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Acute toxicity of 8 antidepressants: What are their modes of action?

Laetitia Minguez [a,b,*], Emilie Farcy [a,b,1], Céline Ballandonne [b], Alban Lepailleur [b], Antoine Serpentini [a], Jean-Marc Lebel [a], Ronan Bureau [b], Marie-Pierre Halm-Lemeille [b]

[a] UMR BOREA (Biologie des ORganismes et Ecosystèmes Aquatiques), CNRS-7208/MNHN/UPMC/IRD-207/UCBN, Esplanade de la Paix, 14032 Caen Cedex, France
[b] CERMN, UFR des Sciences Pharmaceutiques, UPRES EA4258 – FR CNRS INC3M – SF 4206 ICORE, Université de Caen Basse-Normandie, Bd Becquerel, 14032 Caen Cedex, France

HIGHLIGHTS

- Different acute toxicities of the 8 antidepressants on *Daphnia magna.*
- Correlation between *in vivo* and *in vitro* toxicity data.
- Manifold modes of action for the antidepressants.
- A narcotic effect associated to a more specific effect on lysosomal membranes.

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ABSTRACT

Currently, the hazard posed by pharmaceutical residues is a major concern of ecotoxicology. Most of the antidepressants belong to a family named the Cationic Amphipathic Drugs known to have specific interactions with cell membranes. The present study assessed the impact of eight antidepressants belonging to selective serotonin reuptake inhibitors or serotonin norepinephrine reuptake inhibitors by the combination of multi-approaches (*in vivo*, *in vitro*, *in silico*) and gives some insights on the mode of action for these molecules. Antidepressants were from the most to the least toxic compound for *Daphnia magna*: Sertraline (EC_{50} = 1.15 mg L^{-1}) > Clomipramine (2.74 mg L^{-1}) > Amitriptyline (4.82 mg L^{-1}) > Fluoxetine (5.91 mg L^{-1}) > Paroxetine (6.24 mg L^{-1}) > Mianserine (7.81 mg L^{-1}) > Citalopram (30.14 mg L^{-1}) and Venlafaxine (141.28 mg L^{-1}). These acute toxicities were found correlated to Log K_{ow} coefficients (R = 0.93, p < 0.001) and to cytotoxicity assessed on abalone hemocytes through the neutral red uptake assay (R = 0.96, p < 0.001). If narcosis as mode of action is typically expected during acute ecotoxicity bioassays, we showed by molecular modeling that particular interactions can exist between antidepressants and phosphatidylcholine, a major component of cell membranes, leading to a more specific mode of action corresponding to a potential acidic hydrolysis of ester functions.

1. Introduction

The pollution by pharmaceutical residues in surface waters represents a recent preoccupation of the scientific community. Over 3000 different substances used in human and veterinary medicines can be found in aquatic environments (Richardson and Ternes, 2005; Fent et al., 2006). The main pathway for environmental contamination is *via* the sewage network that focuses wastewaters through sewage treatment plants (STP). Compounds not well-degraded in the STP are being discharged in treated effluents and thus end up in rivers, streams and marine ecosystems (Fent et al., 2006; Santos et al., 2010). Contrary to pollutants commonly monitored like metals, pesticides or hydrocarbons, pharmaceutical residues are continuously discharged into superficial waters involving for aquatic organisms an exposure during their entire life cycle. Since they are designed to highly interact with biological systems, the examination on their toxicity appeared relevant (Fent et al., 2006).

In the European Union (EU), the environmental risk assessment on pharmaceuticals is based on a stepwise procedure. The first phase aims to estimate the predicted environmental concentration (PEC) in surface water. If the $PEC_{surface\ water}$ is below 10 ng L^{-1} and no other environmental concerns are apparent, it is assumed that the compound is unlikely to represent a risk for the environment. If the $PEC_{surface\ water}$ is equal or above 10 ng L^{-1}, then a second phase should be performed on environmental fate and effect

* Corresponding author at: UMR-BOREA (Biologie des ORganismes et Ecosystèmes Aquatiques), CNRS-7208/MNHN/UPMC/IRD-207/UCBN, Esplanade de la Paix, 14032 Caen Cedex, France. Tel.: +33 (0)231565294; fax: +33 (0)231565346.
E-mail address: laetitia-minguez@hotmail.fr (L. Minguez).
[1] Current address: Laboratoire Ecologie des Systèmes Marins Côtiers (ECOSYM), Université de Montpellier 2, UMR 5119, F-34095 Montpellier, France.

