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Research Article

Not Sufficiently Revealed Side Effects of Three Largely Used Drugs, i.e. Furosemide, Apranax, Metformin, Studied on Ants as Models

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Abstract

Using ants as models, we examined the side effects of three largely used drugs, i.e. furosemide (a diuretic), Apranax (an anti-inflammatory), and Metformin (a drug used for caring of persons suffering from type 2-diabetes). We found harmful effects, not or not adequately divulgated, which should be known by practitioners. Furosemide affected the ants' locomotion, and leaded to need of water as well as to dependence on its consumption. Apranax impacted the ants' food intake, locomotion, sensory perception, social relationships, cognition, state of stress, short-term memory, and leaded to need of water as well as to dependence on its use. Most ants consuming Apranax died. Metformin affected the ants' food intake, activity, audacity, social relationships, state of stress, cognition, learning, and leaded to dependence. Over time, Metformin was found to allow treating several illnesses. Its use increased and it is now the second more present pollutant in the natural water. This list of side effects is not exhaustive. All of them should be taken into account for safely caring of humans using any of the three here studied drugs.

Keywords: *Addiction; Anorexia; Anti-Inflammatory Drug; Diuretics; Social Interactions; Type-2-Diabetes*

Abbreviations

ang.deg.: Angular Degrees; ang.deg./cm: Angular Degrees Per cm; mm/s: Millimeter Per Second; n°: Number; cm: Centimeter; mm: Millimeter; ml: Milliliter; mg: Milligram; s: Second; min: Minute; h: Hour; t: Time; %: Percentage

Introduction

Having until now studied, on ants as models, the side effects of 51 products used by humans, we can state that, usually, wanted effects of the product are clearly reported while their adverse effects are poorly or even not reported except in specific works not easily available to public. Such doing occurs for any commercialized products, but is particularly obvious for pharmaceutical medicines. Here below, we summarize easily available information on the three examined drugs, and briefly explain why we used ants as biological models and which species we used.

Information on the three examined drugs

Furosemide efficiently acts on the ascending part of the Henle's loop of the kidneys, decreasing its recapture of water [1,2]. Patients suffering from edema are thus well cared using this drug, which is considered as an essential drug by the WHO. The pharmacological properties of furosemide have already been largely examined [3,4 = two reviews]. The notice for use of this drug reports few, not severe

adverse effects, except risks of acute kidney injury [5], deafness [6], and low level of sodium and potassium in the blood [2]. Also, allowing not seeing the consumption of anabolic products, furosemide is considered as a doping agent for people doing competitive sports (notice for use joined to the packages of furosemide). Our aim was to examine the potential effects of this drug on several not yet considered important physiological and ethological traits (see under).

The active substance of Apranax is Naproxen-Natrium. Its use was authorized in 1983, and it has been proved to be an efficient anti-inflammatory drug with few adverse effects [7-10]. It allows caring of persons suffering from, among others, arthritis, rheumatism, periarthritis, tendonitis, low back pain, and stomatological inflammation. The side effects often cited in the notices joined to this drug packages are digestive problems, headache, drowsiness, hearing and eye problems, dyspnea, and edema. Nevertheless, few experimental works exist about the side effects of Naprofen. Let us cite a work on its impact on the upper part of the gastrointestinal track [11], a clinical study made on patients with osteoarthritis [12] and a work on its adverse effect on the kidneys [13]. As a matter of fact, generally, the efficiency of Apranax is advocated, while its side effects are reported without insistence; i.e. there is a contrast between the marketing and the praise for this drug [14]. Let us add that Naproxen has a not negligible ecotoxicity, and systems for reducing this impact should be set up [15,16]. Concerning our own participation to the study of Naprofen (= Apranax), we thought that, according to the mode of action of this drug [17], it could have other physiological and ethological adverse effects than those already known. Therefore, examining this potential not yet revealed harmful impacts of Naprofen was our research aim.

Metformin is a hypo glycerinate which reduces the amount of sugar in blood, the insulin resistance of muscles and fat tissues, the neoglucogenesis and the absorption of glucose [18-20]. It also inhibits the propagation of glucagon into the blood [18]. It is thus used for treating persons suffering from type 2-diabetes. Over time, Metformin was found to be useful for treating polycystic ovary syndrome [21], for preventing and/or reducing some cancers [22] and for losing weight [23,24]. However, Metformin presents a few side effects, the most divulgated being digestive problems, decrease of the amount of vitamin B12, metallic taste in the mouth, and anorexia [e.g. notices joined to the drug packages], as well as kidney dysfunction [25]. No information can be found as for the potential impact of Metformin on other ethological and physiological traits: this is why we conducted our study on ants as models. Due to its increasing use, Metformin is nowadays the second pollutant of the nature water [26]. Nevertheless, it can be (and should thus be) degraded by photocatalysis, and the degradation products are not toxic [27].

Why using ants as biological models and which species we used

Most biological processes are similar for every animal species; invertebrates and vertebrates can thus be used as biological models for physiological and ethological studies. Invertebrates are preferred because they have a small size, can easily be maintained out of their natural environment, and have a short generation time [28]. Insects are often used, and ants can thus be used. They can be the more so since their maintenance is very easy and not at all expensive, and since they detain many evolved biological characters on which the impact of situations and products can be examined [29]. We worked on the species *Myrmica sabuleti*, Meinert 1861 the biology of which we know rather well. We have studied its visual perception, conditioning acquisition, recruitment system [30], ontogenesis (by imprinting or learning) of some of their skills [31], their recognition in a mirror [32], as well as several of their cognitive abilities [33-35]. Among others, they possess a left to right oriented number line, they acquire the notion of zero through experiences, they can make additions and subtractions, they can expect the following element of an increasing or decreasing arithmetic or geometric sequence, and they can acquire symbolisms and use the symbols for adding and subtracting. Also, the distance effect, the size effect and the Weber's law can be applied to their perception [36,37]. They are thus as valuable models as are rats, mice, or monkeys, but their maintenance and their use are far more easy.

In the present paper, we summarize our studies of the potential adverse effects of furosemide, Apranax and Metformin on *M. sabuleti* workers' food consumption, general activity, locomotion, orientation ability, audacity, tactile (pain) perception, social relationships, stress, cognition, learning and memory. We also summarize what we found about potential adaptation to these side effects, habituation to a wanted effect, and dependence on the drug consumption, as well as our exam of the loss of the effects of these drugs after their consumption was stopped.

Materials and Methods

Collection and maintenance of ants

The experiments were conducted on colonies of the ant *Myrmica sabuleti* Meinerts, 1861 collected in 2021 in Belgium from the Aise valley (Ardenne region), Marchin (Condroz region), and Visé (the Basse-Meuse region). Each colony was maintained in one to two glass tubes half-filled with water, a cotton plug separating the ants from the water, deposited in a tray (34 cm x 23 cm x 4 cm) which served as foraging areas. The ants were fed with sugar water permanently provided in small cotton-plugged tubes and with *Tenebrio molitor* larvae delivered three times per week. The lighting of the laboratory varied between 110 and 330 lux, the temperature equaled *ca* 20°C, the humidity *ca* 80%, and the electromagnetic field *ca* 2 μWm^2, these conditions being suitable to *M. sabuleti*.

Drug solution given to the ants

The three studied drugs were furnished by the pharmacist Wera (1170 Bruxelles, Belgium). Humans consume about one liter of water per day. Insects, thus ants, due to their anatomy and physiology consume about ten less water than mammals. Therefore, to set ants under a drug diet similar to that of humans, the usual dose daily consumed by humans were dissolved into 100 ml of the ants' sugar water, and this solution was delivered to them in their usual cotton-plugged small tubes. The cotton plug and the entire drug solution were refreshed or replaced as necessary.

