Acta Scientific Pharmacology

Volume 3 Issue 1 January 2022

Research Article

Side Effects of the Largely Used Drug Indapamide; A Study on Ants as Models

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Received: November 26, 2021

Published: December 31, 2021

Abstract

Indapamide is a drug used since a long time for treating patients suffering from hypertension, edema or heart problems. Its side effects have never been precisely defined though, due to its modes of action, it may have such effects. Working on ants as models, we found that effectively this drug impacted the ants' food intake, general activity, audacity, sensory perception and social relationships. Indapamide did not affect the ants' state of stress, cognition, learning and memorization. The ants did not adapt themselves to the side effects of indapamide and developed no dependence on its consumption. Habituation to the researched effect of the drug could not be examined in ants, and should be studied in humans. In ants, the effect of indapamide slowly decreased after weaning, according to a quadratic function of time, and fully vanished in 24 hours, what allowed approving the general daily consumption of 2.50 mg. A large individual variability seemed to exist in ants. We thus suggest to examine in humans the occurrence of the different side effects observed in ants, some potential habituation to the drug effect, and to propose, case-by-case, the best possible dosage of the drug.

Keywords: Activity; Antihypertensive Drugs; Cognition; Diuretics; Memory; *Myrmica sabuleti*; Social Relationships

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: Hour; t: Time; %: Percentage

Introduction

The drug indapamide is a sulfonamide with an indole ring which increases the excretion of sodium, chlorides and potassium. Doing so, it is used as a diuretic, for decreasing the arterial pressure (the hypertension), and for treating some heart failure. It is thus largely used, under more than twenty labels, e.g. indapamide Sandoz®, Natrixam®, Paraterax®, Triplixam®. Information on its use can be found in several internet links such as: https://www.vidal.fr › Médicaments› Substances; https://sante.journaldesfemmes.fr › medicament-8823; https://sante.lefigaro.fr › medicaments › 3298046-indap.... In the notice for use joined to the package of these drugs, several observed side effects and recommendations are reported: headache, nausea, dry mouth, tiredness, weakness, dizziness, muscle cramps, tottering or staggering of the body, decrease of the sodium and potassium levels in the blood; paying attention when changing from horizontal to vertical position, taking care of oneself while driving a vehicle etc... According to its mode of action, indapamide may impact, among others, the nervous system functioning, the ionic balance of blood and interstitial fluid, and therefore, many physiological and ethological traits, i.e. more than those reported in internet links and the notices for use. For instance, it may affect the brain functioning, the memorization, the learning, the cognitive abilities, the sensory perception, the feeling of hunger, the interactions with congeners, the execution of specific acts and movements, and so on. Also, in the notice for use joined to the drug packages, nothing is reported about consumers' potential adaptation the adverse effects of indapamide, habituation to its wanted effect, dependence on its consumption. In addition, nothing is reported as for the loss of the effects of the drug after its consumption was stopped. Being accustomed to study, on ants as models, effects of products used by humans, we aimed to examine, again on ants as models, the potential adverse impacts of indapamide on several physiological and ethological traits. Before relating our work, we below recall why using ants as models, which species we used and what we know on it, and we list the different traits we tried to examine.

Most of the biological processes (e.g. genetics, muscles contraction, nervous impulses, memorization, and sensory perception) are similar in the different animal species, including humans. Several animals, invertebrates and vertebrates, are therefore used as biological models [1,2]. Invertebrates are small, easily maintained in a laboratory, and have a short generation time: they are thus advantageously used [3]. Insects, among others, are often used: let us cite the locusts, the mealworms, the fruit flies, the bees [4]. Ants can also be used, the more so because their maintenance is not expensive and very easy and because they detain many evolved ethological capabilities on which the effects of substances or environmental conditions used by humans can be studied. Among these capabilities, there are the use of specific pheromones for communicating with congeners, the ability to memorize visual and olfactory cues and to use them for finding their way, the establishing of efficient recruitment systems, the brood caring behavior, several specific territorial markings, nest building, and cemeteries managing [5].

In the present work, we used a species we particularly well know: *Myrmica sabuleti* Meinert, 1861. We know its recruitment system, navigating strategy, visual perception, visual and olfactory conditioning [6], the ontogenesis of several of their knowhow [7,8], their self recognizing in a mirror [9]. Also, they detain many cognitive abilities, such as having a number line, acquiring the notion of zero, counting elements, adding numbers, acquiring numerical symbolisms, expecting future events on the basis of previously experienced ones, associating perceived visual and olfactory cues with their time period of occurrence [10,11]. In addition, the distance effect, size effect and Weber's law can be applied to their perception [12,13]. However, their cognitive abilities remain at a concrete level and never reach abstraction.

In this study, we examined the potential effects of indapamide on the ants' food consumption, general activity, locomotion, orientation ability, audacity, tactile (pain) perception, social relationships, stress, cognition, learning and memory. We studied the ants' potential adaptation to revealed adverse impacts of the drug, and their possible dependence on its consumption. We finally defined how the effect of the drug decreased after its consumption was stopped. The experimental materials and protocols were identical to those previously used e.g. [14-17]: they are thus briefly related, though self plagiarism could not be avoided.

Figure 1: Making the solution of indapamide given to the ants. Successively: the chemical structure of the drug, a package of indapamide, the material used to make the drug solution, one tablet of indapamide duly crushed, an ant drinking the provided drug solution.

