J Appl Physiol 119: 1393–1399, 2015.
First published October 22, 2015; doi:10.1152/japplphysiol.00602.2015.

Fluoxetine stimulates anti-inflammatory IL-10 cytokine production and attenuates sensory deficits in a rat model of decompression sickness

Jean-Eric Blatteau,[1] Sébastien de Maistre,[1] Kate Lambrechts,[1,2] Jacques Abraini,[3,4] Jean-Jacques Risso,[1] and Nicolas Vallée[1]

[1]*Institut de Recherche Biomédicale des Armées, Équipe de Recherche Subaquatique Opérationnelle, Toulon, France;* [2]*Laboratoire Motricité Humaine, Éducation, Sport, Santé (LAMHESS), Université de Toulon UFR STAPS, La Garde, France;* [3]*Département d'Anesthésiologie, Université Laval, Québec, Canada; and* [4]*Normandie-Université, Université de Caen-Basse Normandie, Caen, France*

Submitted 17 July 2015; accepted in final form 15 October 2015

Blatteau JE, de Maistre S, Lambrechts K, Abraini J, Risso JJ, Vallée N. Fluoxetine stimulates anti-inflammatory IL-10 cytokine production and attenuates sensory deficits in a rat model of decompression sickness. *J Appl Physiol* 119: 1393–1399, 2015. First published October 22, 2015; doi:10.1152/japplphysiol.00602.2015.—Despite "gold standard" hyperbaric oxygen treatment, 30% of patients suffering from neurological decompression sickness still exhibit incomplete recovery, including sensory impairments. Fluoxetine, a well-known antidepressant, is recognized as having anti-inflammatory effects in the setting of cerebral ischemia. In this study, we focused on the assessment of sensory neurological deficits and measurement of circulating cytokines after decompression in rats treated or not with fluoxetine. Seventy-eight rats were divided into a clinical ($n = 38$) and a cytokine ($n = 40$) group. In both groups, the rats were treated with fluoxetine (30 mg/kg po, 6 h beforehand) or with a saccharine solution. All of the rats were exposed to 90 m seawater for 45 min before staged decompression. In the clinical group, paw withdrawal force after mechanical stimulation and paw withdrawal latency after thermal stimulation were evaluated before and 1 and 48 h after surfacing. At 48 h, a dynamic weight-bearing device was used to assess postural stability, depending on the time spent on three or four paws. For cytokine analysis, blood samples were collected from the vena cava 1 h after surfacing. Paw withdrawal force and latency were increased after surfacing in the controls, but not in the fluoxetine group. Dynamic weight-bearing assessment highlighted a better stability on three paws for the fluoxetine group. IL-10 levels were significantly decreased after decompression in the controls, but maintained at baseline level with fluoxetine. This study suggests that fluoxetine has a beneficial effect on sensory neurological recovery. We hypothesize that the observed effect is mediated through maintained anti-inflammatory cytokine IL-10 production.

diving; decompression sickness; bubble; neuroprotection; antidepressant

MILLIONS OF PEOPLE WORLDWIDE take part in recreational or professional diving. Decompression sickness (DCS) following return from elevated pressure is initiated by the formation of inert gas bubbles, resulting from nitrogen accumulation during the dive (1). The predominant theory is that bubbles grow from preformed nuclei composed of small stable gas bubbles (6, 23). Massive bubble formation after diving can lead to DCS, which may result in central nervous system disorders or even death (1, 7, 17, 50). It is now accepted that DCS-induced ischemia in the spinal cord or the brain results in neurological damage (11, 18, 19, 21). Bubble formation in the blood activates the vascular endothelium, stimulates prothrombotic phenomena, and induces inflammation: platelet and leukocyte activation have been observed, associated with raised production of cytokines and cell adhesion stimulators (2, 14, 28, 34, 35, 40).

Fluoxetine, a well-known antidepressant, is recognized as having anti-inflammatory effects in the setting of cerebral ischemia (9, 25, 30, 37, 45). Recently, we have shown that fluoxetine dramatically reduces the incidence of lethal and severe neurological DCS in a mouse model after rapid decompression (3). This mouse model of DCS is likely to generate a massive bubble formation, in the body and the brain, with a risk of convulsions and death in the mouse. Treatment with fluoxetine had the effect of limiting the number of deaths from convulsions and allowed better motor recovery in the treated animals.