Assessment of the examined physiological and ethological traits

Relating how the 17 examined traits were assessed would have been really too long for the present summary of previously made works. These assessments are explained in our three previous works [38,39, upcoming published data], and are also detailed in our still previous works (until now, we have studied the effects of 51 products, drugs or situations). They are thus here below only very briefly recalled. The ants' food intake and activity were assessed by counting the ants eating meat, drinking sugar water, and being active at any place of their environment, several times per day during six days, and by establishing the means of the these counts. The ants' linear speed, angular speed, and orientation were assessed by recording ants' trajectories, analyzing them thanks to appropriate software, and by establishing the median and quartiles of the recorded data. The ants' audacity was assessed by counting the ants coming onto an unknown risky apparatus. The ants' tactile (pain) perception was evaluated by quantifying the ants' linear and angular speed while they walked on a rough substrate: indeed, when perceiving the rough character of a substrate, the ants walked on it more slowly and more sinuously than usually. The ants' social relationships were examined through the ants' behavior in front of larvae experimentally removed from the nest, as well as in front of a congener in the course of dyadic encountering. The ants' state of stress and cognition were appreciated through their ability in escaping out of an enclosure, and in crossing a twists and turns path. The ants' learning and memory were assessed by quantifying their acquisition of operant conditioning and their loss of their conditioning after the removal of the conditional stimuli. The ants' adaptation to side effects of the drug was examined by quantifying again a trait impacted by the drug after several days of this drug consumption. The ants' habituation to a wanted effect of the drug was examined by quantifying again, after several days of this drug consumption, a trait changed as wanted by the drug. The ants' dependence on the drug consumption was quantified by assessing the ants' choice between a drug solution and a drug-free solution. The decrease of the effect of the drug after weaning was studied by replacing the drug solution provided to the ants by a drug-free solution, and by quantifying over time a trait impacted by the drug until this trait became as it was before the drug consumption. Every result was statistically analyzed thanks to non-parametric adequate tests.

Results and Discussion

Furosemide

Furosemide did not impact the ants' meat intake, orientation, audacity, tactile (sensory) perception, social relationships, state of stress, cognition, learning and memory. This is quite different from what was observed for a previously studied diuretic, indapamide, which appeared to be far more toxic than furosemide. Nevertheless, furosemide affected the ants' sinuosity of locomotion, induced a need for water and leaded to dependence on this drug consumption. In addition, no adaptation to these side effects occurred. The effect of furosemide became different from its initial one as soon as 3 - 4 hours after its consumption was stopped (and this can be perceived by consumers), but stayed different from the control situation until 12 hours after weaning (what validates the advised daily dose), and fully vanished in a total of about 13 hours after weaning. This rather rapid decrease could be best described thanks to the following quadratic function of the fourth degree:

$E_T = 231.33 – 16.22\ T + 3.15\ T^2 - 0.32\ T^3 + 0.01\ T^4$

With E_T = effect of the drug at the time T, and T = the time in hours.

These results are detailed in [38], briefly summarized in Table 1, second column, and partly illustrated in Figure 1, 1a, 1b, 1c.

No doubt that furosemide is an excellent diuretic, and should be used for patients suffering from edema, but attention must be paid as for these patients' (essentially the elderly ones') locomotion, hydration, dependence on the drug consumption, and potential deafness occurrence.

Examined traits	Furosemide	Apranax	Metformin
Meat intake	No impact	Decrease	Decrease
Sugar water intake	Increase	Decrease, then increase	Decrease
General activity	Slight decrease	Decrease	Decrease
Linear speed	No impact	Decrease	Decrease
Angular speed	Increase	Increase	Increase
Orientation	No impact	Decrease	Decrease
Audacity	No impact	Decrease	Decrease (hesitation)
Tactile perception	No impact	Decrease	No impact
Brood caring	No impact	Decrease	Decrease
Social relationships	No impact	Impact	Slight impact
Stress and cognition	Slight improvement	Impact	Impact
Cognition	Slight improvement	Decrease	Decrease hesitation
Learning and memory	No impact	Decrease	Decrease
Adaptation	No adaptation	No adaptation	No adaptation
Habituation	Not examined	Habituation	No habituation
Need for water	Need for water	Need for water	To not examine
Dependence on the drug	Dependence	Dependence	Dependence
Loss of the effect of the drug after weaning	Loss in 14h according to a 4^{th} degree function	Loss in 13h according to a 2^{nd} degree function	Loss in 13h according to a linear function