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analysis (Directive 2001/83/EC amended by Directive 2004/27/EC). However, highly potent pharmaceuticals which can have adverse effects at trace concentrations, like hormones, directly enter Phase II irrespective of their PEC values (Christen et al., 2010). Even if most of the pharmaceutical compounds would have PEC values above the action limit, their ecotoxicity and behavior in aquatic ecosystems is still poorly understood.

France is one of the most drug-consuming countries in the world especially for psychotropic drugs (Pelissolo et al., 1996). The gap between France and other countries is especially marked for anxiolytics and hypnotics, but is lower for antidepressants (Briot, 2006). Due to the high number of pharmaceutical compounds used, it is necessary to focus on some classes. Based on the drug uptake data (2009) in Basse-Normandie (France) and on the prioritization methodology of Besse and Garric (2008), we focused on 8 antidepressants, all with PEC values higher 10 ng L^{-1} (Table 1). Antidepressants represent an important drug class associated to the treatment of clinical depression and other mental disorders in Humans. They are classified on the basis of the way they interfere with the serotonergic and norepinephrinic neurotransmitter systems. Two classes are defined: (1) Selective Serotonin Reuptake Inhibitors (SSRIs) and (2) Serotonin–Norepinephrine Reuptake Inhibitors (SNRIs). Most studies about ecotoxicological effects of antidepressants have focused on the SSRI Fluoxetine (e.g. Brooks et al., 2003; review in Nentwing, 2007; Santos et al., 2010) and few have examined the aquatic toxicity of other antidepressants (Minagh et al., 2009; Getz et al., 2011; Fong and Hoy, 2012; Fong and Molnar, 2013). Moreover, their mode of action (MOA) on non-target aquatic organisms is not well enough understood. As highlighted by Kar and Roy (2010), too little studies examine interspecies correlations of pharmaceutical toxicities, whereas these correlations can provide a tool for estimating contaminant sensitivity with known levels of uncertainty for a diversity of species. The present study aimed thus to assess the acute toxicity of 4 SSRIs (Fluoxetine, Sertraline, Paroxetine and Citalopram) and 4 SNRIs (Venlafaxine, Amitriptyline, Clomipramine, Mianserine) on the invertebrate *Daphnia magna*, and to propose a MOA using *in vitro* and *in silico* approaches. The *in vitro* approach was performed on primary cultures of abalone hemocytes (*Haliotis tuberculata*), a cell type considered as useful screening strategy for risk and impact characterization of contaminants (Parolini et al., 2011).

2. Materials and methods

2.1. Chemicals

Antidepressants used for the biotests were Venlafaxine hydrochloride, Citalopram HBr, Sertraline hydrochloride, Paroxetine hydrochloride, Amitriptyline hydrochloride, Clomipramine hydrochloride, Mianserine hydrochloride, and Fluoxetine hydrochloride; all supplied in analytical grade by Kemprotec Limited (Maltby, Middlsbrough, U.K).

Table 1
Predicted Environmental Concentrations (PEC) of 8 antidepressants estimated for the Basse Normandie Region (France) in 2009. PEC (ng L^{-1}) = (Total quantity consumed (mg))/(365 × *P* × V × D))*10^6 with *P*: number of inhabitants in Basse Normandie (i.e. 1450000); V: volume of waste water per capita and day (200 L); D: factor of dilution of waste water by surface water flow (i.e. 10) (from Besse and Garric, 2008). SNRI: Serotonin-Norepinephrine Reuptake Inhibitor, SSRI: Selective Serotonin Reuptake Inhibitor.

Drugs	Class	PEC values (ng L^{-1})
Venlafaxine	SNRI	187
Citalopram	SSRI	69
Sertraline	SSRI	66
Paroxetine	SSRI	62
Amitriptyline	SNRI	49
Clomipramine	SNRI	48
Mianserine	SNRI	35
Fluoxetine	SSRI	33

2.2. Daphnia acute immobilization test

Daphnia tests were conducted following the guideline NF EN ISO 6341 (1996) using the water flea *D. magna* Straus (Cladocera, Crustacea). Daphnids were bred in Elendt medium M4, pH 7.4. Experiments were run at temperatures of 20 ± 1 °C and a photoperiod of LD 16:8 in ISO medium. Twenty daphnids younger than 24 h were used for the controls and each treatment subdivided in five replicates each containing five daphnids. Culture volume was 10 mL. Immobility was observed after 24 and 48 h with the latter being the endpoint for effect calculation. A reference test with potassium dichromate ($K_2Cr_2O_7$) was performed to test the sensitivity of the daphnids. The result of this reference test was in accordance with NF EN ISO 6341 (1996) (i.e. 24 h EC_{50}-value = 1.19 ± 0.27 mg L^{-1}).