The purpose of this study was to assess the effect of fluoxetine on another animal model for DCS, by using a slower staged decompression, capable of causing ischemic impairments of the spinal cord with variable severity in the rat (4, 41). This model, therefore, makes it possible to move closer to the DCS observed in humans. In fact, in recreational diving, medullary DCS is frequent and, in most cases, occurs in the absence of a fault in procedure or a fast ascent to the surface (5). Unfortunately, 20-30% of patients admitted to a hyperbaric center for a neurological accident will present residual sequelae, with motor and sensory neurological deficits after treatment with hyperbaric oxygen therapy (5). Treatment in a rehabilitation center often makes it possible to obtain motor recovery, but the sensory sequelae have a tendency to persist and are often responsible for gait disorders due to proprioceptive ataxia (16). In this study for the first time, we measured the intensity of the sensory neurological deficit and evaluated the efficacy of fluoxetine in the experimental DCS model. The clinical study was completed by the evaluation of decompression stress markers, such as the search for circulating bubbles in the right cavities after decompression, as well as counts for cellular blood components and platelets (blood cell counts) before and after hyperbaric exposure. Furthermore, to understand better the neuroprotective processes brought into play by fluoxetine in this DCS model, we performed an analysis of several pro- and anti-inflammatory cytokines in plasma. Indeed, our laboratory previously found that fluoxetine was able to reduce circulating levels of IL-6, a relevant inflammation marker in our mouse model of DCS (3). Since fluoxetine is recognized as having numerous anti-inflammatory effects by suppressing the produc-

Address for reprint requests and other correspondence: J.-E. Blatteau, IRBA-ERRSO, Bd. Sainte Anne, Toulon, VAR 83800 Toulon Armee, France (e-mail: je.blatteau@infonie.fr).

tion of IFN-γ and stimulating that of IL-10 (26), we hypothesized that fluoxetine could act by limiting inflammation processes, resulting from DCS-induced ischemia in our rat model.

MATERIALS AND METHODS

Study Population

Only male Sprague-Dawley rats (Harlan Laboratories) were used in this experiment to avoid fluctuations due to female hormone cycles. Rats were kept at 22 ± 1°C in a 12:12-h light-dark cycle (lights on at 7:00 AM) with food (A03, UAR) and water ad libitum. Before experiments, rats were housed in an accredited animal care facility. All procedures involving experimental animals were in line with European Union rules (directive 2010/63/EU) and French law (decree 2013/118). All experimental procedures were approved by an independent ethics committee at the Institut de Recherche Biomédicale des Armées.

A total of 78 rats were exposed to compressed air to induce decompression stress and bubble formation. The sample size was determined based on the expected incidence of DCS, according to the weight of the animals. Previous studies in our laboratory showed that bubble formation and the incidence of DCS were highly dependent on the body weight of the rat. Rats with a body weight >350 g are generally more prone to present neurological DCS symptoms, whereas a lower weight reduce the occurrence of DCS. We separated the rats into two groups according to their weight with a group > 350 g ($n = 38$) for clinical study [high weight (HW) group] and a group < 350 g ($n = 40$) dedicated to the cytokine analysis [low weight (LW) group]. The establishment of this lightweight group aimed to limit the number of DCS, especially lethal forms, thus enabling a biological assessment over a larger number of animals. This separation into two groups was necessary because the clinical study was conducted over 2 days after decompression, whereas the cytokine study required the death of animals within 60 min after surfacing.

In each group, the rats were randomly divided into two subgroups of equal number between treated rats and controls. The experimental design is detailed in Fig. 1. The mean weight was 380.5 ± 20 g in the HW group and 309 ± 12 g in the LW group. In each of these two groups, weights were not statistically different between treated and control rats. According to the literature, the experimental group received 30 mg/kg of fluoxetine solution (2.5 ml) by gavage in the form of Prozac (Lilly Laboratories) 6 h before surfacing, whereas the control group received a similar saccharine solution (2.5 ml) without fluoxetine in the same conditions (3, 10, 30, 42).

Hyperbaric Procedure

Batches of 10 freely moving rats (5 per cage) were subjected to the hyperbaric protocol in a 200-liter chamber fitted with three ports for observation.