Table 1: *Summary of the observed side effects of furosemide, Apranax and Metformin. The numerical results are details in the three works relative to these drugs [38,39, unpublished data]. They are briefly related in the 'Results and Discussion' section of the present paper, and partially illustrated in Figure 1.*

Apranax

This drug appeared to have far more severe adverse effects than those easily available, e.g. in the notice joined to the drug package. Indeed, Apranax decreased the ants' food intake, activity, speed of locomotion, orientation ability, audacity, and sensory perception, i.e. the wanted effect of the drug. It affected the ants' social relationships, state of stress, cognition, learning and memorization. No adaptation occurred to these side effects, and in addition, habituation to the drug wanted effect occurred. Also, Apranax leaded to need for water and to dependence on its consumption. The effect of this drug became lower than its initial one as soon as 4 hours after weaning, stayed slightly different from the control situation until 12 hours after weaning, and fully vanished in a total of 13 hours. This decrease could be best described thanks to the following quadratic function:

$V_t = V_i + t^{0.3858}$

With V = linear speed (mm/s), t = time (hours), V_i = initial speed, V_t = speed at time t

These results are detailed in [39], briefly summarized in Table 1, third column, and partly illustrated in Figure 1, 2a, 2b, 2c.

Apranax is a very efficient anti-inflammatory drug, but it has many severe adverse effects and should thus be used very cautiously. Practitioners should be acquainted with our findings. They should control their patients' health and potential dependence on Apranax consumption. They should try to use, case by case, the smallest possible dose of Apranax, during the shortest possible time period. Let us add that Apranax (Naproxen) contributes, with plenty of other drugs, to pollute the natural water, affecting thus the life of several vertebrates and invertebrates.