2.3. Toxicity assessment on primary cultures of Haliotis tuberculata hemocytes

Adult abalones with shell length between 9 and 11 cm were sampled by Ormasub® on the Northern Cotentin peninsula (France). Organisms were maintained in natural and continuously aerated seawater at 17 °C and fed with a mixed algal diet (*Laminaria* sp. and *Palmaria* sp.) at the Centre de Recherche en Environnement Côtier (C.R.E.C., Luc-sur-Mer, Basse-Normandie, France). Prior to their use in our study, abalones were acclimated for at least 2 weeks.

Hemocytes were cultured as previously described by Lebel et al. (1996). Briefly, after a medio–lateral incision in the abalone foot, the hemolymph was withdrawn using a 20 mL syringe with a 25G needle. Hemolymph was transferred into a sterile tube and diluted 1:4 in cooled sterile anticoagulant modified Alsever's solution (115 mM glucose; 27 mM sodium citrate; 11.5 mM EDTA; 382 mM NaCl; pH 7.5) (Bachère et al., 1988). Cell cultures were first made in artificial sterile seawater (ASSW) (pH 7.4), to allow cells to adhere onto the bottom of the culture well. Then, the ASSW was replaced by Hank's sterile 199 medium modified by the addition of 250 mM NaCl, 10 mM KCl, 25 mM $MgSO_4$, 2.5 mM $CaCl_2$ and 10 mM Hepes (final pH 7.4), and supplemented with 2 mM L-glutamine, 100 μg mL^{-1} streptomycin, 60 μg mL^{-1} penicillin G and 2 mM concanavalin A. Cell cultures were incubated at 17 °C overnight. The culture medium was then replaced by the different antidepressant solutions. Cells were exposed during 48 h. Each concentration was tested in quadruplicate (i.e. four wells per concentration). The medium was changed every day. The cell exposure was repeated at least thrice, i.e. using at least three abalones (experiment replicates).

2.3.1. MTT assay

Cellular viability was estimated using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay, which is a sensitive and quantitative colorimetric assay measuring the capacity of mitochondrial succinyl dehydrogenase in living cells to convert a yellow substrate (MTT) into a dark blue formazan product (Mosmann, 1983). This test was adapted to molluscan cell cultures by Domart-Coulon et al. (2000). Briefly, 10% (v/v) of the MTT stock solution (5 mg MTT mL^{-1} PBS 1X pH 7.4) was added to the culture dishes. After 24 h incubation at 17 °C, an equal volume of isopropanol containing 0.04 N HCl was added to each culture to dissolve the converted formazan. The absorbance was measured at a wavelength of 570 nm with a 630 nm reference.

2.3.2. Lysosomal membrane stability (LMS)

After treatments, the LMS was assessed using neutral red uptake assay (NRU) following the method employed by Coles et al. (1995) and adapted to microplate cultures. Briefly, 10% (v/v) of the neutral red stock solution (0.5% neutral red in PBS 1X) was added to each well. After 1 h incubation at 17 °C the medium was removed, and wells were washed first with 3% formaldehyde in ASSW to fix cells and then twice with PBS 1X. Neutral red was extracted from lysosomes using 1% glacial acetic acid in 50% ethanol. After 30 min at room temperature, the plates were transferred to a TECAN Infinite M200 microplate reader, and the absorbance was measured at a wavelength of 540 nm with a 650 nm reference.

2.4. Molecular modeling

2.4.1. Initial conformation of phosphatidylcholine (PC)

The Cambridge Structural Database (CSD) was searched to retrieve information on the 3D structure of PC (Chetina and Howard, 2003). The closest structure of PC with available 3D coordinates corresponds to 1-octadecyl-2-methyl-*sn*-glycero-3-phosphocholine (Pascher et al., 1986). From this conformation, the replacement of ether by ester functions led to a first conformation of PC.

2.4.2. Initial conformation of Paroxetine–PC complex

The initial conformation of Paroxetine was extracted from the CSD (Chetina and Howard, 2003). PC and Paroxetine were assembled in a complex by manually orientating the amine function of Paroxetine towards the ester function of PC and the two aromatic groups of Paroxetine towards the ammonium of PC.

