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Effect of Fluoxetine Hydrochloride on the Biochemistry Profile and Complete Blood Count in Healthy Dogs

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ABSTRACT

Objective: The aim of this study is to evaluate the effect of fluoxetine on any hematological and biochemical parameter that could, in turn, affect the physiology and the behavior of the dog.

Methods: Thirteen healthy dogs without medical or behavioral problems were included in the study. All of them received 1 mg/kg q24 h of fluoxetine for 45 days (induction period), and then 1 mg/Kg q 48 h for 15 more days (withdrawal period). Blood was collected on Days 0, 46 and 61. A complete blood count and complete the biochemistry profile were performed.

Results: Regarding the complete blood count, the most important results that were obtained were related to HCT, MCV, MCHC and Mono. HCT and MCV increased during the withdrawal period, whereas MCHC and Mono decreased during the same period. In relation to the biochemistry panel, statistical differences were found in the ALT, AST, and GGT decreased during some of the periods. Cl decreased during induction period, whereas Na increased during the same period. The differences between Gluc and Fruc are noteworthy. Glu decreased during the induction period. In contrast, Fruc increased during the induction period. CK increased during induction period, and the same occurred with prothrombin time. TGlob increased during the withdrawal period, α1-glob decreased during the induction period, whereas α2-glob increased during the withdrawal period. Finally there was a sex-treatment interaction in γ-Glob, so that in female dogs it decreased during induction period and remained low at the end of the study. Most of the parameters remained within the physiological limits during all the study.

Conclusions: Regarding the analyzed parameters, we can conclude that the use of fluoxetine in dogs is safe, and does not seem to change any parameter that could affect the behavior of the animal.

INTRODUCTION

Aggression and anxiety-related disorders account for the vast majority of all behavioral disorders in dogs, and their treatment often includes the use of psychotropic drugs. Selective serotonin reuptake inhibitors (SSRIs) are one of the most commonly used drugs in behavioral medicine, and fluoxetine in particular has become the most commonly used SSRI in dogs.

Fluoxetine is a strong inhibitor of serotonin reuptake and a very weak inhibitor of norepinephrine reuptake. It also has very little binding to muscarinic, histaminergic, and alpha1-adrenergic receptors, as compared with other antidepressants [1]. It is well-

absorbed after oral administration, although food may delay its absorption. Fluoxetine is largely metabolized in the liver by the cytochrome P-450 enzyme system to norfluoxetine, an equipotent SSRI that contributes to the efficacy of fluoxetine [2,3]. After a single dose of approximately 2 mg/Kg body- weight, fluoxetine has a T1/2 of 6.2 h ± 0.8h (mean ± standard error), whereas that of norfluoxetine is of 49.0 h ± 3.0 h. In a 21-day study, after an administration of 0.75 mg/kg/24 h, 1.25 mg/kg/24 h and 3.0 mg/kg/24 h of fluoxetine in laboratory beagles, a steady state appeared to be reached within 10 days [4]. In a one-year study, dogs were administered 1 mg/Kg/24 h dose of fluoxetine, and a continuous increase in trough concentration (plasma concentration of a drug just before the next dose) was observed throughout the year. A similar increase in concentration was observed with norfluoxetine. This phenomenon was not observed at higher doses [4]. Fluoxetine and norfluoxetine are distributed throughout the body, with higher levels found in the lung and liver. CNS concentrations are detected 1 h after dosing [5]. Excretion of fluoxetine is primarily via the kidney. In humans, there is a wide variation in duration of action. Liver, but not renal, impairment will increase clearance time [5].

