



# The multilevel antibiotic-induced perturbations to biological systems: Early-life exposure induces long-lasting damages to muscle structure and mitochondrial metabolism in flies<sup>☆</sup>

David Renault<sup>a, b, \*</sup>, Hesham Yousef<sup>c</sup>, Amr A. Mohamed<sup>c</sup>

<sup>a</sup> Université de Rennes 1, UMR CNRS 6553 EcoBio, 263 Avenue du Gal Leclerc, CS 74205, 35042 Rennes Cedex, France

<sup>b</sup> Institut Universitaire de France, 1 rue Descartes, 75231 Paris Cedex 05, France

<sup>c</sup> Department of Entomology, Faculty of Science, Cairo University, Giza - PO Box 12613, Egypt

## ARTICLE INFO

### Article history:

Received 11 March 2018

Received in revised form

1 June 2018

Accepted 4 June 2018

Available online 7 June 2018

### Keywords:

Antibiotics

Eukaryote

Insect flight muscles

Mitochondrial dysfunction

Metabolomics

Energy metabolism

## ABSTRACT

Antibiotics have been increasingly used over the past decades for human medicine, food-animal agriculture, aquaculture, and plant production. A significant part of the active molecules of antibiotics can be released into the environment, in turn affecting ecosystem functioning and biogeochemical processes. At lower organizational scales, these substances affect bacterial symbionts of insects, with negative consequences on growth and development of juveniles, and population dynamics. Yet, the multiple alterations of cellular physiology and metabolic processes have remained insufficiently explored in insects. We evaluated the effects of five antibiotics with different mode of action, i.e. ampicillin, cefradine, chloramphenicol, cycloheximide, and tetracycline, on the survival and ultrastructural organization of the flight muscles of newly emerged blow flies *Chrysomya albiceps*. Then, we examined the effects of different concentrations of antibiotics on mitochondrial protein content, efficiency of oxidative phosphorylation, and activity of transaminases (Glutamate oxaloacetate transaminase and glutamate pyruvate transaminase) and described the cellular metabolic perturbations of flies treated with antibiotics. All antibiotics affected the survival of the insects and decreased the total mitochondrial protein content in a dose-dependent manner. Ultrastructural organization of flight muscles in treated flies differs dramatically compared to the control groups and severe pathological damages/structures disorganization of mitochondria appeared. The activities of mitochondrial transaminases significantly increased with increased antibiotic concentrations. The oxidation rate of pyruvate + proline from isolated mitochondria of the flight muscles of 1-day-old flies was significantly reduced at high doses of antibiotics. In parallel, the level of several metabolites, including TCA cycle intermediates, was reduced in antibiotics-treated flies. Overall, antibiotics provoked a system-wide alteration of the structure and physiology of flight muscles of the blow fly *Ch. albiceps*, and may have fitness consequences at the organism level. Environmental antibiotic pollution is likely to have unwanted cascading ecological effects of insect population dynamics and community structure.

© 2018 Elsevier Ltd. All rights reserved.

## 1. Introduction

The use of pharmaceuticals has been consistently developed over the past decades (Aminov, 2017), and antibiotics have revolutioned human medicine. Antibiotics are also highly employed for

food-animal agriculture, aquaculture and plant production (Witte, 2000; Le et al., 2005; Cabello, 2006), for disease prevention, or for promoting growth and feed efficiency of livestock animals (Gaskins et al., 2002). However, the use of antibiotics is not without posing significant problems (Finley et al., 2013; Larsson, 2014; Rico et al., 2014), and alternatives to these growth promoters are increasingly discussed (Seal et al., 2013). Besides antibiotic resistance (Neu, 1992; Laxminarayan et al., 2013), the adverse effects of antibiotics on environmental ecosystems is a rapidly emerging concern. Andersson & Hughes (2014) suggested that up to 80% of the active substances of antibiotics can be released into the

<sup>☆</sup> This paper has been recommended for acceptance by Charles Wong.

\* Corresponding author. Université de Rennes 1, UMR CNRS 6553 EcoBio, 263 Avenue du General Leclerc, CS 74205, 35042 Rennes Cedex, France.

E-mail address: [david.renault@univ-rennes1.fr](mailto:david.renault@univ-rennes1.fr) (D. Renault).

environment. Several antibiotics are not degraded within the gut of livestock animals, and the several pathways that permit the wide-scale dissemination of these drugs (Sarmah et al., 2006; Chang et al., 2015) rapidly expand the antibiotic load in the environment (Perry et al., 2014; Amos et al., 2015). The effects of antibiotics on ecosystems and biogeochemical processes have thus become a major research topic (Roose-Amsaleg & Laverman, 2016).

Antibiotics are present in most ecosystems (Daghrir & Drogui, 2013), often as mixtures. Several antibiotics (tetracyclines) are widely used as crop protectants (McManus et al., 2002; Chang et al., 2015), but their exact ecological impacts remain insufficiently explored. Antibiotics have different modes of actions to kill or limit growth of bacteria: they can inhibit/regulate metabolism of nucleic acids (Floss & Yu, 2005), protein biosynthesis (Walsh, 2000; Siibak et al., 2011; Wilson, 2014), activities of certain enzymes (Kohanski et al., 2010), or induce toxic metabolic perturbations (Belenky et al., 2015). Antibiotics are most efficient when cells of the microorganisms are multiplying (Stewart & Costerton, 2001), and can particularly affect bacterial symbionts of organisms, with negative consequences on growth and development of juveniles (Kafil et al., 2013).

Insects are likely to be exposed to environmentally released antibiotics, and more particularly tetracyclines. Tetracyclines are highly persistent into the environment, and can reach very high amounts into winter manure, up to 43 and 183 mg kg<sup>-1</sup> for tetracycline and oxytetracycline, respectively (Hu et al., 2010), and up to 200 µg kg<sup>-1</sup> for tetracycline in the first layers of the soil (Hamscher et al., 2002). Surprisingly, most of the studies have focused on the fitness consequences of adding of these drugs to the food of laboratory cultured insects (Büyükgüzel & Kalender, 2008; Ridley et al., 2013; Xu et al., 2016). At low concentrations of ciprofloxacin (3.33–13.33 µg mL<sup>-1</sup>), the development of the fly *Chrysomya putoria* was not altered (Ferraz et al., 2014), while addition of streptomycin to the food at concentrations of 0.03–0.15% increased the growth of the larvae of the butterfly *Spodoptera litura*, without affecting its survival (Thakur et al., 2016). Dickel et al. (2016) reported that long-term exposure to prophylactic treatments (fumagillin) negatively affects the number of eggs laid by females of the wood tiger moth *Parasemia plantaginis*. Interestingly, metabolic perturbations provoked by antibiotics to insects, and their end-target consequences, have remained unexplored.

Bacteria and mitochondria share many similarities in terms of ribosomal machinery (Zhang et al., 2005), and there are growing evidence that antibiotics can affect mitochondrial protein synthesis and mitochondrial functioning in a wide range of non-bacterial taxa (Kalghatgi et al., 2013; Wang et al., 2015). For instance, doxycycline (tetracycline family) has been demonstrated to affect proteostasis in both invertebrate and vertebrate cells (Houtkooper et al., 2013). Ballard & Melvin (2007) demonstrated that tetracycline reduces ATP production in *Drosophila simulans* and increases mtDNA density in insects free of *Wolbachia*. In parallel, several works have demonstrated that rapid metabolic changes occur after antibiotic treatments in bacteria (Belenky et al., 2015; Hoerr et al., 2016; Zampieri et al., 2017), but there are many unknowns regarding the mechanisms of action of antibiotics, and their impacts, at the intra-organismal level in insects. Hence, the use of extracellular footprints and physiological intracellular fingerprints should improve our understanding of the effects of antibiotics on insects.

Juveniles of dipterans are poorly mobile insects which often thrive in microhabitats near the surroundings of human settlements, including agricultural lands and farms. The probability of exposure of fly larvae and pupae to antibiotics is thus very high, and should greatly affect the biochemistry, physiology and morphology of these developing individuals, with potential long-

lasting effects. At the larval stage, wings and flight muscles are absent from the thorax (Paululat et al., 1999; Roy & VijayRaghavan, 1999), and muscle cells appear and progressively arrange and differentiate at the pupal stage (Roy et al., 1997). Also, in a number of fly species, the maturation of flight muscle mitochondria only occurs after the emergence of adults (Marden, 2000), and this process is associated with a high biosynthetic activity of mitochondrial proteins from flight muscles (Lennie et al., 1967; Tribe & Ashhurst, 1972). Because several antibiotics are inhibitor of protein biosynthesis, the exposure of juvenile flies to these drugs should affect the mitochondrial efficiency and mitochondrial DNA density, as shown in *Drosophila* flies (Ballard & Melvin, 2007).

In this work, we investigated the effects of different antibiotics inhibiting (i) cell wall synthesis (ampicillin and cefradine) and (ii) protein synthesis (chloramphenicol, cycloheximide, and tetracycline) (Table S1) on the survival and ultrastructural organization of the flight muscles of newly emerged blow fly, *Chrysomya albiceps*. Having these phenotypic data, we further examined the effects of these drugs on the physiology and metabolism of the individuals. Considering the prominent importance of mitochondria for flight muscles, we examined the effects of different concentrations of antibiotics on mitochondrial protein content, efficiency of oxidative phosphorylation, and activity of transaminases. Among transaminases that are localized in mitochondria, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are the most active in insects (Gäde, 1985). These enzymes are crucial for amino acid metabolism, but the effects of antibiotics on their activity have received little attention. Finally, we were interested in describing the cellular metabolic perturbations of flies treated with antibiotics, and run metabolomics assays to obtain a system-wide picture of biochemical networks altered by antibiotics.

We expected that moderate to high concentrations of protein synthesis inhibitors would be detrimental to the survival of newly emerged adult *Ch. albiceps*, whose mitochondriogenesis and biosynthetic processes should be affected. Chloramphenicol and tetracycline bind on large and small subunits of the mitochondrial ribosomes, and can inhibit mitochondrial protein synthesis and biogenesis in mammals (Brodersen et al., 2000; Houtkooper et al., 2013), while cycloheximide provokes a complete inhibition of cytoplasmic protein synthesis of eukaryotic cells. We assumed that mitochondrial structures and protein amounts would be altered in muscle tissues of flies injected with these three drugs. Ampicillin and cefradine are cell wall synthesis inhibitors, and these bactericidal antibiotics can strongly reduce the activity of the electron transfer chain of mitochondria (Kalghatgi et al., 2013). At low to moderate concentrations, mitochondrial metabolism of adult *Ch. albiceps* treated with ampicillin, cefradine, chloramphenicol, cycloheximide and tetracycline should be reduced, rendering cells' energy deficient (reduced oxidative phosphorylation). Finally, we hypothesized that metabolic alterations would occur after the flies would be injected by the different antibiotics, and that the effects of the metabolic profiles would mirror the mode of actions of these different drugs.

## 2. Materials and methods

### 2.1. Insect rearing

Adults of the hairy maggot blow fly (GenBank common name: taxid:81419) *Chrysomya albiceps* (Wiedemann, 1819) (Diptera: Calliphoridae) were field-collected from Giza, Egypt (30°01'34.4"N 31°12'21.6"E). Colonies were established at the insectary of the Faculty of Science (Cairo University, Giza, Egypt) as described in Queiroz (1996). Adult flies were placed into wooden cages

Download English Version:

<https://daneshyari.com/en/article/8856135>

Download Persian Version:

<https://daneshyari.com/article/8856135>

[Daneshyari.com](https://daneshyari.com)