Rats underwent the compression procedure at a rate of 100 kPa/min to a pressure of 1,000 kPa (90 m seawater), maintained for 45 min while breathing air. At the end of the exposure period, the rats were decompressed down to 200 kPa at a rate of 100 kPa/min with a 5-min stop at 200 kPa, a 5-min stop at 160 kPa, and a 5-min stop at 130 kPa. Decompression between 200 kPa and the surface was performed at a rate of 10 kPa/min. The decompression rate was automatically controlled by a computer linked to an analog-digital converter (NIUSB-6211, National Instrument), which was itself connected to a solenoid valve (Belino LR24A-SR) and a pressure transmitter (Pressure Transmitter 8314, Burket Fluid Control System). The program used to control the decompression rate was designed on DasyLab (DasyLab National Instrument) by our engineer.

Compressed air was generated using a diving compressor (Mini Verticus III, Bauer Comp) coupled to a 100-liter chamber at 300 bars. The oxygen analyzer was based on a MicroFuel electrochemical cell (G18007 Teledyne Electronic Technologies/Analytical Instruments). Water vapor and CO_2 produced by the animals were captured with secca gel (relative humidity: 40–60%) and soda lime (<300 ppm captured by the soda lime), respectively. Gases were mixed by an electric fan. The day-night cycle was respected throughout. The temperature inside the chamber was measured using a platinum-resistance temperature probe (Pt 100, Eurotherm). The temperature reached 30°C at the end of the compression phase and was stabilized at 25°C during the exposure at 1,000 kPa. During decompression, the temperature dropped to 19°C before returning to the basal values of 24°C.

Behavior and Clinical Observations

At the end of decompression, the rats were transferred to individual cages and observed for 30 min. The possible occurrence and the time

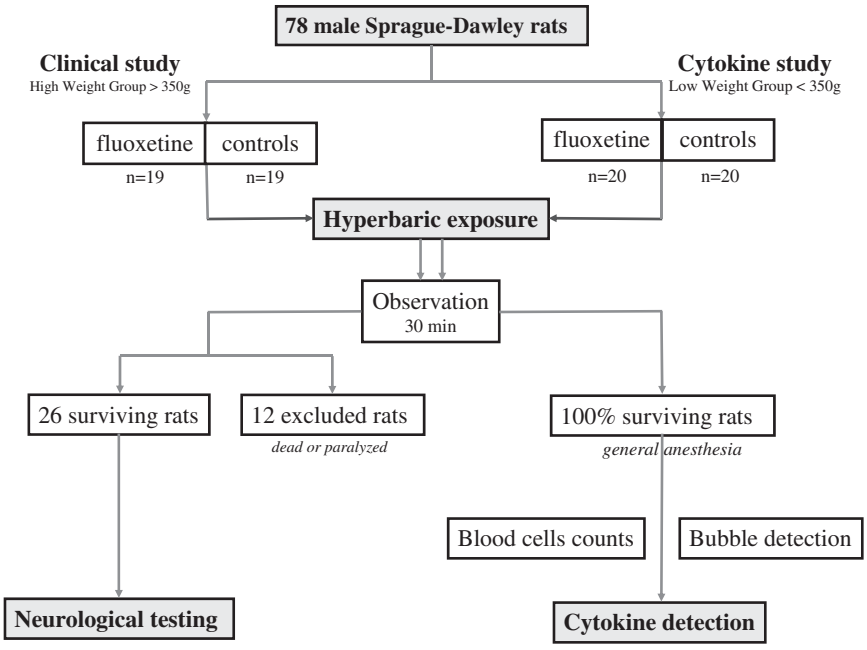

Fig. 1. Flow chart describing the experimental design.

to onset of the following manifestations were recorded: respiratory distress, moving difficulties, convulsions, and death.

At the end of this observation period, all of the rats in the LW group were anesthetized to perform bubble measurements and cytokine analysis. In the HW group, the surviving rats were subjected to various sensory neurological tests and video, apart from rats presenting motor symptoms with paw paresis. These rats were excluded to prevent a methodological bias, because the selected sensory tests are relevant only in the absence of motor deficiency (Fig. 1).

HW Group

Sensory tests. Specific sensory tests were performed on the HW group before and 1 h after the hyperbaric exposure, and there was a third examination 2 days after the hyperbaric exposure. Thermal and mechanical behavioral tests were performed using an automated von Frey and Hargreaves device to evaluate sensory deficit.