Fluoxetine has been widely used for treatment of affective aggression (especially impulsive ones) and anxiety-related disorders (e.g. separation anxiety, fears and phobias and compulsive disorders) in dogs [6,7]. Analgesic activity is another desired effect of fluoxetine which results, in part, from an increase in the activity of the endogenous descending analgesic system and the central opioid pathways [8]. Nevertheless, this analgesic activity is controversial since many studies show that fluoxetine could enhance pain response [9]. Fluoxetine could also have anti-inflammatory effects [10]. The most common side-effects of fluoxetine are vomiting, diarrhea, changes in urine frequency, insomnia, sedation, excitement, seizures, headache, abnormal bleeding, decreased sexual motivation (although in human beings, delayed orgasm or anorgasmia is more common than is a decrease in sexual motivation), anxiety, tremors and changes in appetite [2]. Although it is controversial, some studies show that fluoxetine may increase suicide thoughts in human patients [11]. There are few studies in dogs and human beings about the effect of fluoxetine on the biochemistry panel and complete blood count. However, It is well-known that fluoxetine may alter the metabolism of blood glucose. In particular, hyperglycemia may develop during treatment with fluoxetine, while hypoglycemia may develop upon withdrawal of fluoxetine [1]. Fluoxetine may increase liver enzymes, although there are no reports of liver pathology unless the patient had prior liver disease [5]. Hypernatremia has been described in human medicine, particularly in elderly patients [12-15]. The vast majority of these studies were retrospective and they were made in human patients with some kind of psychiatric disorder. Finally, one prospective study showed a relationship between the use of fluoxetine and changes in thyroid hormones [16], which has an important role in human and animal behavior. A relationship between lipid profiles and changes in behavior also has been suggested in both animals and human beings, such as major depression [17], generalized anxiety [18], Asperger Syndrome [19] and obsessive-compulsive disorders [20,21]. To the best of our knowledge there is no previous study on the effects of fluoxetine on the biochemistry profile and a complete blood count in dogs. Therefore, the aim of this study is to quantify such effects in order to assess if fluoxetine is a safe drug in dogs and whether it can modify any hematologic and biochemical parameter that could, in turn, affect the behavior of the dog.

MATERIAL AND METHODS

Animals

Thirteen animals (n=13) were included **(Table 1)** in the study: two French bulldogs, one Boxer, one Dalmatian, one English bulldog, two Majorcan dog buzzard, one Golden retriever and five crossbred dogs. All of them were patients of a private clinical service (Clínica Veterinaria Balmes) in Palma de Mallorca, Spain. None of them had behavioral problems, and they had come for a routine visit (vaccination). One of them (Table 1–No. 12) had suffered a unilateral Legg-Calvé-Perthes, which was solved with surgical treatment (arthroplasty) at nine months old. Another individual (Table 1–No. 7) had hip dysplasia diagnosed three years before the study, but no treatment was required at the moment of the study.

Table 1. Animals included in the study.

ID	Name	Age (years)	Breed	Sex	Neutered
1	Avalancha	7	French Bulldog	Female	Y
2	Twister	2,5	French Bulldog	Male	Y
3	Hugo	7	Boxer	Male	N
4	Trui	8	Dalmatian	Male	N
5	Truy	5	Crossbred dog	Male	N
6	León	10	Crossbred dog	Male	N
7	Bubi	13	Golden retriever	Male	Y
8	Estrella	12	Crossbred dog	Female	Y
9	Bruixa	10	Crossbred dog	Female	Y
10	Chuski	8	Crossbred dog	Male	N
11	Bruce	3	English Bulldog	Male	N
12	Xima	9	Majorcan dog buzzard	Female	N
13	Clapeta	2	Majorcan dog buzzard	Female	N

Eight dogs (8/13) were purebreds. Eight (8/13) were males and five (5/13) were females. Two males were castrated (2/8)

and three females were spayed (3/5). The mean age was 7.42 years (2 years ≤ x ≥ 13 years). 1 One week after initiating the study, one dog (Table 1–No. 4) was bitten, and he required antibiotic and anti-inflammatory treatment. Fluoxetine treatment was stopped and the animal was discarded from the study. All the methods involving the dogs studied in this project were approved by the ethics committee of the Autonomous University of Barcelona and the Government of Catalonia (Spain).

Methodology

From Day 1 to Day 46 all dogs received 1 mg/Kg q 24 h of fluoxetine (Fluoxetine Cinfa® 20 mg-tablet) in the morning and on an empty stomach. From Day 46 to Day 61 they received 1 mg/Kg q 48 h in the same conditions described above. Blood was collected on Days 0 (t0), 46 (t1) and 61 (t2) through venipuncture of the jugular vein. All samples were processed within the first 48 h after extraction in Vetlab-Idexx Laboratories S.L. (c/ Plom n 2–8, 3rd 08038, Barcelona, Spain).

A complete blood count (CBC) (Sysmex XT-2000i–Laser analyser; Fluorescent flow cytometry) and a complete biochemistry (Olympus AU640-Spectrophotometry) profile were performed for each sample. CBC included red-blood-cell count (RBC), hematocrit (HCT), red-blood-cell distribution width (RDW), mean corpuscular volume (MCV), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white-blood-cell count (WBC), band neutrophils, polymorphonuclear neutrophils (PMN), lymphocytes (Lym), monocytes (Mono), eosinophils (Eos), basophils (Baso), platelet count (PLT) and mean platelet volume (MPV).