2.4.3. Initial conformation of Citalopram–PC complex

The initial conformation of Citalopram was extracted from the CSD (Harrison et al., 2007). From the optimized Paroxetine–PC complex, Citalopram was fitted on the Paroxetine coordinates to obtain the Citalopram–PC complex.

2.4.4. Optimization of the complexes

A rapid optimization was carried out by molecular mechanics modeling (Discovery Studio v3.5 software) with the steepest descent method by considering a CHARMm force field. Further optimization were performed by Density Functional Theory (DFT) calculations, using the local density approximation (LDA) (Kohn and Sham, 1965) with the parameterization of Perdew and Wang (1992) (PWC), and the double numerical (DN) basis set. Effective core potentials were applied corresponding to replace the inner (core) electrons of atomic and molecular systems by an effective potential. In this case, only the valence electrons are treated explicitly in quantum mechanical calculations. A gradient of 0.004 Ha $Å^{-1}$ for the maximum force on atoms was defined as convergence criterion.

2.5. Statistical analyses

To assess the EC_{50} for the different ecotoxicological tests, non-linear regressions using the Hill equation were obtained using the Excel® macro REGTOX (Vindimian, 2012). The interspecies correlations of ecotoxicity of the different antidepressants were also studied by Spearman rank tests. For these tests, EC_{50}-values (mg L^{-1}) were converted to molar basis and then the log-transformed data were used as variables. Two variables were then considered correlated when $p < 0.05$.

3. Results

The acute toxicity was assessed for 8 antidepressants on *D. magna* (Table 2). The toxicity of the tested antidepressants ranged from 1.15 mg L^{-1} (Sertraline) to 141.28 mg L^{-1} (Venlafaxine). Most EC_{50}s were in the range of toxic substances (i.e. EC_{50} between 1 and 10 mg L^{-1}). Citalopram was the only antidepressant classed as harmful substance (i.e. 30.14 mg L^{-1}). Venlafaxine appeared not dangerous to aquatic organisms with an EC_{50} above 100 mg L^{-1}.

Fig. 1 shows the correlation of acute toxicities on *D. magna* with octanol–water partition coefficients of antidepressants (Log K_{ow}). The Log K_{ow} coefficients were obtained from Drugbank database. In the correlation analysis, Paroxetine differed from other antidepressants. By removing this compound, EC_{50}-values in *D. magna* were significantly correlated to Log K_{ow} coefficients ($R = 0.93$, $p < 0.001$).

In order to evaluate whether cytotoxicity may be indicative for *in vivo* acute toxicity, the cytotoxicity in abalone hemocytes was compared to data on *D. magna* (Fig. 2). The acute *Daphnia* toxicity and the *in vitro* to toxicity assessed by MTT assay showed a correlation which tended to be significant ($R = 0.71$, $p = 0.0506$) (Fig. 2A). On the contrary, a strong correlation was found between the acute

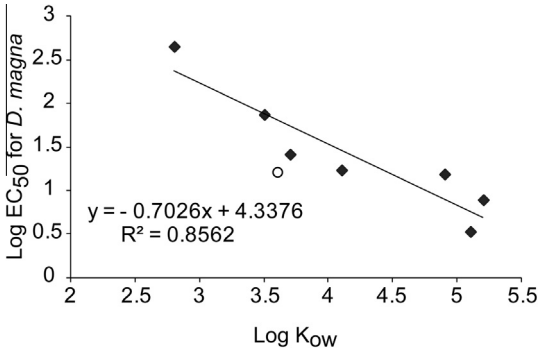

Fig. 1. Correlation between Log EC_{50} on *Daphnia magna* and Log K_{ow}. Paroxetine (O) was not included in the analysis. Log K_{ow} were obtained from Drugbank database and Chemaxon (only for Mianserine).

Table 2
Antidepressant toxicity data on *Daphnia magna* (Immobilization test, 48 h-exposure). Results are given as Effective Concentrations affecting 50% of test organisms (EC_{50}) with 95%-confidence intervals into brackets. The octanol–water partition coefficients Log K_{ow} were obtained from Drugbank database (experimental data) except for Mianserine obtained from Chemaxon (predicted value).