Mechanical stimulation. Mechanical hypersensitivity was measured using an electronic von Frey device (Electronic von Frey Aesthesiometer EVF3, Bioseb, Vitrolles, France). Rats were placed on a wire mesh floor in a plastic enclosure. A computer-driven filament was then extended up through the mesh floor and exerted an increasing amount of pressure (measurement range 0–500 g) onto the rat's hindpaw. The force in grams required until the rat withdrew its hindpaw was defined as the mechanical pain threshold. Both hindpaws were tested in each rat. The stimulus was repeated three times following a 5-min interstimulus interval, and the mean was calculated for each rat's hindpaw.

Thermal stimulation. A thermal stimulus was delivered using the Hargreaves technique (7371 Plantar Test from Ugo Basile S.R.L. Biological Research Apparatus) (22a).

Rats were placed in a clear Plexiglas box resting on an elevated glass plate. Following acclimatization, a radiant beam of light at 60°C was positioned under the hindpaw, and the average time for the rat to remove the paw from the thermal stimulus over three trials was electronically recorded in seconds as the paw withdrawal latency (PWL). The intensity of the beam was set to produce basal PWL's of ~8–10 s. A maximal PWL of 25 s was used to prevent excessive tissue damage due to repeated application of the thermal stimulus. All studies were conducted by an observer blinded to the experimental conditions.

Dynamic weight-bearing distribution assessment. The dynamic weight-bearing (DWB) distribution was assessed by a biometric floor instrumented cage (Dynamic Weight-Bearing, Bioseb Development, Vitrolles, France). The device consisted of a Plexiglas box (width 22 × length 22 × height 30 cm) with a calibrated weight transducer pad composed of 44 × 44 captors (TEKSCAN, Boston, MA). The rat was allowed to move freely within the box for 4 min each. Using a synchronized video recording and a scaled map of the stimulated captors, each of the rat's paws was validated by an observer and identified as a unique paw. DWB was recently validated to evaluate both the severity of central nervous system trauma and the effectiveness of pharmacological strategies (39).

The pressure exerted by each paw (in grams) was only measured when the four paws were in contact with the biometric floor and then normalized by the total weight of the rat. Ratios distinguishing the forepaws vs. hindpaws and the right vs. left side were calculated to assess the weight-bearing distribution: *1*) the sum of the right and the left forepaws (F) was normalized by the sum of the right and the left hindpaws (H) (F/H ratio); *2*) the left forepaw (LF) was normalized by the right forepaw (RF) (LF/RF ratio); *3*) the left hindpaw (LH) was normalized by the right hindpaw (RH) (LH/RH ratio). The time spent on three paws and on four paws (in seconds) was also measured to determine the solicitation of the paws in postural stability. The time period spent on four paws was normalized by the time period spent on three paws (4P/3P ratio). DWB distribution assessment was performed before and 2 days after the hyperbaric exposure in the HW group.

LW Group

The rats from the LW group were anesthetized 30 min after surfacing by intraperitoneal injection of a mixture of 16 mg/kg xylazine (Rompum 2%, Bayer Pharma) and 100 mg/kg ketamine (Imalgene 1000, Laboratoire Rhône).

Bubble detection. A precordial bubble detection was performed at 40 ± 10 min after surfacing using a Micromaxx Sonosite, with a probe of 4–8 MHz. The right ventricle was identified with the two-dimensional mode, and then bubbles were graded according to the Spencer scale using the pulsed Doppler mode (43). Basically grade 0 corresponds to no bubble detected, grade I is a few bubbles, grade II is "some bubbles at each heartbeat," grade III is "many bubbles per heartbeat," and grade IV is "continuous bubbles." A 5-min recording was given to each animal. The experienced operator was blinded to the group allocation of the rat.

Blood cell tests. Blood tests were carried out in an automatic analyzer (ABCvet, SCIL, France) on samples taken before the dive and then again 50 min after surfacing. Red cells, leukocytes, and platelets were counted in 20-μl samples taken from the tip of the tail and diluted in an equivalent volume of 2 mM EDTA (Sigma).

Detection of circulating cytokines. Blood samples were collected 60 min after surfacing from the inferior vena cava to determine the values of plasmatic cytokine levels. Blood (900 μl) was drawn up carefully into a disposable syringe with anticoagulant citrate dextrose (100 μl) and centrifuged immediately. At the end of the experiment, the rats were killed by injecting pentobarbital (200 mg/kg ip, Sanofi Santé). Plasma was obtained within 30 min by a single centrifugation at 1,100 *g* and 4°C for 10 min. The supernatant was stored at −80°C until assay.