Materials and Methods

Collection and maintenance of ants

The experiments were conducted on two colonies of *M. sabuleti* collected from an abandoned quarry of the Aise valley (Belgium, Ardenne) in May 2021. These colonies contained about 500 workers, a queen and brood. In the laboratory, they were maintained in one to three glass tubes containing water and a cotton plug to separate the water from the ants' compartment. The nest tubes of each two colonies were set in a tray (34 cm x 23 cm x 4 cm) serving as foraging area. In this area, *Tenebrio molitor* larvae (Linnaeus, 1758) were provided three times per week, and a tube filled of sugar water and plugged with cotton was permanently set. The lighting of the laboratory equaled *ca* 330 lux while working on ants and 110 lux during the other time periods. Its temperature was *ca* 20°C, its humidity *ca* 80%, and its electromagnetic field *ca* 2 µWm2. These environmental conditions are suitable to *M. sabuleti*. In the present work, the ants are often named 'workers' or 'nestmates'.

Solution of indapamide EG® given to the ants

Humans are advised to consume 1.25 mg of indapamide at the start of their medical treatment, then to consume 2.50 mg of the drug each day. A package of indapamide EG® 2.50 mg (manufacturer: Sanico NV, Veedijk 59, 2300 Turnout, Belgium) was furnished by the pharmacist Wera (1170 Bruxelles, Belgium). Humans as most of the mammals consume about one liter of water per day. Patients treated with indapamide consume thus 2.50 mg of the drug together with one liter of water. Insects, and thus ants, consume about ten less water than mammals due to their anatomy (cuticle) and physiology (excretory system). Therefore, to set ants under a diet with indapamide similar to that of humans, they must be provided with a solution of one tablet of 2.50 mg of indapamide in 100ml of water. Such a tablet was thus crushed then dissolved into 100 ml of the ants' sugar water, and this solution was given to the ants in their cotton-plugged tubes (Figure 1). The plug of the tubes was refreshed every 2-3 days, and the entire solution was renewed every 7 days. We checked each day if ants drunk the indapamide solution, and they did. All the control experiments were firstly made on the two colonies maintained under normal diet. Then, the tubes containing sugar water were replaced by tubes contained the sugared solution of indapamide, and the test experiments were performed as soon as the ants had the drug solution at their disposal since 24 hours.

Meat and sugar water consumption, general activity

During the ants maintenance under normal diet, then during their maintenance under a diet with indapamide, we counted those being on the meat food, those being at the entrance of the sugar water tube, and those being active at any place of their foraging area, nest entrance, and inside the nest, four times during the day and four times during the night (total = 8 counts x 2 colonies = 16 counts per day) during 6 successive days, each day at the same time o'clock. For each kind of count and for each diet, we established the daily mean of the sixteen counts (Table 1, lines 1 - 6). These six dealing means obtained for one and the other kind of diets were compared to one another using the non-parametric test of Wilcoxon [18]. In addition, for each diet and each kind of count, we calculated the mean of the six daily means (Table 1, last line).

Linear and angular speeds; orientation to a tied nestmate

We assessed these traits on ants moving in their foraging area, their linear and angular speeds without stimulating them, and their orientation while stimulating them with a nestmate tied to a piece of paper (Figure 2, A). This tied nestmate emitted its mandible glands alarm pheromone which contains an attractive component. To assess the ants' speeds on one hand and their orientation on the other hand, 40 trajectories were each time manually recorded and were analyzed thanks to appropriate software [19]. This software was set up on the basis of the following definitions. The linear speed (in millimeter per second = mm/s) is the length of a trajectory divided by the time spent to travel it; the angular speed (in angular degrees per centimeter = ang.deg./cm) is the sum of the angles made by successive adjacent segments, divided by the length of the trajectory; the orientation (in angular degrees = ang. deg.) to a location is the sum of successive angles made by the direction of the trajectory and the direction towards the location, divided by the number of measured angles. An orientation value of 0°-90° means that the tested animal has a tendency to orient itself toward the location. An orientation value of 90°-180° means that the tested animal has a tendency to avoid the location. For the three considered variables, the median and quartiles of the 40 recorded values were established (Table 2, lines 1, 2, 3). Each distribution of values obtained for ants under an indapamide diet was compared with each corresponding distribution of values obtained for ants under normal diet using the non-parametric χ^2 test [18].

Audacity

This ethological trait was assessed through the ants' tendency in coming onto a risky unknown apparatus. To do so, a cylinder (height = 4 cm; diameter = 1.5 cm) vertically tied to a squared platform (9 cm^2), both in Steinbach® white paper, was set in the ants' foraging (Figure 2 B), and the ants present on this apparatus were counted 10 times over 10 minutes (number of counts: 10 x 2 = 20). The mean and the extremes of these counts were established (Table 2, line 4). The numbers obtained for the two colonies were added then chronologically summed by two what provided five successive added numbers. These five sums obtained for ants under indapamide diet were compared to those obtained for ants under normal diet using the non-parametric Wilcoxon test [18].

Tactile (pain) perception

Ants which duly perceive the rough character of a substrate walk on it slowly, sinuously, with difficulty, and they often touch the substrate with their antennae (Figure 2 C1). Ants which poorly perceive the rough character of a substrate walk on it frankly, not very sinuously and rather rapidly, seldom touching the substrate with their antennae. Therefore, to evaluate the ants' tactile (pain perception), we assessed their linear and angular speeds on a rough substrate. A piece (3 cm x 2 + 7 + 2 = 11 cm) of n° 280 emery paper duly folded was set in a tray (15 cm x 7 cm x 4.5 cm) in order to divide this tray in a first 3 cm long zone, a second 3 cm long one containing the emery paper, and a last 9 cm long zone. Each colony had its own apparatus. To make an experiment, 12 ants of each colony were deposited in the first zone of their own apparatus, and their trajectories were recorded while they walked on the emery paper. Their linear and angular speeds were assessed as explained in the subsection relative to these traits (see above). The 24 values of linear and of angular speeds obtained for ants under one and the other kinds of diet were characterized by their median and quartiles (Table 2, lines 5, 6), and their distributions were compared using the non-parametric χ^2 test [18].