SSRIs/SNRIs	Acute toxicity on *Daphnia magna* (48 h-exposure)		Log K_{ow}
	EC_{50} (mg L^{-1})	EC_{50} (μM)	
Venlafaxine	141.28 [116.05–170.15]	450.13 [369.74–542.12]	2.8
Citalopram	30.14 [21.19–32.78]	74.36 [52.28–80.88]	3.5
Sertraline	1.15 [0.95–1.28]	3.36 [2.77–3.74]	5.1
Paroxetine	6.24 [4.29–6.77]	16.26 [11.17–17.64]	3.6
Amitriptyline	4.82 [3.43–5.13]	15.36 [10.93–16.34]	4.9
Clomipramine	2.74 [1.79–2.91]	7.80 [5.10–8.28]	5.2
Mianserine	7.81 [7.54–7.95]	25.96 [25.06–26.43]	3.8
Fluoxetine	5.91 [4.42–6.50]	17.09 [12.78–18.80]	4.1

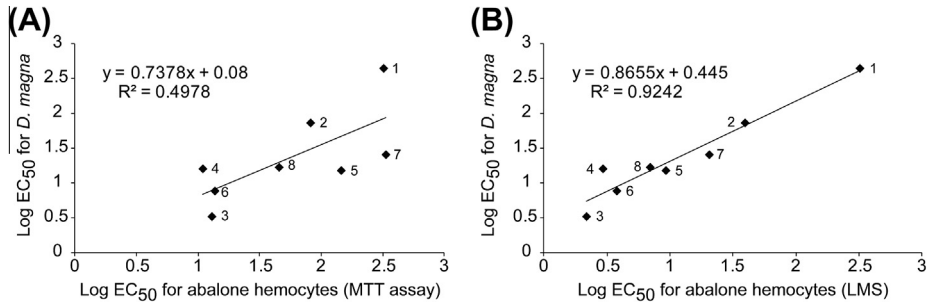

Fig. 2. Comparison of *in vivo* data on *Daphnia magna* with *in vitro* abalone hemocytes toxicity (*Haliotis tuberculata*). (A) Correlation of acute toxicity in *D. magna* and cytotoxicity on abalone hemocytes (MTT assay). (B) Correlation of acute toxicity in *D. magna* and lysosomal membrane destabilization assessed on abalone hemocytes (LMS). Numbers refer to the following antidepressants: 1. Venlafaxine; 2. Citalopram; 3. Sertraline; 4. Paroxetine; 5. Amitriptyline; 6. Clomipramine; 7. Mianserine and 8. Fluoxetine.

Daphnia toxicity and the *in vitro* toxicity assessed on LMS ($R = 0.96$, $p < 0.001$) (Fig. 2B).

In order to explain these toxicities and specific behavior of Paroxetine, we studied the potential intermolecular interactions with one of main molecular component of membranes, the phosphatidylcholine (PC). The first minimization of the Paroxetine–PC complex using the steepest descent method led to the following results (Fig. 3A): (i) distances around 5 Å were observed between the two aromatic rings of Paroxetine and the ammonium group of PC, (ii) a hydrogen bond was formed between the basic center of Paroxetine and the carbonyl group of PC (1.85 Å for the distance between NH. . .O), and (iii) the conformations for Paroxetine and PC in the complex were closed to the initial conformation, except a different torsion of the dihedral angle between the parafluoro phenyl ring and the piperidine of Paroxetine (139° *vs* 90°). The optimization of the complex using a DFT calculation led to a shorter distance between the basic center of Paroxetine and the carbonyl group of PC (1.5 Å) and distances of 4.2 and 5.2 Å between the ammonium group and the two aromatic rings. Concerning the Citalopram–PC complex, higher distances were observed between the basic center of Citalopram and the carbonyl group of PC (1.59 Å), and between the two aromatic rings of Citalopram and the ammonium of PC (5.35 Å and 5.6 Å) (Fig. 3B). Moreover, the force of the interaction between antidepressants and PC can also be defined by the angle between the two aromatic rings of the antidepressant, 62° and 110° for Paroxetine and Citalopram respectively (Fig. 3C and D).