Cytokine and chemokine detection was carried out with a Bioplex100 (Bio-Rad) and a immunoassay kit (Milliplex Rat Cytokine/Chemokine MAGNETIC BEAD, Merck Millipore) that allows simultaneous detection of IFN-γ, IL-1β, IL-6, and IL-10 cytokines. Samples, standards, and quality controls were carried out using two duplicates per point. All standards (10,000 to 16 pg/ml) and quality controls were prepared as recommended in the kit. Baseline levels of cytokines were obtained in a group of eight matched rats, which received no treatment and were not submitted to hyperbaric exposure.

Statistical Analyses

For statistical processing, we used Sigmastat 3.0 (SPSS, Chicago, IL). Numerical results were expressed as means ± SD for parametric data or median ± interquartile range for nonparametric data. Comparisons between multiple groups were analyzed by the nonparametric Kruskal-Wallis test with the post hoc Dunn's method. Comparisons between multiple paired groups were analyzed by one-way ANOVA for repeated measurements with the post hoc Holm-Sidak test or the nonparametric Friedman test with the post hoc Dunn test. Differences between two groups were analyzed by a *t*-test or the nonparametric Mann-Whitney test, whereas matched comparisons within groups used a paired *t*-test or the nonparametric Wilcoxon test. A difference was considered as significant for P values < 0.05.

RESULTS

According to our protocol, no neurological symptoms or death were observed in the LW group; some animals presented minor symptoms, such as prostration or piloerection, with no differences between groups. In the HW group, death occurred after surfacing for four and three rats in the fluoxetine and control groups, respectively. Locomotor impairment, including mono- or paraparesis, occurred within 15 min after surfacing for four and two rats in the fluoxetine and control groups,

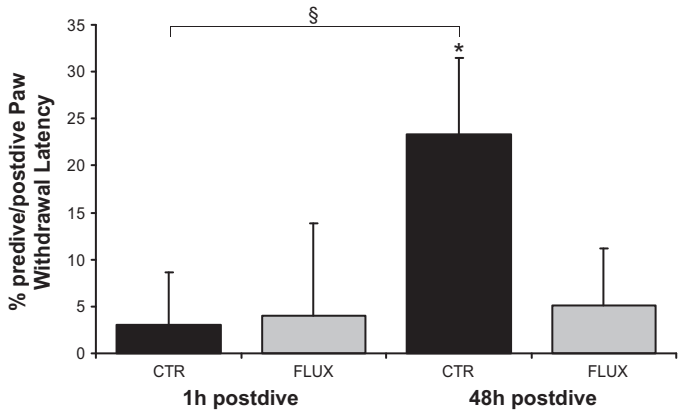

Fig. 2. The sensory impairment of treated rats with fluoxetine (FLUX; shaded bars) and controls (CTR; solid bars) following decompression was evaluated by testing the pain sensitivity to heat. The average time for the rat to remove the paw from the thermal stimulus over three trials was electronically recorded in seconds as the paw withdrawal latency. Values are means ± SE. *Significant difference from baseline, and §difference between 1-h and 48-h postdive measurements: $P < 0.05$.

respectively, with no significant differences between groups. Time to onset of motor deficit and time to death were not different between groups.

HW Group

The sensory tests were performed on 12 rats treated with fluoxetine and 14 control rats.

Mechanical stimulation. The controls presented a significant increase in paw withdrawal force from baseline, 1 h following hyperbaric exposure, indicative of reduced mechanical sensitivity (109.7 ± 4.6 vs. 95.75 ± 3.2 g, $P = 0.005$). In the fluoxetine group, no significant changes were found. Basal paw withdrawal force was comparable between all groups ($P > 0.05$).

Thermal stimulation. Controls presented a significant increase in PWL from baseline, indicative of reduced thermal sensitivity, 48 h following hyperbaric exposure (9.28 ± 3 vs. 7.87 ± 2.67 s, $P = 0.011$) (Fig. 2). In the fluoxetine group, no significant changes were found. Basal PWL was comparable between all groups ($P > 0.05$).

DWB distribution assessment. F/H, LF/RF, AND LH/RH PRESSURE RATIOS. No statistical differences before and after hyperbaric exposure were observed in the groups (Table 1).

TIME SPENT ON FOUR PAWS OR THREE PAWS. We found that the time spent on four paws was increased after hyperbaric exposure in the control group ($P = 0.042$), whereas no differences were found in the fluoxetine group. The time spent on three paws was increased after hyperbaric exposure in the fluoxetine group ($P = 0.014$), whereas no differences were found in the controls. The 4P/3P ratio was also significantly reduced in the fluoxetine group after hyperbaric exposure ($P = 0.049$) (Table 1).

LW Group

Bubble detection. Even though no animals presented clinical symptoms, the presence of circulating bubbles was observed in 19/40 of rats in the LW group. Bubbles were observed in 9/20 of animals treated with fluoxetine and in 10/20 of the controls. 30% of animals presented high bubble grades i.e., Spencer grades II or III in both group. No differences in bubble grades were observed between groups ($P = 0.99$), with a median grade of 0 ± 2 (range: 0–3) in the fluoxetine group and 0.5 ± 2 (range: 0–3) for controls.

Blood cells. Following the dive, blood cell counts were significantly reduced from the baseline only in the fluoxetine group for leukocytes (−13.4 ± 21%, $P < 0.001$) and red cells (−6.5 ± 17.3%, $P = 0.04$). However, no statistical differences between treated and nontreated groups were found (Table 2).

Cytokine detection. Levels of circulating cytokines were compared between groups, with the baseline level obtained in a group of rats maintained at atmospheric pressure. We found no significant differences between groups for proinflammatory cytokines i.e., IFN-γ, IL-1β, and IL-6 ($P > 0.05$).

As shown in Fig. 3, circulating levels of the anti-inflammatory cytokine IL-10 were significantly decreased by −56 ± 30% in the control group from the baseline ($P = 0.024$), whereas no differences were found between the fluoxetine group and the baseline level.

DISCUSSION

The main result of this study is the demonstration in the clinical group of a sensory impairment that is significantly less marked for rats treated with fluoxetine. The sensory impairment was evaluated by three different methods: by testing pain sensitivity to heat, sensitivity to mechanical stimulation, and also a behavioral evaluation of the position of the animal (on 3 or 4 paws) in a rest situation. The results are consistent: although no sensory anomalies were observed in the group treated with fluoxetine, a reduction in sensitivity to mechanical stimulation after surfacing, and a reduction in heat-pain sensitivity on *day 2* in the control rats was observed. Moreover DWB distribution assessment highlights the difference between groups with a longer time spent on four paws after hyperbaric exposure for controls and a longer time spent on three paws after hyperbaric exposure for the fluoxetine group.

Table 1. *Dynamic weight-bearing distribution assessment*

	Fluoxetine			Controls		
DWB Distribution Assessment	Predive	Postdive	*P* values	Predive	Postdive	*P* values
F/H ratios	0.43 ± 0.1	0.34 ± 0.3	1	0.32 ± 0.2	0.28 ± 0.1	0.8
LF/RF ratios	1.08 ± 1.1	0.61 ± 0.9	0.32	1.24 ± 1.5	0.99 ± 0.8	0.13
LH/RH ratios	1.08 ± 1.3	0.72 ± 0.4	0.27	0.8 ± 0.6	0.88 ± 0.7	0.8
4P/3P ratios	6.88 ± 11.7	2.58 ± 3.9*	0.049	5.75 ± 20.2	3.47 ± 3.9	0.42

Values are means ± SD. Dynamic weight-bearing (DWB) distribution was assessed by a biometric floor instrumented cage. The pressure exerted by each paw in contact with the biometric floor was measured. Ratios distinguished the forepaws (F) vs. hindpaws (H) and the right (R) vs. left (L) side as follows, i.e., F/H ratio, LF/RF ratio, LH/RH ratio, and the time period spent on 4 or 3 paws (4P/3P ratio). *Significant difference between predive and postdive values.

Table 2. *Blood cell counts*

	Fluoxetine	Controls	*P* Values (Controls vs. Fluoxetine)
Platelets	−3.6 ± 16.6	−11.5 ± 21	0.56
White cells	−13.4 ± 21*	−1.5 ± 44	0.09
Red cells	−6.5 ± 17.3*	−4.8 ± 24	0.84
Hematocrit	−6.4 ± 17.4*	−4.8 ± 22	0.62

Values are means ± SD in %difference of predive vs. postdive values. Blood tests were carried out before and after the dive. *P* values are related to the difference between the treated group and controls. *Significant difference between predive and postdive values.

This result might be explained by a lack of stability on three paws for controls after hyperbaric exposure, resulting in a longer time spent on four paws, and a better stability on three paws for the fluoxetine group. This reduced stability is probably related to an alteration in proprioceptive sensibility.

Concerning the pressure ratio during DWB distribution assessment, no differences were found. This result might be explained by a lack of motor impairment for rats included in this neurologically sensitive testing, according to our protocol.

In this study, we have not observed any difference in terms of mortality and paralysis between the controls and the rats treated with fluoxetine. In fact, the aim was specifically to consider changes in sensitivity. The purpose of the protocol was not to induce and assess severe forms of DCS, which would have required more staff; moreover, our laboratory has previously demonstrated the clinical benefit of fluoxetine on mortality and motor recovery (3). In addition, we chose to reserve a group of lighter animals (LW group) to avoid any lethal form of DCS and allow measurements of the bubbles and an assay of cytokines on the largest possible number.

In this LW group, the presence of circulating bubbles was observed in ~50% of animals, attesting to the effect of decompression on the rats' bodies, even those that did not present clinical symptoms of DCS. It is interesting to note that fluoxetine does not seem to influence the formation of bubbles, with bubble levels equivalent for the treated and the nontreated rats. We were expecting a possible action on bubble formation. In fact, fluoxetine, the active compound in Prozac, prevents the re-uptake of serotonin (5-hydroxytryptamine) and increases the concentration of circulating serotonin (8) by inhibiting serotonin transporters located in neurons, platelets (29), and leukocytes (15, 31, 51). 5-Hydroxytryptamine is usually a vasodilator, becoming a vasoconstrictor when the endothelium is damaged, being taken up from plasma and stored in platelet granules. Past studies have suggested that bubble formation after decompression in rats can be reduced when production of vasodilator agents such as endothelial nitric oxide is increased (47). Conversely, nitric oxide inhibition increases peripheral vascular resistance and bubble formation in rats with a reduction in survival after decompression (48). The vasodilator effect of fluoxetine mediated by serotonin would, therefore, be able to encourage the elimination of gas nuclei in the blood vessels and limit the production of bubbles during decompression. This was not observed in this study. The effect on bubble formation does not seem to be dominant with fluoxetine. However, further investigation, including repeated bubble measurements, could be of interest to determine whether fluoxetine may alter the elimination kinetics of vascular bubbles.

Animal experiments strongly suggest a role for the involvement of blood components in DCS (28, 40). We found that white and red cell counts were significantly reduced before/after decompression in the treated rats. However, the reductions were moderate because we have not shown a statistical difference between the groups of treated and nontreated rats.

Previous animal studies reported that the platelet count falls following decompression (40) and can be considered to be a relevant index for evaluating decompression stress (41). The drop in the platelet count is usually attributed to clotting activity following exposure of the collagen under bubble-damaged endothelial cells in the blood vessels (34, 38, 46), or direct interaction between bubbles and platelets (20, 22). Contrary to the previous study conducted on an explosive decompression model in mice, which showed a possible anti-aggregant effect for fluoxetine, this study did not reveal a significant drop in the platelet count following decompression in treated and nontreated animals. This result is certainly linked to the animal DCS model used in this study, which aimed to limit severe forms of DCS.

We observed a reduction in red cells after decompression in the group treated with fluoxetine, to be related to the hematocrit decrease observed in this group. This reduction could be linked to the properties of fluoxetine, which is likely to cause hemodilution. Previous studies found that fluoxetine may have a positive impact on hemorheological measurements of stress-hemoconcentration by improving the increased blood viscosity (49). This effect could be mediated by fluoxetine inhibition of volume-regulated anion channels, which are important regulators of various cell functions and have been described in the neuronal and endothelial cells of the blood-brain barrier. Volume-regulated anion channels are critically involved in volume regulation and maintain the osmotic composition of the fluid compartments in the central nervous system (32, 44).

The fall in the leukocyte count after DCS is usually attributed to diapedesis (13, 24, 35). We found that the leukocyte count decreased after decompression, mainly in the fluoxetine group. This result, previously observed (3), suggests that fluoxetine may modulate leukocyte recruitment, presumably with a specific activation of anti-inflammatory pathways.

Contrary to the previous study conducted on a DCS model with rapid decompression (3), we did not observe a rise in

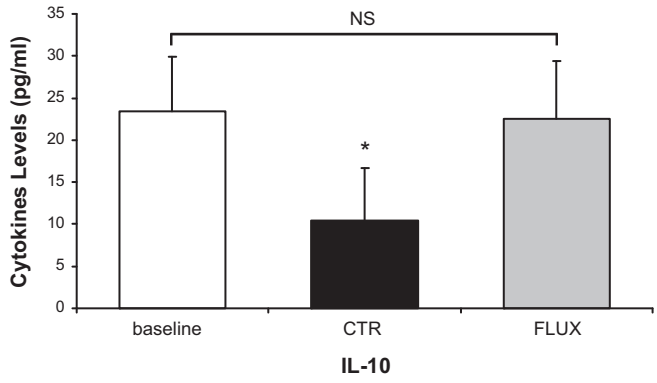

Fig. 3. Postdive levels of circulating IL-10 cytokine were compared in the CTR group (solid bar) and FLUX group (shaded bar) with the baseline level (open bar) obtained in a group of rats maintained at atmospheric pressure. Values are means ± SE. *Significant difference, $P < 0.05$. NS, nonsignificant.

proinflammatory cytokines like IL-6. The presence of this cytokine in the plasma does, in fact, seem to be observed in the most severe forms of DCS. This result may be explained by the choice of using a lightweight group, which was intended to limit the severe and lethal forms of DCS. The absence of an increase in proinflammatory cytokines in the plasma post-decompression is not, therefore, surprising.

On the other hand, we found that fluoxetine maintained cytokine IL-10 baseline levels, despite the diving-induced IL-10 decrease. The IL-10 family represents a family of cytokines that inhibits inflammation and immune responses. Mice deficient in IL-10 develop spontaneous diseases, such as inflammatory bowel disease and arthritis. IL-10 was outstanding in treating a variety of autoimmune diseases as IL-10-suppressed IFN-γ, IL-1, TNF-α, and IL-6 production (12). It is suggested that the antidepressant efficacy of selective serotonin re-uptake inhibitors, such as fluoxetine, could be related to their immunoregulatory effects by increasing the production of IL-10 by peripheral blood leukocytes and suppressing the IFN-γ-to-IL-10 production ratio (26, 27, 33, 36). Moreover, anti-inflammatory effects in the setting of cerebral ischemia are also described. Fluoxetine attenuates kainic acid-induced neuronal cell death in the mouse hippocampus and suppresses proinflammatory markers (25). In a rat cerebral model of middle cerebral artery occlusion, fluoxetine reduced infarct volumes and improved motor impairment. The fluoxetine-treated brain was found to show marked reduction in microglia activation, neutrophil infiltration, and proinflammatory marker expressions (30).

We think that this anti-inflammatory effect from fluoxetine mediated by IL-10 could also be related to the beneficial clinical effects observed in the DCS animal model by reducing the most serious forms with paralysis and death in the mouse (3), and also by limiting the post-decompression sensory impairments seen in the treated rats in this study. Further investigations are required to determine whether the specific action of fluoxetine on IL-10 is an essential factor in the clinical benefit observed, e.g., by trying to block this cytokine selectively or by using knockout animals.

In conclusion, for the first time, we can show that fluoxetine exerts a beneficial effect on sensory neurological recovery. So fluoxetine could be proposed and evaluated in humans as an adjuvant treatment to hyperbaric oxygen to limit sequelae of the proprioceptive ataxia type, which are responsible for gait disorders in injured divers.

ACKNOWLEDGMENTS

We are very grateful to Bruno Schmid and Myriam Dalous for excellent technical assistance.

GRANTS

This work was supported by institutional grants from Direction Générale de l'Armement and the Foundation "Les Gueules Cassées."

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.-E.B. and N.V. conception and design of research; J.-E.B., S.d.M., K.L., and N.V. performed experiments; J.-E.B., S.d.M., K.L., and N.V. analyzed data; J.-E.B., S.d.M., J.H.A., J.-J.R., and N.V. interpreted results of experiments; J.-E.B. prepared figures; J.-E.B. drafted manuscript; J.-E.B. edited and revised manuscript; J.-E.B. approved final version of manuscript.

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