Brood caring behavior

A few larvae of each two colonies were removed from the nest and set in front of its entrance. For each colony, five of these larvae were observed during five minutes. The number of them not yet re-entered after 30 seconds, 1, 2, 3, 4, and 5 minutes were counted (Table 3, line 1), and, at the same time, the workers' behavior towards these larvae was carefully observed (Figure 2 D). No more than five larvae per colony were so treated because we had to look at all of them simultaneously during 5 minutes. Also, the experiment was not repeated because removing larvae out of the nest causes great disruption in the society. The six numbers of larvae obtained for the two colonies were correspondingly added, and the six sums obtained for ants consuming indapamide were compared to the six ones obtained for ants under normal diet using the non-parametric test of Wilcoxon [18].

Social relationship towards nestmates

Ants belonging to the same colony are usually not aggressive towards one another. For studying the effect of indapamide on this peaceful social behavior, five dyadic encounters were performed for each two colonies maintained firstly under normal then under indapamide diet (number of encounters for each diet = 10). These encounters were made in a cup (diameter = 2cm, height = 1.6cm), the borders of which having been covered with talc to prevent ants from escaping. For each encounter, one of the two ants was carefully observed during 5 minutes, and its behavior was characterized by the numbers of times it did nothing (level 0 of aggressiveness), touched the other ant with its antennae (level 1), opened its mandibles (level 2), gripped and/or pulled the other ant (level 3), and tried to sting or stung the other ant (level 4) (Table 3, line 2; Figure 2 E). The numbers obtained for each five ants and each two colonies were correspondingly added, and the distribution of values obtained for ants under indapamide diet was compared to that obtained for ants under normal diet using the non-parametric χ^2 test [18]. In addition, for each diet, a variable assessing the ants' social interaction was calculated: 'a' = the number of aggressiveness levels 2 + 3 + 4 divided by the number of aggressive levels 0 + 1 (Table 3, line 2).

State of stress and cognition through escaping from an enclosure

In order to escape from an enclosure, an ant (or any individual) must be calm, not stressing, and efficiently look for an exit. It must also have some intact cognitive ability. To quantify these traits on ants under normal then indapamide diets, six ants of each colony were enclosed under a reversed cup (made of polyacetate; height = 8cm, bottom diameter = 7 cm, ceiling diameter = 5 cm) set in their foraging area. The inside surface of these cups had been covered with talc to prevent ants climbing on it. A notch (3 mm height, 2 mm broad) had been made in the rim of the bottom of the cup, the ants having so the possibility to escape from the enclosure (Figure 2, F). The ants escaped after 2, 4, 6, 8, 10 and 12 minutes were counted for each two colonies and the obtained numbers correspondingly added (Table 3, line 4). The six sums obtained for ants living under one and the other kinds of diet were compared using the non-parametric Wilcoxon test [18].

Cognition

This trait was evaluated by assessing the ants' ability in crossing a difficult path, i.e. a twists and turns way. Two pieces (two times 4.5 cm x 12 cm) of strong white paper (Steinbach®) duly folded were inserted in a tray (15 cm x 7 cm x 4.5 cm) for creating a twists and turns path between a first 2cm long zone in front of this 'difficult' path and a 8 cm long zone beyond it (Figure 3 A). Each colony had its own device. To make a test on a colony, 15 ants were set in the zone lying in front of the twists and turns path, and those ants still there as well as those having reached the zone located beyond the 'difficult' path were counted after 2, 4, 6, 8, 10 and 12 minutes. The numbers obtained for the two colonies were correspondingly added (Table 3, line 3), and the added numbers obtained for each of the two considered zones for ants consuming indapamide were compared to the two corresponding added numbers obtained for ants under normal diet using the non-parametric Wilcoxon test [18].

Visual conditioning and memory

At a given time, a yellow hollow cube was deposited above the entrance of the tube containing sugar water (Figure 3 Ba). This cube was built in strong paper (Canson®). This being done, the ants underwent operant visual conditioning. The control experiment, on ants living under normal diet, cannot be done on colonies A and B. It was previously performed using another similar colony of *M. sabuleti*, collected on the same site, maintained under normal diet. Indeed, as soon as an ant has acquired conditioning to a stimulus, it keeps this conditioning during two to three days, and after having lost it, the ant more quickly than usually acquires it again. Therefore, it becomes impossible to quantify a second time the ant's conditioning acquisition. Over the ants' conditioning acquisition, then after the removal of the yellow cube, i.e. over the ants' loss of conditioning, the ants of the two colonies were tested in an own Y-maze built in strong white paper, with its sides slightly covered with talc, and set in a separated tray. A yellow hollow cube was inserted in randomly the left or the right branch of each Y-maze. To make a test on a colony, 10 ants were one by one deposited in the maze, before its division into two branches, and the ants' choice of one or the other branch of the Y-apparatus was recorded (Figure 3 Bb). Choosing the branch containing the yellow cube was of course giving the correct response. To avoid testing twice the same ant, after having been tested, each ant was kept in a glass until 10 ants of its colony were tested, and after the entire test, all the ants were set back in their foraging area. Six successive tests were performed while ants acquired condition, and six ones were made while they started losing their conditioning (Table 4). For each of these twelve tests, the responses obtained for the two colonies were added and the proportions of correct responses was established. These twelve proportions obtained for ants under normal diet (previously made experiment) were compared to those obtained for ants consuming indapamide (presently made experiment) using the non-parametric Wilcoxon test [18].