4. Discussion

The pharmaceutical MOA is evaluated for the treatment of human pathologies. However, desired pharmacological properties in Humans might possibly provide an undesired adverse effect on non-target species (Minagh et al., 2009). Antidepressants raise

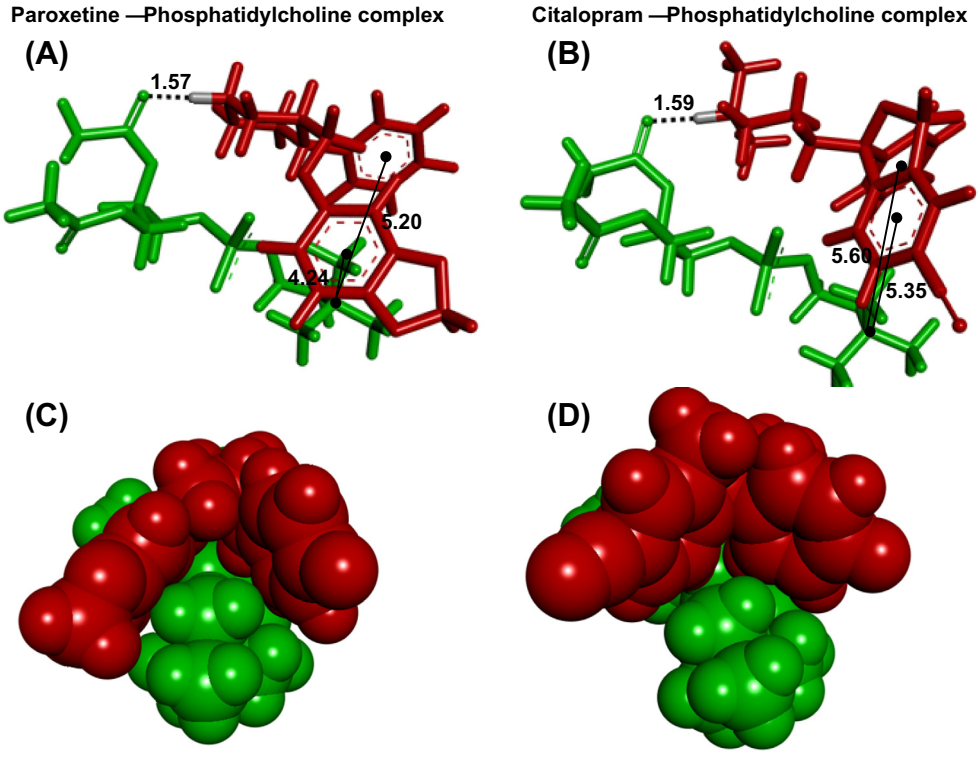

Fig. 3. Molecular modeling of two antidepressant–phosphatidylcholine complexes. (A, B) 3D-representations of the complexes between phosphatidylcholine (PC, green) and (A) Paroxetine or (B) Citalopram (red). The hydrogen bonds between the amine and the ester function are represented by black dotted lines. Distances between the two aromatic rings of the antidepressants and the ammonium group of PC (cation-π interactions) and between the amine and the ester function are indicated. (C, D) 3D-representation of the complex between PC (green) and (C) Paroxetine or (D) Citalopram (red). This representation illustrated the importance of a correct angle between the two aromatic rings (around 70°) to optimize the intermolecular interactions with the ammonium group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