The biochemistry profile included bile acids (BileA), alanine transaminase or alanine aminotransferase (ALT), aspartate transaminase or aspartate aminotransferase (AST), total bilirubin (TBil), total calcium (Ca), chlorine (Cl), cholesterol (Chol), creatinine (Crea), creatine kinase (CK), alkaline phosphatase (ALKP), phosphorous (P), gamma-glutamyl transpeptidase (GGT), glucose (Glu), sodium (Na), potassium (K), sodium-potassium ratio (Na/K), triglycerides (Tryg), blood urea nitrogen (BUN), fructosamine (Fruc), total T4 (T4t) and thyrotropin or thyroid- stimulation hormone (TSH). A coagulation profile (Stago STart System - Bichromatic optics technology) including prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT) was obtained. Also, a total-protein test (Sebia Hydrasys - Electrophoresis) was performed including total protein (TP), total albumin (ALB), total globulins (TGlob), α1- globulins (α1-Glob), α2-globulins (α2-Glob), β-globulins (β-Glob) and γ-globulins (γ-Glob).

All side effects were daily monitored by the owners (e.g. vomiting, diarrhoea, anorexia, drowsiness, etc.).

Statistical Analysis

Data from the complete blood count and biochemistry profile (except ALT and BileA) were analysed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) for repeated measures. A log transformation was applied to those parameters whose residuals were not normally distributed (eight parameters: PMNN, Eos, AST, CK, ALKP, Trig, T4, TSH). ALT and BileA were analysed by means of a GENMOD procedure for repeated measures. A negative-binomial distribution was used according to the value of the deviance. The models studied the evolution of each biological variable over treatment and accounted for the effect of the gender as well as the interaction time of extraction by gender. The residual maximum likelihood was used as a method of estimation and the least square means of fixed effects (LSMEANS) was used when analysis of variance indicated differences ($P<0.05$).

RESULTS

Regarding the complete blood count **(Table 2)**, the most important results that we obtained were related to HCT, MCV, MCHC and Mono. HCT remained constant after the first 45 days (t0–t1) of treatment (induction period), and then it increased during the withdrawal period (t1–t2). No differences were found between t0 and t2. MCV increased during the withdrawal period, whereas it showed no statistically significant changes during the induction period. In contrast, MCHC decreased during the withdrawal period and remained constant during the induction period. Mono decreased in the withdrawal period, but no differences were found between t0 and t1, nor between t0 and t2. Only MCV and MCHC slightly moved out of the physiological range at t2. The mean of MCV at t2 was 78.85 fl ± 3.79 fl (Reference value: 62 fl–74 fl), and of MCHC was 30.46 g/dl ± 1.17 g/dl (Reference value: 31.5 g/dl–36.5 g/dl). The rest of the CBC parameters were always within the physiological range.

Table 2. Complete blood count parameters–Mean ± Standard Deviation.

Parameter (units)	T0	T1	T2	Reference value
RBC (x1000.000)	7.29 ± 0.74	6.85 ± 0.95	7.00 ± 0.78	5.6 – 8.5
HCT (%)	52.18 ± 5.62^{ab}	49.63 ± 6.09^{b}	55.03 ± 5.08^{a}	38.7 – 57
RDW (%)	16.28 ± 1.83	15.72 ± 1.80	15.50 ± 2.34	12 – 15.5
MCV (fl)	71.72 ± 5.63^{b}	72.67 ± 3.48^{b}	78.85 ± 3.79^{a}	62 – 74
HGB (g/dl)	17.35 ± 1.81	16.38 ± 2.34	16.78 ± 1.88	13.4 – 19.1
MCH (Pg)	23.81 ± 1.23	23.90 ± 1.08	24.00 ± 1.08	21.7 – 26
MCHC (g/dl)	33.28 ± 1.53^{a}	32.93 ± 1.06^{a}	30.46 ± 1.17^{b}	31.5 – 36.5
WBC (x1.000)	8.38 ± 1.54	9.01 ± 2.03	8.65 ± 1.54	5.95 – 17.20
BandN (cls/µl)	0 ± 0	0 ± 0	0.5 ± 1,73	0 – 500
PMNN (cls/µl)	5775.33 ± 1405.71	6114.67 ± 1554.75	6216.08 ± 1060.95	3380 – 11530

Lym (cls/µl)	1758.00 ± 528.60	1942.00 ± 622.62	1654.67 ± 478.05	900 – 4300
Mono (cls/µl)	466.92 ± 203.10[ab]	616.58 ± 311.03[a]	340.00 ± 162.63[b]	100 – 1700
Eos (cls/µl)	378.25 ± 226.29	330.91 ± 168.14	431.16 ± 294.36	100 – 1250
Baso (cls/µl)	6.25 ± 7.89	6.66 ± 8.30	7.64 ± 7.74	0 - 100
PLT (x1000)	295.92 ± 108.87	268.58 ± 76.92	267.25 ± 104.85	145 – 493
MPV (fl)	11.83 ± 1.33	11.75 ± 1.01	11.13 ± 1.73	8.4 – 13.2

In relation to the biochemistry panel **(Table 3)**, statistical differences were found in the ALT, AST, Cl, CK, Glu, Fruc and Na values.