Adaptation (tolerance) to indapamide adverse effects

An individual becomes adapted to a drug when it less and less suffers over time from the side effects of this drug. Adaptation to a drug can thus be examined by quantifying an impact of the drug soon after the individual has consumed the drug, then later, after it has consumed the drug for a time, and the two assessments must be compared. In the present work, the ants' locomotion appeared to be largely affected by indapamide consumption. Therefore, for studying potential adaption to the side effects of indapamide, the ants' linear speed, a trait very easy to assess, was again quantified after the ants consumed that drug during eight days. The median and the quartiles of the obtained values were established (Table 5), and the obtained distribution of values was compared to the corresponding one previously obtained after the ants had consumed the drug during one day and to that obtained for ants living under normal diet using the non-parametric χ^2 test [18].

Ants' potential habituation to indapamide

An individual becomes habituated to a drug when it becomes less and less sensitive to the expected effect of that drug over its consumption. For examining an individual's habituation to a drug, a trait favorably modified by the drug has to be assessed soon after the drug consumption, then later after some time of its consumption, and the results of the two assessments should be compared. Concerning the present work, we found no pronounced favorable impact of indapamide on any of the considered traits. We could thus not study if habituation on indapamide exists. However, in humans, this occurrence should be imperatively examined since, if it exists, patients will be tempted to increase their daily dose of the drug, and consequently the impact of this drug adverse effects.

Dependence on indapamide consumption

An individual becomes dependent on a drug when it tried to have this drug at its disposal at any time, it continuously consumes the drug whatever its adverse effects, and finally it can no longer live without consuming the drug. In the present work, the ants' dependence on indapamide consumption was examined after the ants had consumed it during 12 days. For assessing such a potential dependence, 15 ants of each colony were set in an own tray (15cm ×7 cm × 5cm) inside of which two cotton-plugged small tubes (h = 2.5 cm, diam. = 0.5 cm) had been deposited. One tube contained sugar water, the other containing the sugared solution of indapamide used over all the conducted experiments. The tube containing the drug was deposited on the right of the tray for colony A and on the left for colony B (Figure 3 C). Half a minute after the ants were in their trait devoted to dependence assessment, those of the two colonies present at the entrance of each tube were counted 15 times over 15 minutes. For each colony, the 15 counts were added, then, the two sums obtained for each two colonies were correspondingly added. These two added sums allowed calculating the proportion of ants sighted near the tube containing the drug and that of ants sighted near the tube free of drug. Also, the two added sums were compared to the two numbers expected if the ants randomly approached each tube using the non-parametric χ^2 goodness-of-fit test [18].

Decrease of the effects of indapamide after its consumption was stopped

This decrease was examined after the ants had consumed indapamide during 14 days. The ants' linear speed was the physiological trait impacted by the drug chosen for making this study. Twelve hours before the start of the study, the ants were provided with a fresh solution of indapamide, and after these 12 hours, the ants' linear speed was assessed as it had been after 1 and 8 days of the drug consumption, except that 20 instead of 40 ants' trajectories were recorded and analyzed. Reducing the number of analyzed trajectories allowed assessing all the recorded trajectories over the decrease of the effect of indapamide and so evaluating permanently the current situation. After this initial assessment (= that made at t = 0), the tubes containing the sugared drug solution were replaced by tubes filled of sugar water, and this constituted the weaning. The ants' linear speed was thereafter assessed every three hours until this speed became similar to the control one (= that of ants living under normal diet). For each assessment, the median and quartiles of the 20 recorded values were established (Table 6) and the distributions of these successively recorded values were compared to the corresponding distributions obtained at t = 0 and and to that obtained for ants under normal diet using the non-parametric χ^2 test for independent samples (Table 6) [18]. In addition, we provided a mathematical function which described the best the decrease of the impact of indapamide on the ants' linear speed (used as a model trait). The results were also graphically presented in Figure 4, what allowed visualizing the provided mathematical function.

Results and Discussion

Food consumption, general activity

These three physiological traits were affected by indapamide consumption (Table 1). When consuming this drug, the ants eat far less meat (N = 6, T = -21, P = 0.016), drunk somewhat less sugar water (N = 6, T = -20, P = 0.031), and were far less active (N = 6, T = -21, P = 0.016) than while living under normal diet. These decreases of food intake and general activity could lead to impact on other physiological and ethological traits, a presumption the following ‘experiments aimed to examine.

Days	Under normal diet			Under a diet with indapamide		
	Meat	Sugar water	Activity	Meat	Sugar water	Activity
I	1.44	1.69	16.50	0.75	1.38	6.13
II	1.37	1.75	15.94	0.50	1.50	8.25
III	1.37	1.62	18.19	0.50	1.75	9.00
IV	1.50	1.62	12.25	0.63	0.88	7.75
V	1.37	2.00	15.25	0.50	0.88	7.63
VI	1.50	1.87	16.00	0.44	1.38	7.50
I-VI	1.42	1.76	16.06	0.55	1.29	7.71

Table 1: Effect of indapamide on the ants' food consumption and activity. The six first lines give the mean numbers of ants sighted, over 6 days, on their meat and sugar water, as well as of those in activity. The last line gives the means of these six daily means for the three kinds of traits. Indapamide appeared to impact the three considered physiological traits.

Linear and angular speeds

These traits were impacted by indapamide consumption (Table 2, lines 1, 2). Ants consuming this drug had difficulties for walking. They often stopped, left their head and antennae, presented jerky movements, and sometimes horizontally bent their body. Their linear speed was significantly lower, and their angular speed significantly larger than while living under normal diet (linear speed: χ^2 = 54.89, df = 2, P < 0.001; angular speed: χ^2 = 72.38, df = 1, P < 0.001). We examined if the ants could adapt themselves to this worrying effect of the drug after they consumed it during eight days (see the subsection relative to the ants' adaptation).

Orientation

Indapamide affected this trait (Table 2, line 3). While under normal diet, the ants rapidly and very well oriented themselves towards a tied nestmate, when they consumed indapamide, they could no longer do so (Figure 2 A). Their orientation was obviously and significantly of poor quality (χ^2 = 37.98, df = 2, P < 0.001). This probably resulted from their difficulty in moving, but may also be due to some decrease of their perception and/or to some alteration of their social relationships, two presumptions examined in three subsequent experiments (see below the subsections relative to the ants' tactile perception and to their social interactions).

Audacity

This ethological trait was impacted by indapamide consumption (Table 2, line 4). While ants under normal diet came for a time on the risky apparatus and even climbed on the tower, when consuming indapamide, they were reluctant to do so. Under the drug diet, the ants seldom came onto the apparatus and never climbed on the tower (Figure 2 B). The difference of behavior between the ants under one and the other kinds of diet was statistically significant: N = 5, T = 15, P < 0.031. This difference was in agreement with that previously observed between the activity of ants under one and the other kinds of diet (see above the subsection relative to the ants' general activity).

Tactile (pain) perception

This physiological trait appeared to be impacted by indapamide consumption (Table 1, lines 5, 6). Under normal diet, the ants perceived the uncomfortable character of the rough substrate, and walked there very slowly and sinuously, touching the substrate with their antennae (Figure 2 C). Their linear and angular speeds statistically differed from those presented on a usual substrate (linear speed: χ^2 = 60.07, df = 1, P < 0.001; angular speed: χ^2 = 64.00, df = 1, P < 0.001). While consuming indapamide, the ants walked on the rough substrate at the same linear and angular speeds as on a usual substrate (linear speed: χ^2 = 2.31, df = 2, 0.30 < P < 0.50; angular speed: χ^2 = 3.85, df = 3, P ~ 0.30). Such an impact of the drug on the ants' perception was unexpected, may lead to impacts on other traits (a presumption we examined thanks to the following experiments), and should be considered while treating humans with this drug.

Traits	Under normal diet	Under a diet with indapamide
Linear speed (mm/s)	10.2 (9.2 - 11.6)	6.9 (6.2 - 7.5)
Angular speed (ang.deg./cm)	112 (98 - 126)	223 (200 - 242)
Orientation (ang.deg.)	34.1 (24.5 - 42.6)	74.0 (47.0 - 91.3)
Audacity (n°)	2.10 [1 - 3]	0.70 [0 - 1]
Tactile (pain) perception:		
Linear speed (mm/s)	4.4 (4.1 - 4.8)	6.6 (5.8 - 7.8)
Angular speed (ang.deg./cm) on a rough substrate	326 (305 - 343)	210 (201 - 227)

Table 2: Effect of indapamide on five ants' ethological and physiological traits. Are here successively reported the median (and quartiles) or the mean [and the extremes] of the recorded data relative to five examined traits. Indapamide impacted the five considered traits.

Traits	Normal diet	Diet with indapamide
Brood caring: n° of not re-entered larvae over 5 minutes	t: 30s 1 2 3 4 5 min 9 7 5 2 0 0	t: 30s 1 2 3 4 5 min 10 10 10 9 8 7
N° of aggressiveness 0-4 levels towards nestmates, variable 'a'	levels 0 1 2 3 4 'a' 66 39 8 0 0 0.07	levels 0 1 2 3 4 'a' 25 39 69 0 0 1.08
Stress and cognition: n° of ants escaped over 12 minutes	t: 2 4 6 8 10 12 minutes 3 4 7 7 11 12	t: 2 4 6 8 10 12 minutes 1 3 5 8 10 12
Cognition: n° of ants in front and beyond a twists and turns path over 12 minutes	t: 2 4 6 8 10 12 min in front 20 16 15 11 10 7 beyond 0 1 1 3 5 8	t: 2 4 6 8 10 12 min in front 24 16 12 11 9 8 beyond 0 0 2 4 6 8

Table 3: Effect of indapamide on four ants' ethological and physiological traits. Explanation is given in the text (0-4 levels, 'a', etc...). Indapamide affected the ants' brood caring behavior and aggressiveness against nestmates, and thus their social relationships, but did not impact their ability in escaping from an enclosure nor that in crossing a twists and turns path, and thus their stressing and their cognition.

Brood caring behavior

Indapamide appeared to affect this ethological trait (Table 3, line 1; Figure 2 D). Ants living under normal diet rapidly found the larvae removed from the nest, took them between their mandibles, hold them and carried them back inside the nest. While consuming indapamide, the ants stayed a few seconds near the larvae, did not took them between their mandibles and moved away without caring of the larvae anymore. The difference of behavior between the ants maintained under one and the other kinds of diet was significant: N = 6, T = 21, P = 0.016. This may be due to the ants' difficulty of lifting the larvae while being under the drug diet, but may also result from an impact of indapamide on the social interactions, a presumption examined in the next experiment (see the here below subsection).

Social relationship towards nestmates

Indapamide affected this behavioral trait (Table 3, line 2; Figure 2 E). Ants under normal diet presented no aggressive behavior when encountering congeners; the two nestmates often stayed side by side, touching themselves with their antennae, and they sometimes slightly opened their mandibles. While living under a diet with indapamide, encountering nestmates had a tendency to avoid each other and often largely opened their mandibles. The difference of behavior between ants maintained under one and the other kinds of diet was significant: $\chi^2 = 65.67$, df = 2, P < 0.001. This unexpected side effect of the drug is not mentioned in the notice for use, but should be considered as being likely to occur in humans and consequently to impact their social life.

Escaping from an enclosure

Indapamide did not affect this behavioral trait, and therefore the ants' stressing and probably cognitive ability (Table 3, line3; Figure 2 F). Under normal diet, enclosed ants firstly walked erratically all around the enclosure, then more calmly along its rim, saw the notch, and often went through it for escaping. While consuming indapamide, the ants behave in a similar way, but presented these behaviors more slowly, over a somewhat longer time period. Therefore, between the ants maintained under one and the other kinds of diet, the numbers of ants escaped during the first minutes of the experience differed, but finally, these numbers were identical at the end of the 12 experimental minutes. The difference of behavior between the ants living under one and the other kinds of diet was not statistically significant: N = 5, T = +2, -13, P = 0.094. The ants consuming indapamide did not stress more than those living under normal diet. Apparently, their cognitive ability was not affected by the drug consumption, but this presumption must still be examined thanks to the two following experiments (see the two here below subsections).

Cognition

Indapamide did not impact the ants' ability in crossing a twists and turns path, and therefore very probably their cognition (Table 3, line 4; Figure 3 A). A little difference could be observed at the start of the experiment between the ants maintained under one and the other kinds of diet, but thereafter, the ants consuming the drug were as able as those not consuming it in navigating the difficult path. The difference of behavior between the ants living under one and the other kinds of diet was not statistically significant: in front of the path: N = 4, T = +5.5, -4.5, P = 0.500; beyond the path: N = 4, T = -2.5, +7.5, P = 0.250. It remained now to examine if indapamide affected the ants' learning and memorization, what was done thanks to the following experiment (see the here below subsection).

Figure 2: Some views of six experiments made to study the side effects of indapamide. 1: ants under normal diet; 2: ants under a diet with indapamide. A: ants stimulated by a tied nestmates, coming towards it while under normal diet, and doing so poorly while consuming indapamide; B: ants in presence of a risky apparatus, coming on it while under normal diet, poorly doing so while consuming indapamide; C: ants on a rough substrate, walking with difficulty and touching the substrate with its antennae while under normal diet, not doing so and thus less perceiving the uncomfortable character of the substrate while consuming indapamide; D: an ant under normal diet taking a larva in its mandibles and not doing so while consuming indapamide; E: two nestmmates staying peacefully near each other while under normal diet, avoiding each other and presenting aggressive behavior (opening their mandibles) while consuming indapamide; F: an ant under normal diet as well as under a diet with indapamide escaping from an enclosure, the drug thus not impacting the ant's stressing and cognition. Details are given in the text.

Figure 3: Some views of the experiments relative to the impact of indapamide on the ants' cognitive abilities (A), on their conditioning acquisition (B), and relative to dependence on its consumption (C). 1: ants under normal diet; 2: ants under a diet with indapamide. A: ants under one or the other kinds of diet having similarly crossed a twists and turns path; the drug did not impact the ants' cognition. B: a: ants' training to a yellow cube; b: a trained ant giving the correct response when tested in a Y-maze provided with a yellow cube in one of its branch. C: ants similarly visiting a tube containing the drug (red dot) and a tube containing a drug free solution; the ants thus developed no dependence on indapamide consumption.

Visual conditioning and memory

Numerical results are given in Table 4, and two photos can be seen in Figure 3. Indapamide did not affect the ants' conditioning acquisition, and thus very probably their learning ability: the ants reached similar conditioning scores at an equivalent rapidness, and this was statistically confirmed (N = 3, NS). After the cue removal, the ants consuming the drug still presented a high conditioning score during 72 hours, just like ants living under normal diet. There was no statistical difference between the memorization ability of ants maintained under normal diet on one hand and indapamide diet on the other hand (N = 2, NS). It could thus be concluded that indapamide did not affect the ants' memorization.

Adaptation (tolerance) to indapamide adverse effects

The ants did not adapt themselves to the effect of indapamide on their locomotion (Table 5). After eight days under that drug diet, the ants still walked at a lower linear speed and a larger sinuosity than while living under normal diet, and this was statistically significant (linear speed: $\chi^2 = 54.89$, df = 2, P < 0.001; angular speed: $\chi^2 = 72.38$, df = 1, P < 0.001). Moreover, there was no significant difference between the ants' locomotion after one and eight days of the drug consumption: linear speed: $\chi^2 = 2.49$, df = 2, 0.20 < P

Time	Normal diet	Diet with indapamide
7 h	6 *vs* 4 60%	6 *vs* 4 and 6 *vs* 4 60%
24 h	6 *vs* 4 60%	6 *vs* 4 and 7 *vs* 3 65%
31 h	7 *vs* 3 70%	7 *vs* 3 and 7 *vs* 3 70%
48 h	7 *vs* 3 70%	8 *vs* 2 and 8 *vs* 2 80%
55 h	8 *vs* 2 80%	8 *vs* 2 and 8 *vs* 2 80%
72 h	9 *vs* 1 85%	9 *vs* 1 and 9 *vs* 1 90%
Cue removal		
7 h	9 *vs* 1 85%	9 *vs* 1 and 8 *vs* 2 85%
24 h	8 *vs* 2 80%	9 *vs* 1 and 9 *vs* 1 90%
31 h	8 *vs* 2 80%	8 *vs* 2 and 8 *vs* 2 80%
48 h	8 *vs* 2 80%	8 *vs* 2 and 9 *vs* 1 85%
55 h	8 *vs* 2 80%	8 *vs* 2 and 8 *vs* 2 80%
72 h	8 *vs* 2 80%	8 *vs* 2 and 8 *vs* 2 80%