environmental concern since they are now detected in aquatic environments (reviewed in Santos et al., 2010). Numerous studies have assessed the SSRI toxicity on non-target organisms like algae and crustaceans (Brooks et al., 2003; Christensen et al., 2007; Minagh et al., 2009; reviewed in Santos et al., 2010). The reported EC_{50} with *D. magna* acute toxicity tests are quite different from one study to another but the SSRI toxicity range is the same. The acute EC_{50} assessed in the present study on *D. magna* were of the same order of magnitude than those obtained by Christensen et al. (2007). Our results followed the general toxicity pattern with Sertraline being the most toxic SSRI and Citalopram the least toxic SSRI (Henry et al., 2004). SNRI effects on non-target organisms are less documented. Our study provided new insights on the acute toxicity of this antidepressant class on *D. magna*. Acute toxicities have been found to be correlated to Log K_{ow} coefficients, translating an action partly related to the hydrophobic properties of compounds. A non-specific toxicity or narcosis would thus explain the observed toxicities. Paroxetine exhibited a clearly lower EC_{50}-value than expected from their Log K_{ow}-value. Indeed, Paroxetine has the same log K_{ow}-value than Citalopram but a higher toxicity whereas it has a 1.5 logarithmic unit difference with Sertraline for the Log K_{ow}-value but the same toxicity. A more specific mode of toxic action probably occurred for this compound. *In vivo* toxicity tests are generally not designed to determine the specific MOAs of toxic compounds (Fent et al., 2006). Other approaches like *in vitro* assays would allow the understanding of mechanisms involved in the cellular response to pollutants. However, to assess the acute toxicity of pharmaceuticals, *in vitro* approaches are rarely considered (Parolini et al., 2011). In the present study, we used another invertebrate, the abalone *H. tuberculata* to assess the cytotoxicity of antidepressants. Abalones are considered sensitive indicator species (Lin and Liao, 1999; Gorski and Nugegoda, 2006) and one abalone can provide up to 20 mL of hemolymph allowing the number of test organisms to be considerably reduced. Herein, cytotoxicity of 8 antidepressants has been assessed in abalone hemocytes by applying the MTT and LMS assays. The most toxic compound was Sertraline exhibiting EC_{50}-values of 4.40 ± 1.89 mg L^{-1} (MTT) and 0.74 ± 0.11 mg L^{-1} (LMS) and the least toxic compound was Venlafaxine with EC_{50}-values above 100 mg L^{-1} for both toxicity tests. Despite the slightly different cytotoxic endpoints of the two assays (functional integrity of mitochondria and stability of lysosomal membrane, respectively), the effects measured in the MTT as well as in LMS indicate membrane damages. The two membranes of mitochondria, each composed by a phospholipid bilayer, could explain the lower values obtained with the MTT assay compared with lysosomes which are single-membrane organelles. Moreover, the inner mitochondrial membrane is known to be a chemical barrier (i.e. lower permeability) (Krauss, 2001). Besides this non-specific binding to membrane phospholipids, antidepressants as basic lipophilic compounds are trapped by acidic subcellular compartments like lysosomes (Daniel and Wójcikowski, 1997). This mechanism is called lysosomotropism (Lemieux et al., 2004). To be lysosomotropic, compounds should possess a Log $P > 2$ and a basic pKa of between 6.5 and 11 (Nadanaciva et al., 2011). Despite their structural differences, most antidepressants share this physicochemical property.

Our *in vivo* data on *Daphnia* were found to correlate to the log K_{ow}, except for Paroxetine. This correlation is often linked with a non specific toxicity (narcosis) due to alteration processes mainly on cell membranes. Nevertheless, the *in vivo* data on *Daphnia* were also correlated with *in vitro* data on abalone hemocytes (LMS), suggesting a same toxicity mechanism *in vivo* and *in vitro*. Most of the antidepressants belong to a family named the Cationic Amphiphilic Drugs (CAD). These CAD have specific interactions with cell membranes (Sheetz and Singer, 1974; Lemieux et al., 2004). Molecular modeling allows the interactions between pharmaceuticals and their molecular targets to be understood. Since the eukaryotic cell membrane is mainly composed of glycerophospholipids such as PC, the theoretical interactions of SSRIs with PC group were analyzed. One of the main characteristic of PC is the presence of a trimethylammonium moiety. In several intermolecular interactions observed in proteins, the ammonium group is able to develop specific binding through cation-π interactions where the ammonium moiety is the center of a box with the faces of 2–4 aromatic residues located within, 4–5 Å of the amine (Cheng et al., 2013). Selectivity of SSRIs is tightly related to specific 3D characteristics. In Humans, their pharmacophore corresponds to two aromatic groups, a basic center and a hydrophobic group directly connected to one of the aromatic ring. Differences between the conformations of these derivatives translate differences in terms of affinities (Bureau et al., 2002). For our structures, aromatic rings could be involved in cation-π interactions with the ammonium group of PC. The study of Paroxetine–PC complex showed short distances (4.2 and 5.2 Å) between the two aromatic rings (Paroxetine) and the ammonium group (PC). These values are in agreement with distances recorded for cation-π interactions. Moreover, for cation-π interactions, the optimum angle between two aromatic rings associated to this interaction must be around 70°. For Paroxetine in the complex with PC, the angle between the two aromatic rings is 61.9°. So, all the optimum characteristics were obtained for the complex between Paroxetine and PC by considering cation-π interactions (Liu et al., 2004; Cheng et al., 2008) and the hydrogen bond between the amine group (Paroxetine) and the ester function (PC). The degradative transport of CAD across phospholipid bilayers has been recently characterized (Baciu et al., 2006). For the mechanism, the protonated groups on CAD catalyze the acid hydrolysis of the ester function of PC producing a fatty acid and a single-chain lipid which destabilizes the membrane. