twice a day.

To the authors' knowledge, MTZ concentrations are not usually determined in intoxicated dogs. Fitch et al. (1991) measured the MTZ concentration in an intoxicated dog one week after the withdrawal from the MTZ. The determined concentration was 47 mEq/ml, but they did not compare that value to other dogs (human therapeutic concentrations are less than 20 mEq/ml) and did not describe the utilised laboratory technique. In general, MTZ concentrations are frequently measured using chromatographic techniques (Das et al. 2015). In our patient, we used HPLC to determine the serum and CSF concentrations of MTZ. These results were compared to the serum concentrations in a clinically healthy dog and one patient treated with appropriate doses of the drug. Due to the necessity of general anaesthesia for CSF collection, this was not collected in the control dogs. The MTZ serum concentration in our patient was more than twice as high as in an appropriately treated dog, had a rapidly decreasing tendency after the withdrawal from MTZ and was equal to the concentration in CSF (Table 2). Identical MTZ concentrations in the serum and CSF support the presumption that MTZ readily penetrates the blood-CSF and blood-brain barrier (Jokipii et al. 1977; Warner et al. 1979; Hoffmann et al. 1984; Nau et al. 2010). Nevertheless, while HPLC can be beneficial for monitoring the drug concentrations in the intoxicated dogs, it is not sufficient to establish the final diagnosis without further investigations. In humans, some studies have shown that neurotoxicity does not seem to correlate with MTZ concentrations in the serum or CSF but further studies in veterinary medicine are warranted (Kusumi et al. 1980; Dow et al. 1989; Wright and Tyler 2003).

Whenever an animal presents with central vestibular and cerebellar signs, a thorough drug history should be obtained. MTZ neurotoxicosis can develop suddenly and if unrecognised, may be fatal. The MTZ dose should be chosen following the current recommendations and tailored to the individual animal (Wright and Tyler 2003). During the four-week follow-up period, no neurological symptoms or signs of colitis were observed in our patient. One year after the intoxication, the patient is clinically healthy without any neurological deficits or symptoms of colitis.

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