Table 4: Effect of indapamide on the ants' learning and memory. The control experiment, i.e. on ants under normal diet, has been previously made on another similar colony. The experiment on ants consuming indapamide was made on colonies A and B. Under the drug diet, the ants acquired conditioning just like as when living under normal diet: the drug did not affect the learning. Also, after the cue removal, the ants consuming the drug retained their conditioning for more than 72 hours just like ants under normal diet. The drug did not impact the memorization.

Traits	Norman diet	The drug diet for 1 day	The drug diet for 8 days
Linear speed (mm/s) Angular speed (ang.deg./cm)	10.2 (9.2 - 11.6) 112 (98 - 126)	6.9 (6.2 - 7.5) 223 (200 - 242)	7.0 (6.1 - 8.0) 231 (197 - 253)
Ants walking in their foraging area			

Table 5: Adaptation to the adverse effects of indapamide. Ants did not adapt themselves to the impact of indapamide on their locomotion: after 8 days of the drug consumption, the ants' locomotion was affected just like it was after 1 day of consumption.

< 0.30; angular speed: $\chi^2 = 2.14$, df = 3, $0.50 < P < 0.70$. However, after eight days of indapamide consumption, a few old ants seemed to become less affected by the drug. A large individual variability seemed to occur over the drug consumption, the young ants being apparently more (and during a longer time) affected by indapamide than the older ones (personal observation, not quantified). Nevertheless, globally, the ants' locomotion stayed affected by indapamide during a long time period after the start of its consumption. We thus selected the ants' linear speed as the model physiological trait for examining the decrease of the effect of the drug after its consumption was stopped (see below the subsection relative to this decrease).

Dependence on indapamide consumption

Two photos of this study are shown in Figure 3 C. Ants of colony A visited 38 times the drug free solution and 35 times that containing the drug. Ants of colony B visited 30 times the solution containing indapamide and 28 times the drug free solution. In total, 66 visits occurred on the drug free solution and 65 visits occurred on the drug solution. There was no statistical difference between these two counted numbers of visits and those expected if ants randomly went onto the two provided solutions ($\chi^2 = 0.008$, df = 1, $0.90 < P < 0.05$). The ants thus developed no dependence at all on indapamide consumption, what is in favor of that drug use.

Time (hours)	Linear speed mm/s	Statistics					
		Versus t = 0			Versus control		
		χ^2	df	P	χ^2	df	P
t = 0	5.5 (5.2 - 6.6)				36.14	1	< 0.001
3 h	5.9 (5.2 - 6.4)	0.96	1	< 0.50	26.46	1	< 0.001
6 h	6.2 (5.6 - 6.6)	1.66	1	< 0.20	24.14	1	< 0.001
9 h	6.6 (5.4 - 7.7)	5.33	1	< 0.05	15.97	1	< 0.001
12 h	7.2 (6.3 - 7.7)	12.38	1	< 0.001	6.31	1	< 0.02
15 h	8.0 (7.0 - 8.9)	21.54	1	< 0.001	16.87	1	< 0.001
18 h	8.8 (8.5 - 10.5)	10.02	1	< 0.001	1.66	1	~ 0.20
21 h	9.2 (8.6 - 10.0)	21.54	1	< 0.001	0.57	1	< 0.50
24 h	10.3 (9.6 - 11.0)	36.19	1	< 0.001	1.14	1	< 0.30
Control	10.2 (9.2 - 11.6)						

Table 6: Decrease of the effect of indapamide after its consumption was stopped. The decrease was slow, without time period of abrupt decrease. The drug kept an effect similar to its initial one during about 8 hours after weaning, had a slight effect and still differed from the control until about 16 hours after weaning, and fully lost its effect in a total of 24 hours. Details are given in the text, and the here given numerical results are graphically presented in figure 4, what reveals the quadratic character of the decrease of the effect of indapamide as a function of time.

Decrease of the effects of indapamide after its consumption was stopped

Numerical and statistical results are given in Table 6; they are also graphically presented in figure 4. Indapamide slowly lost its effect after weaning, with no period of brutal decrease. Its effect stayed similar to its initial one during about 8 hours after weaning. Then, its effect was lower than its initial one but still different from the control situation until about 16 hours after weaning. It then went on decreasing and fully lost its effect in a total of 24 hours after weaning. The decrease of the effect of indapamide, initially very slow, was accelerating over time. It could thus be best described by a quadratic polynomial function of time. It could be stated that:

Effect at time 't' = initial effect - time $^{\text{a specific contance}}$

This allowed deducing:

$E_i - E_f = t^c$ with E_i = initial effect; E_f = final effect; t = running time; c= constant and therefore:

$10.3 - 5.5 = 4.8 = 24^c \rightarrow \text{Log } 4.8 = c \text{ Log } 24 \rightarrow c = \text{Log } 4.8/24 = \text{Log } 0.2 = 0.03$

The decrease of the effect of indapamide could thus be described by the function:

$E = t^{0.03}$

Such a decrease accounted for the absence of dependence on indapamide consumption. Using 2.50 mg as a daily dose is perfect. However, as for most of the examined traits, there exist a large individual variability for the decrease of the effect of the drug, and the daily dose should be adapted case-by-case according to each patient's physiology.

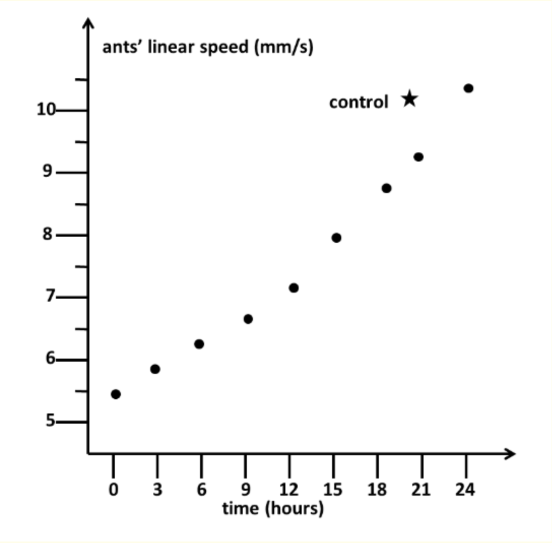

Figure 4: Decrease of the effect of indapamide after its consumption was stopped. The ants progressively, without brutal change, acquired again their usual linear speed after they stopped consuming indapamide. The effect of indapamide stayed similar to its initial one during ca eight hours, still differed from the control during ca 16 hours, and fully vanished in a total of 24 hours. The decrease of the effect of the drug approached a quadratic function of the running time (effect = time$^{0.03}$). Details can be found in the text; numerical and statistical results are given in table 6.

Discussion

Indapamide is a drug largely used for treating patients suffering from hypertensive, edema, and cardiac problems. Little information about its potential adverse effects can be found, though several recommendations are given in the notices joined to the packages of this drug. We thus studied, on ants as models, the impact of indapamide on several physiological and ethological traits and observed that this drug affects the ants' food intake, general activity, audacity, sensory perception, social relationships, but not their state of stress, cognition, learning and memory. The ants did not adapt themselves to the adverse effects of indapamide, and developed no dependence on its consumption. The effects of indapamide slowly decreased according to a quadratic function of time in a total of 24 hours after weaning. A rather large individual variability seemed to exist among the ants; this was not quantified, but is based on personal observations of the ants' behavior and of the obtained distributions of recorded values. Here below we compare our findings with the bibliographical information available.

Our finding of no impact of indapamide on the ants' cognition, learning and memorization can be attributed, among others, to the low diuretic effect of the drug [20]. In fact, indapamide has essentially a lower effect in several forms of hypertension, doing so through several activities, including the production of vasoconstrictor substances, with less severity than other similar drugs [21]. Globally, this not severe multiple modes of action of indapamide protect organs (heart, kidneys, brain) from damage. See also the review of Moore., *et al*. [22] in which the authors also reported that this drug has a low toxicity. Concerning this toxicity, no detail can be found. Even Campbell and Brackman [23] who very well explained how indapamide acts on the cardiovascular system and why this drug should be used for treating hypertensive patients and elderly ones, did not precisely mention the potential side effect of the drug. All what we could find is two works, one relative to the ionic effect of the drug, and one concerning its impact on cognition (see below). Indapamide decreases the amount of calcium and potassium present in the blood, and this leads to electrolytes disturbance, essentially in elderly patients [24]. This unwanted effect of indapamide may be the cause of its impact on the ants' sensory perception, food intake, activity, locomotion and social relationships, but lowering the daily dose may reduce this side and dangerous effect. A not initially seen effect of indapamide is its improved impact on the cerebral functioning of patients having some cerebrovascular disease: indapamide efficiently reduces the risk of dementia and cognitive decline in such patients [25]. This last found effect of indapamide is in agreement with its here observed no or even improved impact on the ants' cognitive, learning and memorization abilities.

On the basis of what we found about the side effects of indapamide and on the few information we collect in the literature, we would advice practitioners to examine the food intake, the activity, the movement, the audacity, the sensory perception, the social relationships of their patients continuously treated with that drug, and on the basis of this exam, to adapt the initial daily consumed dose. We also think that three traits should be examined in humans: the adaptation to the side effects of indapamide, the habituation to its wanted effects (if this occurs, patients will be tempted to increase their drug consumption), and an eventual patient's dependence on indapamide consumption.

Finally, we defined the decrease of the effect of indapamide after weaning: in ants, indapamide lost its effect slowly in a total of 24 hours, but a large variability seemed to exist among them (see the first alinea of this Discussion section). We recommend that the lost of the effect of indapamide after weaning be studied in humans, case by case, in order to perfectly adapt the individual dosage of the drug.

Conclusion

Indapamide has been shown to be efficient for treating patients, essentially elderly ones, suffering from hypertension and heart functioning problems [26-29]. Also, it can serve as a soft diuretic. It must thus be considered as a valuable drug. However, our work on ants revealed that this drug presents several side effects leading to pronounced tiredness, weakness, and locomotion difficulties. These adverse effects, as well as adaptation to side effects, habituation to wanted effect, potential dependence on its consumption, and decrease of its effect after weaning should be cautiously examined in humans, and then, case-by-case, the dosage providing the best health improvement with the less possible side effects should be defined for each treated patients.

Conflict of Interest

We affirm having no conflict of interest at all as for the use of indapamide, or any other similar drugs, for treating concerned patients. We work on ants and receive no money for making our research.

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