Table 3. Biochemistry profile – Mean ± Standard Deviation.

Parameter (units)	T0	T1	T2	Reference value
BileA (µmol/l)	3.00 ± 3.31	3.67 ± 3.67	3.00 ± 2.26	0.1 – 10
ALT (IU/L)	53.00 ± 25.72 [a]	47.40 ± 24.38 [ab]	39.22 ± 19.47 [b]	26 – 89
AST (IU/L)	31.92 ± 15.40 [a]	20.75 ± 9.15 [b]	26.33 ± 11.88 [ab]	<109
TBil (mg/dl)	0.16 ± 0.03	0.21 ± 0.05	0.18 ± 0.04	0.01 – 0.31
Ca (md/dl)	10.71 ± 0.34	10.20 ± 0.36	10.48 ± 0.50	8.2 – 11.9
Cl (mmol/L)	119.00 ± 4.88 [a]	110.83 ± 2.08 [b]	111.54 ± 2.84 [b]	105 – 121
Chol (mg/dl)	220.50 ± 68.98	239.42 ± 90.58	249.25 ± 95.21	112 – 326
Crea (mg/dl)	0.99 ± 0.20	1.00 ± 0.19	1.01 ± 0.23	0.7 – 1.5
CK (IU/L)	35.41 ± 16.52 [b]	80.67 ± 24.34 [a]	73.33 ± 41.05 [a]	69 – 309
ALKP (IU/L)	45.75 ± 15.83	52.73 ± 28.34	48.89 ± 15.00	13 – 105
P (mg/dl)	4.41 ± 0.58	4.22 ± 0.67	4.05 ± 0.60	2.7 – 6.7
GGT (IU/L)	5.54 ± 1.97 a	4.36 ± 1.86 [b]	5.45 ± 1.50 a	0.5 – 10
Glu (mg/dl)	103.00 ± 10.68 [a]	93.08 ± 4.54 [b]	107.83 ± 16.63 [a]	60 – 120
Na (mEq/L)	143.09 ± 2.43 [b]	146.25 ± 1.91[a]	146.82 ± 2.09 [a]	142–153
K (mEq/L)	4.70 ± 0.41	4.75 ± 0.35	4.89 ± 0.58	3.9–5.6
Na/K	30.27 ± 2.30	30.92 ± 1.99	29.34 ± 2.13	>27
Trig (mg/dl)	71.75 ± 21.77	102.33 ± 100.29	119.42 ± 130.97	34–136
BUN (mg/dl)	27.72 ± 6.54	33.09 ± 9.30	31.25 ± 10.68	21–59
Fruc (µmol/l)	218.40 ± 39.59 [c]	274.09 ± 29.92 [a]	245.45 ± 19.53[b]	187–386
T4 (µg/dl)	1.01 ± 0.38	1.16 ± 0.38	1.31 ± 0.42	1.0–2.4
TSH (ng/ml)	0.20 ± 0.11	0.18 ± 0.10	0.18 ± 0.09	0.03–0.6

Different letters within row means significant differences between extractions at p<0.05.

Regarding the hepatic values, no statistical differences were found in ALT values between t0-t1 and t1-t2, but ALT decreased between t0-t2. AST values decreased in the induction period, but no differences were found between t1-t2 and t0-t2. GGT decreased during the induction period and recovered its initial values during the withdrawal period.

In relation to the ion panel, Cl decreased in the induction period, and it remained low during the withdrawal period. In contrast, Na increased during t0-t1 and remained high between t1-t2.

Concerning the glucaemic panel, the differences between Gluc and Fruc are noteworthy. Glu decreased during the induction period and recovered its initial value after the withdrawal period. In contrast, Fruc increased during the induction period and recovered its initial value in t2.

CK increased during the induction period and remained high at t2. No differences were found in the thyroid panel. In fact, in our sample, CK was outside the normal limit at the beginning of the study, and moved within the physiological range when the treatment was initiated. The CK mean at t0 was 35.41 ± 16.52 UI/L (Reference value: 69 UI/L–309 UI/L).

Regarding the coagulation profile **(Table 4)**, PT was the only parameter which showed a treatment effect: it increased in the induction period and remained high at t2.

Table 4. Coagulation profile – Mean ± Standard Deviation.

