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Research Article

Safety of Meloxicam Compared to that of Diclofenac, using Ants as a Model

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Abstract

We here examine, using ants as a model, the ethological and physiological effects of meloxicam, an anti-inflammatory drug, in the same way we previously examined the effects of diclofenac and compare them. Meloxicam, like diclofenac, somewhat impacted the ants' food consumption, decreased their activity, tendency in crossing a difficult path and in escaping from an enclosure. Like diclofenac, it did not negatively affect the ants' tactile perception and their relationships with nestmates and brood. Such as for diclofenac, the ants did not adapt themselves to the effect of meloxicam. However, contrary to diclofenac, meloxicam did not affect the ants' sinuosity of displacement, orientation ability, conditioning capability and memory, and did not induce any dependence. While the effect of diclofenac very rapidly vanished in about 7 hours after weaning, that of meloxicam stayed intact for 6 hours after weaning, and then very slowly decreased and vanished in a total of 51 hours after weaning. As far as ants are concerned, meloxicam presents thus fewer and less severe adverse effects than diclofenac and does not induce dependence. This suggests using meloxicam preferentially rather than diclofenac for animals' and humans' care.

Keywords: Activity; Dependence; Food Intake; Memory; *Myrmica sabuleti*

Abbreviations

ang.deg.: Angular Degrees; ang.deg./cm: Angular Degrees per cm; mm/s: Millimeter per Second; χ^2: Chi-square; vs: Versus; n°: Number; cm: Centimeter; mm: Millimeter; ml: Milliliter; mg: Milligram; s: Second; min: Minute; h: Hours; t: Time; %: Percentage.

Introduction

Among medicines, anti-inflammatory drugs are among the most sold ones, and diclofenac is presently one of the most used. However, this drug is not entirely safe. Indeed, Asian and African vulture populations have drastically declined due to the consumption of carcasses of animals treated with diclofenac [1,2]. A Danish meta-analysis has shown that patients treated with diclofenac have a probability of presenting a severe cardiovascular problem increased by 20%, 30% and 50% in comparison with patients treated with paracetamol or ibuprofen, with naproxen, and having received no treatment [3]. Toxicological studies also pointed out and defined adverse effects of diclofenac [4,5]. Using ants as models, we recently examined the potential physiological and ethological effects of diclofenac and found that it impacted their locomotion, cognition and memory, and could lead to dependence [6].

In the Indian subcontinent, the veterinary use of diclofenac is prohibited since 2006 [7]. Consequently, in this region of the world, meloxicam, another anti-inflammatory drug, proved to be less toxic for vultures, is nowadays used for livestock instead of diclofenac [8], although the illegal use of the latter continues to threat the survival of vultures [9].

As expected, many works have been and are still devoted to the comparative study of these two anti-inflammatory drugs. They concern essentially or exclusively the impact of meloxicam alone or of meloxicam and diclofenac and sometimes other anti-inflammatory drugs, on the digestive track, the liver, the kidneys, and some of these works examine these drugs' efficiency [e.g. 10-17]. This allows concluding that meloxicam may be safer that diclofenac. Nevertheless, serious side effects remain possible with meloxicam

[18]. Moreover, the above cited clinical and toxicological studies do not examine the impact of meloxicam on traits such as the general activity, food consumption, locomotion, cognition, memory, social relationships, habituation, adaptation, and dependence. Consequently, we intended to examine on ants, an animal model presenting no inflammatory reaction, the impact of meloxicam on some ethological and physiological traits exactly as we did for the effects of diclofenac [6]. Here below, we briefly recall why we used ants as models, which species we used and what we know on it, and which traits we intended to examine.

Why we used ants as models?

The fundamental physiological and ethological traits of animals and humans are similar [19]. They are generally firstly examined on animals as models (e.g. fruit flies, cockroaches, bees, mice, monkeys) before being studied in humans [20]. Invertebrates are preferentially used because they have a rapid development and are easily maintained in a laboratory [21,22]. Insects, and hymenoptera among others, are often used [23]. Ants could thus be used [24]. These insects are eu-social. They present colonial regulation, labor division and exchange of information thanks to tactile and chemical signals (pheromones) [25-27]. They take care of their brood, construct sophisticated nests, and chemically mark the different parts of their habitat [25]. They can navigate, recruit congeners, relocate their nest, clean the inside of it, and create cemeteries [26]. According to such a complex biology, they can serve as biological models. The impact of substances and environmental changes can be examined on them, and hypothesis about the impact of such factors on other organisms including the humans can be emitted.

Which species we used?

We know well the biology of some species of the genus *Myrmica*, having looked, among others, to their ecology, eyes morphology, angle of vision, visual perception, recruitment strategies, navigation systems, conditioning [28], to the ontogenesis of some of their abilities [29], and to the limit of their cognitive abilities [30,31]. Studying the effect on them of manmade electromagnetism showed that they could be good biological models [32,33]. Effectively, they proved to be good models all along our studies of the impact of numerous products used by humans [e.g. 34-37]. We have recently used again the ant *M. sabuleti* Meinert 1861 as a model for studying the effect of diclofenac consumption on several physiological and ethological traits [6]. We here used again *M. sabuleti* for examining, as we did for diclofenac, the physiological and ethological potential impacts of meloxicam.

Which traits we examined?

First on ants under normal diet (controls), then on the same ants consuming meloxicam (tests), we examined the 19 following traits: meat consumption, sugar water consumption, general activity, speed of locomotion, sinuosity of movement, orientation ability, audacity, tactile perception, brood caring, aggressiveness against nestmates, aggressiveness against aliens, cognition, escaping ability, conditioning capability and short term memory, adaptation to adverse effects of the drug (if any), habituation to beneficial effects of the drug (if any), dependence on the drug consumption, and decrease of the effect of the drug (if any) after weaning.

Adaptation to a product occurs when adverse effects caused by that product decrease over time. Habituation to a product occurs when beneficial effects caused by that product decrease over time. Dependence on a product develops when individuals using this product prefer a life including this product than a life without it.

Material and Methods

Collection and maintenance of ants

The experimental work was performed on two colonies (A and B) of *M. sabuleti* collected, in September 2018, in an abandoned quarry located in the Aise valley (Ardenne, Belgium). A third colony, collected the same day in the same valley, was used for performing the control experiment of the conditioning study. A fourth colony, collected in June 2018 at Marchin (Condroz, Belgium), also in an abandoned quarry, furnished the alien ants used to study the ants' aggressiveness. These colonies contained about 500 workers, brood and a queen. They were maintained in the laboratory in two to three glass tubes half filled with water, a cotton plug separating the ants from the water. The nest tubes of each colony were set in a tray (34 cm x 23 cm x 4 cm). These trays served as a foraging areas; pieces of *Tenebrio molitor* larvae (Linnaeus, 1758) were deposited in them three times per week, cotton plugged tubes filled of sugar water were permanently set in them. The ambient temperature was ca 20°C, the humidity 80%, the lighting 330 lux while working on ants, and the electromagnetism 2 μWm^2. These environmental conditions are optimum for the species. The ants of a same colony are here often named 'nestmates' as researchers on social insects commonly do.

Solution of meloxicam given to the ants

The instructions for use joined to meloxicam packages, as well as information about this drug available on several internet sites tell us that humans treated with meloxicam usually consume 7.5 mg up to 15 mg of that drug per day according to the level of their health problem [https://base-donnees-publique.medicaments.gouv.fr/]. We opted for the lower dose of 7.5 mg per day. Tablets containing 7.5 mg of meloxicam were furnished by the pharmacist Wera (1170 Bruxelles). A human commonly consume one liter of water each day. Consequently, humans treated with 7.5 mg

of meloxicam per day consume this dose together with one liter of water. Insects, and thus ants, need about 10 times lesser water than mammals, this resulting from their different skin, respiratory apparatus and excretory system [19]. Therefore, for being under a meloxicam diet equivalent to that of a human treated with 7.5 mg of the drug per day, the ants must be provided with a concentration of 7,5 mg of the drug in 100 ml of water (or any liquid). We gave thus to the ants such a concentration using their usual sugar water as liquid, the solution being delivered in their usual tubes plugged with cotton. The ants drank that solution *ad libitum* according to their size and request. We checked many times per day that they effectively did so.

Assessment of the examined traits

These assessments have been many times explained in previous studies [such as 35-37], what allows briefly relating them. Let us recall that these traits were firstly assessed on ants under normal diet, then on the same ants consuming meloxicam, except for the study of adaptation, dependence and loss of the effects which were of course assessed only on ants under meloxicam diet.

Food consumption, general activity

While ants were under normal diet then while they consumed meloxicam, we counted six times per day during six days, at the same times o'clock each day, those eating the pieces of *T. molitor* larvae, those drinking the sugar water, and those being active in their foraging area, on their food sites, and in their nest (as in [35-37]). The mean of the daily counts was established for each kind of count (Table 1), and for information only, the average of these six means was each time calculated (Table 1, last line).

Experimental Days	Normal diet			Diet with meloxicam		
	Meat	Sugar water	Activity	Meat	Sugar water	Activity
I	1.67	2.17	9.33	1.33	2.33	9.83
II	2.00	1.50	12.50	1.50	1.50	8.50
III	1.17	1.17	9.33	1.33	1.83	8.67
IV	1.50	1.67	12.33	1.16	2.33	7.67
V	1.50	1.67	11.17	1.33	2.17	7.82
VI	1.83	1.33	10.50	1.38	1.83	6.66
I – VI	1.61	1.58	10.86	1.38	2.00	8.19

Table 1: Impact of meloxicam on food consumption and general activity. The table gives the mean of 6 daily counts of the ants seen on the meat, on the sugar water, and being active, as well as the mean of these 6 means (last line) for each trait. Details and statistics are given in the text. The drug slightly decreased the meat consumption, increased the sugar water consumption, and decreased the general activity.

Linear and angular speeds, orientation

These traits were quantified on foraging ants, the linear and angular speeds without stimulating them and the orientation by stimulating them with a nestmate tied to a piece of paper (Figure 2 A). Such a nestmate emits its attractive mandibular glands alarm pheromone [35-37]. For the speeds on one hand and for the orientation on the other hand, the trajectory of 40 workers was recorded, and these 40 trajectories were then analyzed thanks to appropriate software [38] which is based on the three following definitions. The linear speed (in mm/s) is the length of a trajectory divided by the time spent to travel it; the angular speed (in angular degrees/cm = ang.deg./cm) is the sum of the angles made by successive adjacent segments, divided by the length of the trajectory; the orientation (in ang. deg.) towards a location is the sum of the successive angles made by the direction to the location and the direction of the trajectory, divided by the number of angles measured. When the mean angle value is lower than 90°, the animal tends to orient itself towards the location; when the value is larger than 90°, it tends to avoid the location [38]. The median and quartiles of each distribution of 40 values were established.

Audacity

As in previous works [35-37], a cylindrical tower (height = 4 cm; diameter = 1.5 cm) tied to a squared platform (9 cm^2), made of white Steinbach® paper, was deposited in the ants' foraging area, and those present at any place on this apparatus were counted 10 times over 10 min (Figure 2 B). The counts obtained for the two colonies were added, and the mean and the extremes of these added counts were established. The counts obtained for two successive minutes were added for statistical analysis.

Tactile perception

On a rough substrate, ants normally walk slowly, sinuously, and with difficulties (Figure 2 C). If they weakly perceive the uncomfortable character of such a substrate, they walk more quickly and less sinuously on it. Therefore, to evaluate the ants' tactile perception, their linear and angular speeds on a rough substrate were assessed as usually (see above 'Linear and angular speeds'). As in previous works [35-37], a folded piece (3 cm x 2 + 7 + 2 = 11 cm) of emery paper n° 280 paper was tied to the bottom and the borders of a tray (15 cm x 7 cm x 4.5 cm), and the tray was so divided in a first 3 cm long zone, a second 3 cm long zone containing the emery paper, and a last 9 cm long zone. Such an apparatus was constructed for each colony. To make an experiment, 12 ants of each colony were deposited in the first zone of their apparatus, and 24 trajectories of ants walking on the emery paper were recorded. From them, the ants' linear and angular speeds could be assessed, and the median and quartiles of the obtained distributions of values established.

Brood caring behavior

For each colony, a few larvae were removed from the nest and deposited in front of the entrance. Five of these larvae, and the ants'

behavior towards them, were observed (Figure 2 D). The larvae among the 5 + 5 = 10 observed ones which were not yet re-entered in the nest after 5 seconds, 2, 4, 6, 8, and 10 minutes were counted.

Aggressiveness against nestmates and against aliens

Ants' aggressiveness was assessed, as previously [e.g. 35-37], in the course of 10 dyadic encounters between an ant of colony A or B and a nestmate or an alien ant (Figure 2 E, F). Each encounter performed in a cylindrical cup (diameter = 2.5 cm, height = 1.8 cm) lasted 5 minutes during which the number of times the observed ant did nothing (level 0 of aggressiveness), contacted the opponent with its antennae (level 1), opened its mandibles (level 2), gripped the other ant (level 3), and tried to sting or stung the opponent (level 4) was counted. The numbers obtained for the 10 encounters were added. The ants' aggressiveness was also evaluated by "a" = n° of aggressiveness levels 2 + 3 + 4/n° of levels 0 + 1.

Cognition

The protocol set up while studying the effects of nicotine was used [39]. For each colony, the following apparatus was build. Two pieces of white paper (Steinbach®, 12 cm x 4.5 cm) duly folded were inserted in a tray (15 cm x 7 cm x 4.5 cm) which became then divided into a first small zone, a zone with twists and turns, and a large zone in which a piece of wet cotton was deposited (Figure 2 G). To conduct an experiment, 15 ants of each colony were transferred into the first small zone of their apparatus, and then, the ants present in that zone and in the large one beyond the twists and turns were counted after 30 seconds, 2, 4, 6, 8, 10 and 12 minutes. The numbers obtained for the two colonies were added.

Escaping ability

For each colony, 6 ants were enclosed under a reversed polyacetate glass (h = 8 cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) set in their tray [35-37]. A notch made in the bottom rim of the glass (3 mm height, 2 mm broad) allowed the ants escaping (Figure 2 H). After 30 seconds, 2, 4, 6, 8, 10 and 12 minutes, the ants still enclosed and those escaped were counted. The numbers obtained for the two colonies were added. The ants' ability or tendency in escaping could be evaluated by the proportion of ants escaped among 12 initially enclosed.

Conditioning and memory

The control experiment had been made four months before on a similar colony (see the subsection 'Collection and maintenance of ants'). The present test experiment was made using exactly the same protocol. A green hollow cube was set above the entrance of the ants' sugar water tube, the ants going so through visual conditioning. Their acquisition of such a conditioning was evaluated by making tests over time. To make a test, 10 ants of each colony were individually tested in a Y-apparatus provided with a green hollow cube in one of its branch. This Y-apparatus was made of strong white paper and was deposited in a separate tray (30 cm x 15 cm x 4 cm). The green cube was randomly located in the right or the left branch of this Y-apparatus. Moving into the branch containing the cube was considered as giving the correct response (Figure 2 I). Each test furnished the response of 20 ants, and therefore the proportion of correct responses, i.e. the ants' conditioning score.

Adaptation to potential adverse effects of meloxicam

The ants' general activity was again assessed in the course of the twelfth day of the ants' meloxicam consumption, at six different times of the day, as it had been after 1 to 6 days of the drug consumption. The six mean numbers obtained were compared to the control ones and to those obtained during the six first days of the drug consumption.

Habituation to potential beneficial effects of meloxicam

No beneficial effect could be revealed, and this drug characteristic could not be examined on ants.

Dependence on meloxicam consumption

Dependence was examined after the ants consumed the drug since 12 days. As in previous works [35-37], for each colony, 15 ants were transferred into a tray (15 cm × 7 cm × 5 cm) containing two tubes (h = 2.5 cm, diam. = 0.5 cm), one filled with pure sugar water, the other filled with the sugar solution of meloxicam used throughout the experimental work. The tube containing the drug was set on the right in one tray, and on the left in the other tray (Figure 2 J). The ants coming onto each tube were counted 15 times over 15 min. The counts corresponding to each kind of liquid were separately added.

Decrease of the effect of meloxicam on the ants' general activity, after its consumption was stopped

Such a decrease was examined after the ants consumed meloxicam during 15 days, using a previously set up protocol [e.g. 35-37]. Since the ants' locomotion was not affected by meloxicam consumption, this decrease was examined using the ants' general activity as assessed trait. The ants received a fresh solution of the drug 12 hours before the weaning time. After these 12 hours (t = 0), the ants' general activity was assessed. Then, weaning started: the solution of meloxicam was replaced by sugar water. Since this change, the ants' general activity was assessed over time as it had been during 6 days on ants under normal diet, during 6 days on ants consuming meloxicam, after the ants consumed the drug for 10 days, and after they consumed it for 13 days. In fact, during the decrease, each three hours, the active ants were counted 6 times over 12 minutes. At each of these 6 times, six counts were made. The results are numerically given in Table 5 and graphically presented in Figure 3. The experiment ended when the ants' general activity no longer increased and became similar to that observed under normal diet.

Statistical analysis

The numerical results concerning the ants' linear speed, angular speed, orientation, tactile perception, aggressiveness against nestmates and against aliens were ranked. Those obtained on ants under meloxicam diet were statistically compared to those obtained under normal diet using the non-parametric χ^2 test [40]. The ants' food consumption, general activity, audacity, brood caring, cognition, escaping behavior, conditioning capability, adaptation, as well as the decrease of the effect of meloxicam were statistically studied using the non-parametric test of Wilcoxon [40]. Ants' dependence on meloxicam consumption was statistically analyzed using the non-parametric goodness of fit χ^2 test [40].

Results

Food consumption, general activity

These three physiological traits were affected by meloxicam consumption (Table 1). Ants consuming this drug were somewhat less numerous on the *T. molitor* larvae than ants living under normal diet, and this observation was statistically significant (N = 6, T = -19.5, P = 0.039). On the contrary, ants consuming meloxicam were substantially more numerous in drinking the provided sugar water than ants living under normal diet, and this observation was also significant (N = 5, T = +15, P = 0.031). Meloxicam affected thus the ants' food intake. In comparison, diclofenac induced the ants to drink somewhat less sugar water and to eat a little less meat [6]. As for the general activity, except during their first day of meloxicam consumption, the ants consuming this drug became far less active than when living under normal diet, and this result was statistically significant (N = 6, T = -20, P = 0.031). Meloxicam decreased thus the general activity of the consumers, a result also found, although less significantly, for diclofenac [6]. These three results are important and should be taken into account when treating humans or animals with meloxicam.

Linear and angular speeds

These locomotion parameters were not affected by meloxicam consumption (Table 2, Lines 1, 2). This was obvious to observers and confirmed by the numerical and statistical results (linear speed: $\chi^2 = 3.77$, df = 3, $0.20 < P < 0.30$; angular speed: $\chi^2 = 1.82$, df = 3, $0.50 < P < 0.70$). This was an advantage of meloxicam use compared to that of diclofenac which significantly increased the ants' angular speed [6].

Orientation to an alarm signal

This ability was not affected by meloxicam consumption (Table 2, line 3; Figure 2 A1A2). Ants which consumed or did not consume meloxicam oriented themselves similarly towards a tied nestmate. Due to their reduced general activity, they were less numerous in doing so while consuming meloxicam, but each active ant approaching the tied nestmate perceived the emitted alarm pheromone and oriented itself towards this source. There was no statistical difference between the orientations of ants under one and the other kinds of diet ($\chi^2 = 0.65$, df = 3, $0.80 < P < 0.90$). This was a second advantage of the use of meloxicam instead of diclofenac since the latter decreased the ants' orientation capability [6].

Audacity

Meloxicam somewhat affected this trait (Table 2, line 4; Figure 2 B1B2). This was probably due to the effect of the drug on the ants' general activity (see above). Under meloxicam diet, the ants were rather reluctant in coming onto the presented unknown apparatus. The difference of behavior in front of such an apparatus between the ants under one and the other kinds of diet was significant (N = 5, T =-15, P = 0.031). A same reduced inclination to come onto an unknown apparatus was observed under diclofenac diet [6].

Tactile perception

This trait was not affected by meloxicam consumption (Table 2, the two last lines; Figure 2 C1C2). On a rough substrate, ants under normal or meloxicam diet walked slowly, sinuously, with difficulties, and seemed to have more difficulties while consuming the drug (personal observation). Even if the sinuosity on a rough substrate was higher for ants under meloxicam diet than for those under normal diet, this difference was only near the limit of significance (angular speed: $\chi^2 = 7.48$, df = 3, $0.05 < P < 0.10$), and there was no statistical difference between the linear speeds of the two kinds of ants (linear speed: $\chi^2 = 0.24$, df = 1, $0.50 < P < 0.70$). Meloxicam is not an anti pain, as was not diclofenac [6].

Traits	Normal diet	Diet with meloxicam
Linear speed in mm/sec	11.8 (10.7 – 12.9)	11.6 (10.5 – 12.9)
Angular speed in ang.deg./cm	126 (113 – 141)	116 (104 – 130)
Orientation in ang. deg.	35.6 (26.4 – 50.1)	34.2 (22.8 – 53.1)
Audacity (n°)	4.03 [3 – 5]	1.95 [1 – 3]
Tactile perception assessed on a rough substrate:		
linear speed in mm/s	5.9 (5.4 – 7.3)	5.6 (4.6 – 6.2)
angular speed in ang.deg./cm	221 (200 – 272)	269 (249 – 301)

Table 2: Impact of meloxicam on five traits. Details and statistics are given in the text. The drug did not affect the ants' linear speed, angular speed, orientation ability and tactile perception, but reduced their audacity. ang.deg. = angular degrees, ang.deg./cm = angular degrees per cm, mm/s = millimeters per second, n° = number.

Brood caring

This trait was not significantly affected by meloxicam (Table 3, line 1; Figure 2D1D2). While being under this drug diet, the ants found and hold the larvae experimentally removed from the nest as quickly as ants living under normal diet. After the 10 experimental minutes, all the observed larvae had been re-entered, whatever the kind of diet, with no significant difference (N = 3, NS). A same behavior was observed for diclofenac [6].

Traits	Normal diet	Diet with meloxicam
Brood caring: n° of larvae not re-entered over 10 min	at 30'' 2' 4' 6' 8' 10' 8 7 5 3 1 0	at 30'' 2' 4' 6' 8' 10' 9 7 5 4 2 0
Aggressiveness: levels and its variable 'a'	levels 0 1 2 3 4 var. 'a'	levels 0 1 2 3 4 var. 'a'
against nestmates (n°)	64 58 19 0 0 0.15	56 71 7 0 0 0.06
against aliens (n°)	6 27 51 48 19 3.58	7 33 59 30 36 3.13
cognition: n° of ants in front (*f*) and beyond (*b*) a difficult path over 12 min	at 30'' 2' 4' 6' 8' 10' 12' *f:* 26 23 20 15 13 10 9 *b:* 0 0 1 2 5 6 7	at 30'' 2' 4' 6' 8' 10' 12' *f:* 27 25 23 22 21 21 20 *b:* 0 0 0 0 0 0 1
escaping behavior: n° of ants in and out of the enclosure over 12 min.	at 30'' 2' 4' 6' 8' 10' 12' in: 12 11 9 8 7 4 3 out : 0 1 3 4 5 8 9	at 30'' 2' 4' 6' 8' 10' 12' in: 12 11 10 9 9 8 6 out: 0 1 2 3 3 4 6
conditioning ability: score (in %) over time (in hours)	time: 7 24 31 48 55 72 score:70 80 85 90 85 85	time: 7 24 31 48 55 72 score: 70 80 85 90 90 90

Table 3: Impact of meloxicam on six traits. Details and statistics can be found in the text. Brood caring, aggressiveness against nestmates and aliens, as well as conditioning ability were not affected and even somewhat improved by the drug. The tendency in crossing a twists and turns path and in escaping from an enclosure was affected by meloxicam. min = minutes; t = time; '= minutes; '' = seconds; n° = number; % = proportion, 'a': variable assessing the aggressiveness = n° levels 2 + 3 + 4 / n° levels 0 + 1

Aggressiveness against nestmates

This ethological trait was lowered by meloxicam consumption (Table 3, line 2; Figure 2 E1E2). Indeed, ants consuming this drug less often opened their mandibles in front of a nestmate than ants living under normal diet. This difference was significant: $\chi^2 = 7.23$, df = 2, $0.02 < P < 0.05$. The social relationships between the members of a colony were thus not impacted but somewhat reinforced by meloxicam consumption, what is in favor of the use of that drug. No difference in the relationship with nestmates was observed under diclofenac diet [6].

Aggressiveness against aliens

This ethological trait was somewhat improved by meloxicam consumption (Table 3, line 3; Figure 2 F1F2). Indeed, ants consuming that drug stung an alien more promptly than ants maintained under normal diet, a significant difference of behavior ($\chi^2 = 9.95$, df = 3, $P \sim 0.02$). The ants' usual tendency to defend their colony by attacking and even killing any alien or intruder was thus somewhat improved by meloxicam consumption. This result was in agreement with a reinforcement of the social relationships (see above 'Aggressiveness against nestmates') and is in favor of the use of that drug. On the contrary, a diet with diclofenac somewhat reduced the ants' tendency to attack an intruder [6].

Cognition

This trait was impacted by meloxicam consumption (Table 3, line 4; Figure 2 G1G2). Under normal diet, 3 ants of colony A and 4 of colony B succeeded in crossing the path with twists and turns

and in reaching the area beyond this difficult path. While consuming meloxicam, only one ant of colony A could do so. The level of cognitive ability required for making the difficult crossing significantly differed between ants maintained under normal and under meloxicam diet. For ants present over time in front of the difficult path, the difference was highly significant: N = 7, T = +28, P = 0.008; for ants present over time beyond the difficult path, the difference was significant: N = 5, T = -15, P = 0.03. This result is in agreement with that concerning the ants' audacity (see above 'Audacity'), may reveal some impact of the drug on the brain functioning, and was checked by the following experiment relative to the ants' escaping ability (see below). Diclofenac also reduced the ants' ability (or motivation) to cross a difficult path [6].

Escaping ability

Meloxicam consumption somewhat reduced this ability (Table 3, line 5; Figure 2H1H2). While under normal diet, most ants succeeded in escaping from the experimental enclosure (in 12 minutes, 9 ants among 12 could do so), while consuming meloxicam, half of them succeeded in doing so (in 12 minutes, only 6 ants among 12 could escape). The difference of escaping ability between ants under one and the other kinds of diet was significant (N = 5, T = +15 for ants enclosed, -15 for ants escaped, P = 0.031). Many enclosed ants having consumed meloxicam walked in front of the exit of the enclosure, stopped, and did not escape. This observation, which is in agreement with those made about the ants' audacity and cognition (see above), leads to presume a potential effect of the drug on some cerebral function, and was thus checked by a following experiment relative to the capability in acquiring conditioning (see below). Diclofenac also reduced the ants' escaping ability [6].

Conditioning capability, short memory

Meloxicam not at all impacted this important trait (Table 3, last line; Figure 2 I1I2). Ants under normal diet as well as those consuming the drug reached a conditioning score of 70%, 80%, 85% and 90% after 7h, 24h, 31h and 48h of training respectively. After 55h and 72h of training, the ants under normal diet presented a score of 85%, while ants consuming meloxicam presented a score of 90% and 90% respectively. This small difference between the ants under one and the other kinds of diet was not statistically significant (N = 2, NS). As a matter of fact, ants under the drug diet could perfectly be conditioned and their short term memory stayed thus fully intact. Such a result was important since diclofenac had been shown to drastically decrease the ants' short term memory [6]. Concerning the effect on memory, the use of meloxicam is thus preferable to that of diclofenac. For evaluating the impact of meloxicam on the ants' middle term memory, the green cubes were removed from the ants' foraging area, and four hours later, these ants' conditioning score was again assessed. It equaled 90%. Consequently, the drug also did not affect the middle term memory. Since ants consuming meloxicam went on easily finding their nest entrance and their food sites, we could moreover affirm that their long term memory was also perfectly intact. Finally, meloxicam had thus no impact on the ants' memorization capability.

Adaptation to potential adverse effects of meloxicam

Over the first 12 days of meloxicam consumption, the ants did not adapt themselves to the impact of the drug on their general activity (Table 4, upper part). The six mean numbers of active ants counted during the twelfth day of the ants' meloxicam consumption statistically differed from the numbers obtained for ants under normal diet (N = 6, T = -21, P = 0.016), and not from those obtained during the six first days of the ants' this drug consumption (N = 6, T = +3, -18, P = 0.078). It was obvious to the observer that the ants' general activity did not continue to decrease in the course of their drug consumption, and stayed at the low level reached after a few days of meloxicam consumption. Under a diclofenac diet the ants also did not adapt to the drug impact, this time assessed on their locomotion speeds [6](Table 4).

Habituation to potential beneficial effects of meloxicam

Our experiments on ants could not point out any beneficial effect of meloxicam sufficiently demonstrable so that, through repeated assessments, it could allow revealing some habituation of the ants to this effect. However, meloxicam is an anti-inflammatory drug and should thus be consumed only over the occurrence of an inflammatory problem, i.e. a rather short time period. Such a use is free of habituation. For the same reason, ants under diclofenac diet could also not be examined as for their habituation to that drug [6]

Dependence on meloxicam consumption

Ants did not developed dependence on meloxicam consumption (Table 4, lower part, Figure 2 J). Indeed, 23 ants of colony A were counted on the liquid free of the drug and 13 on that containing the drug while 40 ants of colony B were counted on the liquid containing the drug and 24 on that free of the drug. These two results did not statistically differ from the numbers resulting from a random choice of the ants (18, 18: $\chi^2 = 0.91$, df = 1, $0.30 < P < 0.50$, and 32, 32: $\chi^2 = 0.11$, df = 1, $0.70 < P < 0.80$ respectively). In total, 53 ants were seen on the meloxicam sugar solution and 47 ones on the sugar water free of the drug ($\chi^2 = 0.08$, df = 1, $0.70 < P < 0.80$). The fact that meloxicam did not lead to any dependence is in favor of this drug use. This was not the case for diclofenac: this drug may lead to dependence [6]. At least on the basis of these two observations, meloxicam is far more preferable than diclofenac.

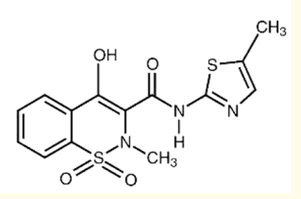

Figure 1: Chemical structure of meloxicam, an anti-inflammatory drug, the adverse effects of which are here examined on ants.

Adaptation						
time at which ants' general activity was assessed	Means of six counts of ants active anywhere in their environment					
during six days under normal diet	9.33	12.50	9.33	12.33	11.17	10.50
during their six first days of the drug consumption	9.83	8.50	8.67	7.67	7.83	6.66
during their 12th day of the drug consumption	7.00	6.17	7.00	7.33	7.50	7.67
Dependence						
colony	Number of ants counted on the liquid					
	containing the drug			free of the drug		
A	13			23		
B	40			24		
A + B	53			47		

Table 4: Adaptation to the impact of meloxicam on the general activity, and dependence on meloxicam consumption. Details and statistics are given in the text. The ants went on being less active after 12 days of meloxicam consumption: no adaptation occurred thus as for the impact of the drug on the general activity. The ants did not prefer a liquid containing the drug presented together with a liquid free of it: they thus did not become dependent on meloxicam consumption.

Figure 2: Some views of the experiments. **1**: ants under normal diet; **2**: ants under meloxicam diet. **A**: ants having reached a tied nestmate, and being less numerous under meloxicam diet. **B**: ants having come onto an unknown apparatus, and being less numerous under meloxicam diet. **C**: ants walking with difficulty on a rough substrate, essentially while consuming meloxicam. **D**: ants taking care of larvae, whatever their diet. **E**: two nestmates presenting no aggressiveness, essentially while consuming meloxicam (the ants under normal diet slightly opened their mandibles). **F**: ants stinging an alien, whatever their diet. **G**: ants having to cross a twists and turns path, 5 among 15 succeeding (red circles) under normal diet, and none under meloxicam diet. **H**: enclosed ants in the process of escaping under normal diet and failing to do so under meloxicam diet. **I**: ants trained to a hollow green cube and giving the correct response, whatever their diet, in a Y-apparatus provided with the cue in one branch. **J**: ants under meloxicam diet in front of sugar water and sugar water containing meloxicam (red spot), and not preferring the latter.

Decrease of the effect of meloxicam on the ants' general activity, after its consumption was stopped

Let us recall (see 'Material and Methods') that the trait considered to study this decrease was the ants' general activity quantified through its mean value. Numerical results are given in Table 5; mean values obtained over time are graphically presented in Figure 3. During the first six hours after weaning, the effect of meloxicam did not decrease. Then, it slowly, nearly linearly decreased and fully vanished in a total of 51 hours after weaning. In details, (1) let us compare the values obtained over time with those obtained at t = 0 (just before weaning), (2) then with the control values obtained before ants consumed meloxicam, (3) and on the basis of these two comparisons, let us explain the entire decrease of the effect of meloxicam after its consumption was stopped.

Time (hours)	Mean of six counts of active ants						Mean of the six means
t = 0	6.17	5.67	5.33	5.67	5.83	5.50	5.69
3	6.17	6.00	6.17	5.50	5.50	6.00	5.89
6	6.00	6.00	5.83	6.00	5.83	5.83	5.91
9	5.50	5.67	5.00	5.00	5.83	5.83	5.47
12	5.33	5.67	5.50	5.33	5.83	5.50	5.53
15	5.67	5.83	6.00	5.67	5.50	5.17	5.64
18	6.33	6.50	6.33	6.50	6.67	6.33	6.44
21	6.33	6.67	6.17	6.67	6.33	6.50	6.45
24	6.83	6.83	6.67	6.67	6.67	7.00	6.78
27	7.50	7.33	7.17	7.33	7.50	7.67	7.42
30	7.50	7.33	7.67	7.50	7.50	7.67	7.53
33	8.83	8.67	8.67	8.50	8.50	8.33	8.58
36	8.83	9.33	9.17	9.00	9.33	9.50	9.19
39	9.83	10.00	10.17	10.00	9.83	10.00	9.97
42	10.17	9.83	10.00	10.17	10.00	10.50	10.11
45	9.83	10.00	10.00	10.00	10.17	10.33	10.06
48	10.83	10.83	10.83	10.67	10.67	11.00	10.80
51	10.67	11.00	11.83	11.33	11.00	11.00	10.97
control	9.33	12.50	9.33	12.33	11.17	10.50	10.86

Table 5: Decrease of the effect of meloxicam on the ants' general activity after weaning. Experimental details and statistics are given in the text. The means of the six means obtained over time are graphically presented in Figure 3. The effect of meloxicam first stayed intact for 6 hours, then slowly decreased for 48 hours, and vanished in a total of 51 hours. Such a slow decrease allows an absence of dependence on the drug. t = 0 corresponds to weaning.

Figure 3: Decrease of the effect of meloxicam on the ants' general activity after weaning. Numerical values are given in Table 5, details and statistics in the text. The effect of meloxicam stayed intact for 6 hours after weaning, then slowly decreased during 48 hours and fully vanished in a total of 51 hours.

(1) Three hours after weaning, the ants' activity was similar to that at t = 0 (N = 5, T = +12 -3, P = 0.156). Even if six hours after weaning, the ants' activity was slightly higher (N = 5, T = +14 -1, P = 0.063) then, 9 hours, 12 hours and 15 hours after weaning, it remained similar to that at t = 0 (successively: N = 4, T = +1.5 -8.5, NS; N = 3, NS; N = 5, T = +6 -9, P = 0.400). Then, 18 hours after weaning, the ants' activity became significantly higher than that at t = 0 (N = 6, T = +21, P = 0.016), this difference, with P = 0.016, remaining until the end of the experiment, i.e. during a total of 51 hours. So, after weaning, the effect of meloxicam did not differ from its initial one during about 16 or 17 hours, what is in favor of that drug use.

(2) From 3 hours until 36 hours after weaning, the ants' activity was lower than that observed before giving meloxicam to the ants, each time, with the statistical result of N = 6, T = -21, P = 0.016. Such a long lasting effect of the drug after weaning is in favor of its use. From 36 hours after weaning, the ants' activity slowly and progressively increased until reaching that presented under normal diet. The statistical analysis of the obtained values of activity clearly revealed this slow decrease. Indeed, at t = 39 hours after weaning, it became statistically similar to that under control condition (N = 6, T = +4.5 -16.5, P = 0.133). At t = 42, 45 and 48 hours after weaning, the statistical results were successively: N = 5, T = +3 -12, P = 0.156; N = 6, T = +5 -16, P = 0.156; N = 6, T = +8 -13, P = 0.344. Ultimately, at t = 51 hours after weaning, the ants' activity reached that presented under normal diet (N = 6, T = +11.5 -9.5, P = 0.461). Such a slow decrease is beneficial and avoids developing dependence. It is thus in favor of the use of meloxicam. In comparison, the effect of diclofenac rapidly decreased after weaning, vanishing in a total of about 6 hours, what was not in favor of the latter drug use [6].

(3) In conclusion, after weaning, the effect of meloxicam stayed intact for about 17 hours after weaning, being still statistically similar to that before weaning and different from that under a drug-free diet. After that, from 18 hours to about 37 hours after weaning, the effect of meloxicam was lower than its initial one but still present since the results statistically differed from that under a drug-free diet. Finally, 39 hours after weaning, such a difference with a drug-free diet no longer existed, and the effect of meloxicam continued to slowly decrease during 12 hours, fully vanishing after a total of 51 hours after weaning.

Discussion and Conclusion

Having previously found that diclofenac, one of the most largely used anti-inflammatory drugs, is not safe, and being so in agreement with other research (see the introduction section), we examined here, on ants as models, the potential adverse effects of another anti-inflammatory drug, meloxicam. We found that meloxicam decreased the ants' meat consumption, increased the sugar water consumption, and decreased their general activity, audacity, tendency in crossing a twists and turns path, and tendency in escaping from an enclosure. Meloxicam did not affect the ants' locomotion characteristics, orientation ability, tactile perception, and social relationships such as brood caring and aggressiveness against nestmates, as well as their conditioning ability, and their short term, middle term and long term memory. The ants did not (or very poorly) adapt themselves to the effect of the drug on their general activity. They developed no dependence on meloxicam consumption. The effect of that drug on the ants' general activity decreased very slowly after weaning, what can explain the non development of dependence. Meloxicam presented thus some adverse effects, but these effects were not very severe and did not concern the most important examined traits (such as the locomotion, the orientation, the memory, and dependence). Used in small amounts and only when necessary, meloxicam may be an anti-inflammatory drug with a low toxicity.

It is now required to compare the harmful impact of meloxicam and of diclofenac, first using our results on ants (present paper and previous one [6]), secondly using the information for humans' use joined to the packages of the drugs, and thirdly using research works published on the subject. A summary of these comparisons is given in Table 6.

Source of information	Diclofenac	Meloxicam
Works on ants: diclofenac: [6], meloxicam: [present work]	Decrease of meat consumption Decrease of sugar consumption Some decrease of general activity No change in linear speed Increase of sinuosity Decrease of orientation ability Decrease of audacity No change of tactile perception No change of brood caring No change of social relationships Decrease of cognition Decrease of escaping ability Large decrease of conditioning Large decrease in memorization No adaptation Some dependence Rapid decrease of effects after weaning	Decrease of meat consumption Increase of sugar consumption Decrease of general activity No change in linear speed No change in sinuosity No change of orientation ability Decrease of audacity No change of tactile perception No change of brood caring Improvement of social relationships Decrease of cognition Decrease of escaping ability No change of conditioning No change in memorization No adaptation No dependence Slow decrease of effects after weaning
Instructions for humans' use joined to the drug package (relating some undesirable effects)	Digestive problems (infrequent) Liver problems (infrequent) Kidneys inflammation (infrequent) Cardiovascular risk (low) Headache (infrequent) Sleepiness (rarely)	Digestive problems (frequent) Liver problems (infrequent) Kidneys inflammation (rarely) Cardiovascular risk (very rarely) Headache (frequent) Sleepiness (infrequent)
Research on vertebrates including humans	Lesser GI tolerability [11,14,15] More toxic for liver [4,5] More toxic for kidneys [10] More adverse effects and laboratory abnormalities when cared for osteoarthritis [12] Less tolerated in osteoarthritis care [13] Half-Life ~ 1 -3 h in mammals and 12 h in vultures [41]	Higher GI tolerability [11,14,15] Rarely toxic for liver [18] Less toxic for kidneys [10] Less adverse effects and laboratory abnormalities when cared for osteoarthritis [12] Better tolerated in osteoarthritis care [13] Half-life ~ 6 – 120 h in mammals and 0.5 h in vultures [41]

Table 6: Comparison of adverse effects of diclofenac and meloxicam observed on ants, related in the instructions supplied to the drugs and reported by research on vertebrates including humans.

Our results clearly showed that diclofenac presented the few adverse effects of meloxicam, and moreover impacted the ants locomotion (increased their sinuosity of movement), decreased their orientation ability, and largely decreased their conditioning capability and their memory. Also, contrary to meloxicam, diclofenac induced dependence, and like for mammals [41], its effects very quickly vanished after weaning. All this is not in favor of diclofenac humans' use. Therefore meloxicam seems preferable to diclofenac, even if it is not without undesirable effects.

Concerning the information for human's use joined to the packages of these two drugs, even if adverse effects are reported for meloxicam, they seem less severe and less often observed than those reported for diclofenac (see details in Table 6).

Let us look to research papers on the subject. The works considered at the start of the present study [10-18] slightly induced to think that meloxicam may be safer than diclofenac though presenting some adverse effects (see also Table 6). Moreover, given to humans, the half-life of meloxicam largely exceeds that of diclofenac (ca 14 h against 1.5 h: [41]), what should avoid dependence to the drug. Other works must be reported. An early toxicology overview of studies mainly performed on non-human mammals concluded to the low toxicity of meloxicam for stomach and kidneys among others [42]. In 2000, Van Hecken., *et al*. made a comparative study of the COX-2 versus COX-1 inhibitory activity of five anti-inflammatory drugs, rofecoxib, meloxicam, diclofenac, ibuprofen and naproxen. They concluded that meloxicam and diclofenac act almost similarly, being the most specific against COX-2 activity although remaining active against COX-1, but that rofecoxib uniquely inhibits COX-2 without affecting COX-1. Rofecoxib was therefore considered to have some advantage over the use of meloxicam and diclofenac, although these three drugs were similar by their absence of influence on the bleeding time [43]. Yet, in 2004, rofecoxib has been retired from the pharmaceutical market because leading to severe cardiovascular problems [44]. Naido and co-authors examined in 2010 the toxicity on vultures of diclofenac, meloxicam and ketoprofen. They concluded that the less toxic drug (the only alternative to diclofenac, as they said) was meloxicam [45]. A following work, also performed on vultures in 2010, again pointed out the toxicity of ketoprofen and proposed the hypothesis of species-specific differences in the cytochrome P450 pathway to explain this toxicity [46]. The work of Adawaren., *et al*. [41] should aware us that birds, mammals and probably also ants may use de-toxification metabolic pathways of their own and therefore that they can react differently to the same drug.

We can conclude that to treat animals or humans, meloxicam seems preferable to diclofenac because appearing somewhat less toxic. However, according to the lack of absolute physiological safety of the nowadays anti-inflammatory used drugs and to the absence of research on their effect on e.g. cognition and memorization, medicinal, toxicological and neurological studies should be pursued in order to find a best possible medicine.

Conflict of Interest

We affirm having no conflict of interest concerning the use of meloxicam as an anti-inflammatory drug. We work on the ants' biology, ethology and physiology, and receive no money for performing our research.

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