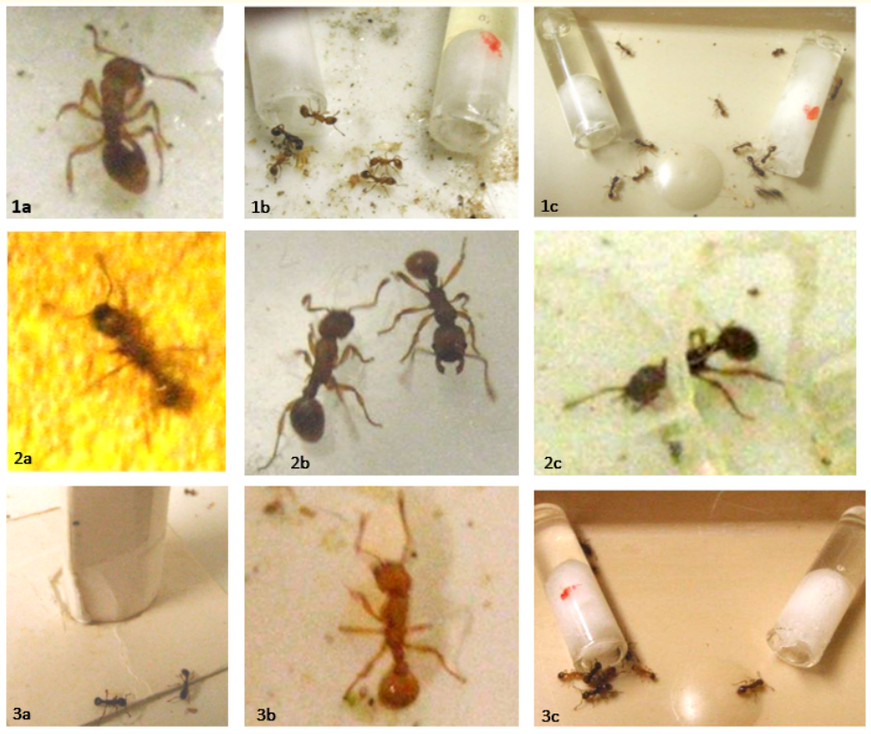

Figure 1: *Photos taken during some of the experiments made for examining the adverse effects of furosemide (1), Apranax (2), and Methformin (3). Ants under furosemide diet, among others, walked with difficulty (1a), need for water (1b), and became dependent on that drug consumption (red dot) (1c). Ants under Apranax diet, among others, had their sensory perception decreased, walking as usually on a rough substrate (2a), had their peaceful social relationships impacted, opening their mandibles near a congener (2b), and hesitated to escape from an enclosure (2c). Ants under Metformin diet, among others, were not inclined to come onto an unknown risky apparatus (3a), delayed in taking care of their larvae (3b), and developed dependence on this drug consumption (red dot) (3c). These photos are not those published in our three previous works on the subject [38, 39, upcoming published data], but are different ones taken during the same experimental times.*

Metformin

On ants, Metformin appeared to decrease their food intake, general activity, audacity, cognition and learning skill. This drug also impacted the ants' social relations and induced some stress. No adaptation occurred to these side effects, but no habituation was observed for the wanted effect of the drug, i.e. a low amount of sugar consumption. Ants developed obvious dependence on this drug consumption. After weaning, Metformin kept an effect similar to its initial one during 4 hours, and totally lost its effect in a total of 13 hours. This decrease could be best described thanks to the following linear function:

$$E_t = E_I - a \times t \text{ or } E_t = 254 - 10.21\ t$$

With E_t = effect at time 't'; E_I = initial effect; t = time (in hours).

These results will be detailed in an upcoming publication, are briefly summarized in Table 1, fourth column, and partly illustrated in Figure 1, 3a, 3b, 3c.

Metformin is a drug very useful for caring of persons suffering from type 2-diabetes (its initial use), as well as those suffering from obesity and some cancers (novel uses discovered over time). However, according to the side effects we found on ants, we advise to cautiously monitor the patients treated with this drug as for the occurrence of these side effects, and particularly of anorexia and dependence on this drug consumption. It must also be pointed that, due to its increasing use, Metformin is nowadays the second more abundant pollutant of the nature water, and that system for eliminating it from waste water should be set up.

Remark

Knowing the adverse effects a product has on ants as models, researchers and practitioners must then conduct experiments on vertebrates (rats, mice, dogs, monkeys) and on humans for more adequately and safely treat patients with this product. For instance, they should look at the impact of the product on the experimented models' locomotion, audacity, sensory perception, social relationships, cognitive abilities, learning and memorization. They should also examine the potential occurrence of adaptation to, of habituation to, and of dependence on the product consumption, as well as how the effect of the product decreases after weaning. In other words, experiments on ants (easily and rapidly made, at low cost) reveal possible side effects of products, but they are a first step to the knowledge of the products side effects, and further researches should check the occurrence of such side effects *in fine* in humans.

Conclusion

Using ants as models, we studied the side effects of three largely used drugs: a diuretic: furosemide, an anti-inflammatory drug: Apranax, and a drug used for treating type 2-diabetes: Metformin. For each of them, we found severe adverse effects which should be known and taken into account by practitioners. Among others, and above all, these side effects are, for furosemide, locomotor problems, dehydration and dependence on the drug (however, furosemide is far less toxic than the diuretic indapamide). For Apranax, the side effects we found were, among others, decrease of food consumption, activity, cognition, learning, as well a, dehydration, dependence on the drug. Ants consuming this drug died in a few days. The lowest daily dose of Apranax consumption and the shortest time period of the treatment should thus be used. As for Metformin, the most important side effects were hesitation to perform tasks, anorexia, and dependence on the drug consumption. More information can be found in our three published works on each of these three drugs [38,39, upcoming published data]. Let us add that these drugs, as several other products used by humans, nowadays pollute the natural environmental water, affecting so the maintenance of the initial beneficial biodiversity. While going on studying the ants' skills and the side effects of drugs, we also aim to examine the effects of low amounts of insecticides and herbicides (two kinds of pollutants) on ants as models.

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Conflict of Interest

We affirm having no conflict of interest as for the use of the three here studied drugs. We work on ants, on their behavior, cognitive abilities and ontogenesis of their skills, and we receive no money for conducting our research.

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