Parameter (units)	T0	T1	T2	Reference value
PT (s)	8.54 ± 0.49 [b]	9.30 ± 0.80 [a]	9.55 ± 0.76 [a]	6.0–10.8
aPTT (s)	11.34 ± 0.97	11.08 ± 1.04	11.54 ± 0.83	10.1–13.5
TT (s)	14.48 ± 0.89	13.26 ± 1.29	13.64 ± 1.32	13.3–15.7

Different letters within row means significant differences between extractions at p<0.05.

In relation to the total-protein test **(Table 5)**, differences were found in α1-glob, α2-glob and TGlob. α1-glob decreased during the induction period and remained low after the withdrawal period, whereas α2-glob increased between t1-t2 (no differences were found between t0-t1 and t1-t2). However, it is important to highlight that α2-glob were slightly high respect the reference values during all the study **(Table 5)**. Finally there was a sex-treatment interaction in γ-Glob, so that in female dogs it decreased during t0-t1 and remained low in t2.

Table 5. Total protein test–Mean ± Standard Deviation.

Parameter (units)	T0	T1	T2	Reference value
TP (g/L)	67.58 ± 4.78	65.00 ± 4.77	67.42 ± 4.34	52–76
ALB (g/L)	31.32 ± 3.81	30.01 ± 3.70	30.16 ± 4.35	25.4–40.6
TGlob (g/L)	37.00 ± 5.23 [ab]	34.97 ± 5.24 [b]	37.25 ± 6.33 [a]	20.6–50.6
α1-Glob (g/L)	3.37 ± 0.33 [a]	3.00 ± 0.34 [b]	3.12 ± 0.47 [b]	1.3–4.5
α2-Glob (g/L)	10.51 ± 2.02 [b]	11.02 ± 2.37 [ab]	12.07 ± 2.79 [a]	4.6–9.9
β-Glob (g/L)	14.81 ± 2.30	14.73 ± 2.32	15.53 ± 2.21	13.5–23.5
γ-Glob (g/L)	6.95 ± 1.21 [a]	5.71 ± 0.95 [b]	6.01 ± 1.55 [b]	1.2–20

Different letters within row means significant differences between extractions at $p<0.05$.

All the parameters, except the mentioned exceptions, were within the physiological range during all the study.

None of the owners reported any side effect during all the study.

DISCUSSION

CBC values

Red cells

Hematocrit: We found differences between t1 and t2, t2 being significantly higher than t1, but it must be stressed that all values were always within the normal limits.

There are few scientific data about the effect of fluoxetine on the Hematocrit value. Hematocrit depends mainly on Red Blood Count (RBC) and the hydration state. In turn, RBC depends on the production of red cells by the bone marrow (it could be decreased or increased), their losses from the body (e.g. external hemorrhage), and their destruction in the body (e.g. hemolysis). The hydration state depends on many factors, the most important being water balance including water losses [22]. We assume that all of these variables remain within normal limits in healthy animals, and therefore the Hematocrit changes observed in our study would be caused by fluoxetine.

In depressed elderly human patients treated with fluoxetine [23], the Hematocrit changed after 42 days, on average. The Hematocrit was higher in non-respondent patients than in respondent ones, showing that Hematocrit may change in response to the use fluoxetine, as has been found in our study. To the best of our knowledge this is the only article that provides some evidence concerning the relationship of fluoxetine treatment and Hematocrit, although the mechanism responsible for such an effect is not known.

MCV–Mean Corpuscular Volume

We found normocytosis in t0 and t1 and macrocytosis in t2: 78.85 fl ± 3.79 fl (mean ± standard error) (Reference values: 62 fl–74 fl). The most common cause of macrocytosis is reticulocytosis, especially 4–5 days after the onset of anaemia [22]. Other causes are stomatocytosis, breed-associated stomatocytosis (e.g. poodles, miniature and standard Schnauzers, and Alaskan Malamute) [24], and artifactual swelling of RBCs in EDTA tubes during prolonged storage (from 6 to 24 h in non-refrigerated samples, and from 24 h in refrigerated ones) [25]. The macrocytosis of storage is common in samples mailed to laboratories or samples analysed the day after collection [22].

In our study, none of the animals had anemia or stomatocytosis.

In conclusion, it cannot be ascertained if this macrocytosis was due to a storage problem or to the effect of fluoxetine. Indeed, although all samples were analysed within 48 h after collection, we could not control whether the analyses were performed during the first or second 24 h.

Mean Corpuscular Hemoglobin Concentration–MCHC

MCHC is calculated using the following formula, MCHC=(Hemoglobin × 100)/Packed Cell Volume. Hemoglobin was constant between the extractions. Indirectly, we know that PCV is higher in t2 because we had macrocytosis in t2 and RBC remained constant during t0–t2 (MCV=(PCV × 10)/RBC) [25]. Thus, if hemoglobin and RBC were constant between the three extractions, then MCHC is inversely proportional to MCV. For that reason, low MCHC in t2 could be explained as a result of macrocytosis in t2.

White cells

Monocytes (Mono): There are no previous studies about the effect of fluoxetine on total count of monocytes nor on the other cells of the leukogram.

A decrease was found between t1 and t2, but always within the reference values. There are no data available concerning the underlying mechanism of action, and further investigation would be needed.

In our study, this variation in monocytes does not modify the total count of white cells, perhaps due to the low decrease.

White Blood Count (WBC)

We did not find any variation in WBC. However, in one study with depressed human patients [26] the authors found that fluoxetine could have some effect on WBC, because after two months of treatment they found a decrease in WBC. In that study, the authors used WBC as an inflammatory marker, and they found that the depressed group (treatment group) had higher levels of WBC, when compared to controls in the first part of the study, whereas after two months of treatment no differences were found between groups.

In conclusion, fluoxetine could have a profound effect on WBC in animals with medical conditions or stress-related disorders due to its anti-inflammatory and anxiolytic effect, but not in healthy ones, as can be concluded from our study.

Biochemistry panel

Hepatic function-related parameters: Fluoxetine undergoes hepatic metabolism after its absorption, being transformed into norfluoxetine (active metabolite) and a number of other metabolites [2]. CYP 450 2D6 plays a major role in its metabolism, but it is not the only enzyme involved. Fluoxetine can inhibit some of the enzymes involved in its own metabolism [27]. Moderately or highly elevated levels of aminotransferases have been observed in clinical trials in human beings [28] and in laboratory studies in rats [29]. Acute and chronic hepatitis have also been documented in human beings [30,31], and hepatic enzyme elevation has been found in 0.5% of humans treated with fluoxetine [28]. Unlike these studies, we found decreased values of transaminases (ALT and AST) after treatment with fluoxetine (without pathological significance). Nevertheless, the vast majority of these studies have no basal values, and they are clinical trials with other uncontrolled variables (patients with anxiety-related problems, or with multi-drug therapy, etc.). In one controlled study in rats [29], fluoxetine was administered orally at dosages of 8 mg/Kg and 32 mg/kg, and ALT and AST values were compared with a control group. In contrast with our study, they found that fluoxetine induced dosage-dependent liver damage. However, they had no information about basal ALT and AST. We also found a slight decrease in GGT values at t1.

In addition, no statistical differences were found in other hepatic values, neither structural nor functional (TBil, ALKP, BileA, BUN and ALB).

In conclusion, unlike all previous studies, our results reveal that there is no evidence of liver impairment due to the use of fluoxetine in healthy dogs. Nevertheless, in spite of our results, we cannot advise the use of fluoxetine in dogs with liver damage, because it has not been specifically analyzed in our study and further studies are needed.

Renal function-related parameters: Crea, BUN, P and Alb were included as renal function-related parameters, and no treatment effect was found in any of those parameters. There is a paucity of data regarding the effect of fluoxetine on renal function. In one study [32], the authors did a systematic review of randomized clinical trials and observational studies examining antidepressants in patients with renal failure. The authors concluded that, unlike other antidepressants, the pharmacokinetic parameters of fluoxetine were similar between patients with renal impairment and healthy controls. Although there are no other studies about the effect of fluoxetine in those parameters, our results, together with the conclusions of Nagler and colleagues [32], suggest that the use of fluoxetine in healthy animals does not affect renal function, and it could be safe even in animals with renal impairment.

Ion Panel: Na, K, Na/K, Cl and Ca were included in this panel. No statistical changes were observed with Na/K and Ca, whereas Cl decreased in the induction period and remained low during the withdrawal period, and Na increased during t0-t1 and remained high between t1-t2.

Although it is uncommon, hyponatraemia has been reported in 0.1% of humans treated with fluoxetine [2,12-15,33], especially in the elderly [15], during the first two weeks of treatment with fluoxetine. The mechanism underlying this effect is not well understood, but it has been attributed to reduced antidiuretic hormone secretion. A recent study in rats provides evidence that this decrease in the plasmatic sodium level can be attributed, at least in part, to the intrinsic capacity of fluoxetine to increase water permeability in the inner medullary collecting duct (IMCD), leading to an increase in water absorption [34]. In our study, fluoxetine leads to a slight increase in the serum sodium level (within the physiologic limits).

The differences between our data and the previous studies, increases of natraemia in contrast with hyponatraemia observed in few patients, could be due to the mean of age the dogs involved in our study (7.42 years), since they were not geriatric, and the fact that hyponatraemia is a really uncommon side-effect. We did not observe hyponatraemia in any of the dogs studied. However, further studies are needed in order to understand the underlying mechanism of that increase.

Potassium levels remained unchanged after the treatment, as has been found in other studies [34].

Chloride decreased in t1 and t2, as compared with t0, but it was always within the physiologic limits. Further studies are needed in order to know the underlying mechanism. Evaluation of chloride concentration must be performed in conjunction with evaluation of sodium concentration. Changes in water balance alter chloride and sodium concentrations proportionately, a phenomenon known as artifactual hypochloraemia [35]. Changes in the permeability of IMCD caused by fluoxetine [34] may lead to excessive loss of chloride.

Calcium did not change during the treatment, and there is no previous data available dealing with this issue.

Thyroid function: Thyroid hormones have an important role in animal and human behaviour.

Additionally, a relationship between thyroid function and behavioural problems has been suggested. Hypothyroidism has been associated with aggressive and fear-related behaviours in dogs [36], and with many psychiatric disorders in human patients [37,38]. Nevertheless, the mechanism underlying such effects has not been elucidated.

Total T4 (tT4) and TSH were analysed in order to evaluate thyroid function. None of them changed during treatment in our study. Several studies in human patients with major depression have examined the effects of selective serotonin reuptake inhibitors (SSRIs) on thyroid function and have yielded ambiguous results [39-43]. In the only prospective study that evaluates the effect of fluoxetine (and sertraline) on thyroid function in humans, 67 subjects were involved [16]. Twenty-eight patients with major depression and hypothyroidism on adequate levothyroxine therapy were randomized for treatment with fluoxetine (n=13) or sertraline (n=15); 29 patients with major depression and normal thyroid function were treated with fluoxetine (n=15) or sertraline (n=14) and 10 control patients with hypothyroidism were put on adequate levothyroxine treatment without depression. The authors found that patients with major depression and normal thyroid function who were treated with fluoxetine demonstrated a significant reduction of T3 after 15 and 30 days of treatment, and tT4 after 15 days, 30 days and 3 90 days of treatment (all of the intervention period) respectively. However, all thyroid parameters remained within the euthyroid range. In the control group and in the group of depressed patients with primary hypothyroidism, no changes were observed in T3 and tT4. TSH did not change in any group. Our results support part of the conclusions of this study. Fluoxetine does not change TSH levels and, in our case, tT4 concentrations.

Glycaemic-related parameters: Both glucose and fructosamine blood concentration undergo significant statistical changes during (t1) and after (t2) treatment. In a review article [44], the authors analysed 17 published case reports of glucose dysregulation associated with antidepressant agents. They concluded that hypoglycemia is associated with fluoxetine. We observe the same dysregulation in glucose levels, which decrease in t1 and increase after treatment (t2). However, the levels of fructosamine undergo the opposite change in our study. Fructosamine increases during treatment (t1) and decreases at the end of the withdrawal period (t2). Fructosamine levels correlate with the glucose blood levels during the preceding two to three weeks [45] and is not affected by acute increases in blood glucose concentrations, as occurs with stress hyperglycaemia.

Long-term use of SSRIs is associated with an increased risk of diabetes [46-48]. This could be attributed to weight gain, a frequent side-effect of treatment with SSRIs. Weight gain that leads to obesity is associated with an increased incidence of hypertension, dyslipidaemia, coronary artery disease, insulin resistance and overt diabetes in humans [49]. Despite these findings little is known about the pathophysiology of SSRIs as direct inducers of insulin resistance. A recent study [49] demonstrates that SSRIs induce insulin resistance in cultured Min6 cells and isolated murine islets. That result could explain why fructosamine remained high during treatment in dogs.

The differences between serum levels of glucose and fructosamine observed in our study, and seen in other studies in human beings separately, could be due to the fact that fructosamine remains unaffected by other circumstances that modify acute concentration of glucose.

From a clinical point of view, glucose levels should be carefully monitored when administering fluoxetine, particularly in diabetic animals, due to its potential effect on glucose metabolism and insulin resistance.

Lipid-related parameters: As mentioned above, the use of SSRIs is associated with weight gain, which could lead to dyslipidaemia in human beings. There are two clinical cases in the literature of severe and moderate hypertriglyceridaemia secondary to citalopram and fluoxetine [50] and to venlafaxine and fluoxetine [51] in human medicine. One retrospective study reports a correlation between the use of fluoxetine, sertraline or fluvoxamine (n=131) and abdominal obesity and hypercholesterolaemia [46]. The authors concluded that patients taking SSRIs should be carefully monitored for obesity and dyslipidaemia. In our study, no significant statistical differences were found in cholesterol and triglyceride serum levels. The differences between these human studies and our report could be explained in three different ways. The length of the study, the number of dogs involved in conjunction with the fact that a severe or moderate dyslipidaemia is a rare side-effect and, finally, it could be that the effect of fluoxetine on lipids in dogs is different from human beings. Further studies are needed in order to clarify this.

Finally, these results are especially important because some behavioural changes have been associated with variations in lipid profiles, such as Asperger syndrome [19], generalized anxiety [18], major depression [17], and bulimia nervosa [52] in human beings, and obsessive-ompulsive disorders in both humans and animals [20,21].

Moreover, the fact that fluoxetine does not modify the cholesterol level is important for three additional reasons. First, adequate levels of cholesterol are crucial for serotonin metabolism and myelination in the brain [19]. Second, cholesterol is required for the development of serotoninergic CNS neurons and for the catabolism and transport of serotonin [19]. Finally, studies in humans indicate a positive correlation between cholesterol and serotonin levels [53].

Others–Creatine Kinase: CK increases after 45 days (mean=80.67 IU/L) of treatment and remains significantly higher at

60 days (mean=73.33 IU/L). These changes had no clinical evidence because they moved into 3 the physiological range (69 IU/L–309 IU/L). CK is a sensitive indicator of muscle damage and only large increases (>10,000 IU/L) or persistent increases, even if moderate (>2,000 IU/L), are generally of clinical significance [54]. One study evaluates the effect of fluoxetine on CK activity in the brain after 28 days of treatment, but not in muscle or heart [55], nor serum concentration. The study mentioned found that after 28 days of treatment, the CK activity decreases two hours after the last injection of fluoxetine but not after 24 h. However, in dogs and horses they did not find a relationship between WBC counts, serum CK, or cerebrospinal fluid (CSF) total protein and CSF CK [56,57]. In fact, it is known that CK, a large macromolecule, does not cross the blood-brain-barrier (BBB); therefore, increased activity of this enzyme in CSF is considered to be of CNS origin (if the BBB is intact). Thus, it is likely that these slight changes in serum CK due to the use of fluoxetine do not directly affect a dog's behaviour.

To the best of our knowledge, there are no other studies that evaluate the effect of fluoxetine on CK serum concentration. The most important conclusion is that fluoxetine may change CK serum concentration, but further studies are needed in order to clarify the exact relationship and the underlying mechanism.

Coagulation profile

The coagulation profile included prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin time (TT). Differences were found only in PT time. PT increased after 45 and 60 days of treatment, but always within the physiologic range. Because bleeding abnormalities are common among patients treated with fluoxetine, many studies have been performed. The general conclusion is that decreased platelet aggregation and activity, and prolongation of bleeding time (primary haemostasis) are common, but modification of platelet count, PT, aPTT and TT (coagulation cascade) are much less frequent [58]. In fact, other studies [59,60] evaluated the effect of fluoxetine on PT, aPTT and TT (among others), and no differences were found in any case.

To conclude, bleeding abnormalities in association with the use of fluoxetine are common. These abnormalities are more likely to be due to primary haemostasis alterations more than to changes in the coagulation cascade.

Total protein test

We found no changes in total protein serum and albumin concentration. Significant results were found in total globulin serum. This change responds to significant statistical variations in α1-glob that decreased during and after treatment and in α2-glob, which were higher at the end of the study (Day 60). γ-Glob decreased at 45 and 60 days of study, but only in females. Variations in α1-glob and γ-Glob were always within the physiologic range and were minor changes. Although in the case of α2-glob the variations were minor too, the means of concentrations were slightly higher than was the physiologic range in each sample. Means were 10.51 g/L, 11.02 g/L and 12.07 g/L in T0, T1 and T2, respectively, the normal range being 4.6–9.9 g/L. We did not find any controlled factor that could explain that results. Otherwise, the variations were minor in all cases, and more studies would be needed in order to corroborate our findings with α2-glob.

There are few studies in the literature that have evaluated the effect of antidepressant drugs on serum proteins. Van Hunsel et al. [61] found differences in the major, electrophoretically separated protein fractions (α1-glob, α2-glob and γ-Glob) between human patients with major depression and the control group. They did not find differences due to the treatment with any antidepressant used in the study (fluoxetine, trazodone and pindolol). There are no studies carried out in veterinary medicine or in human patients without psychiatric disorders.

CONCLUSIONS

Regarding the analysed parameters, we can conclude that the use of fluoxetine in dogs is safe, and does not seem to change any parameter that could affect the behaviour of the animal.

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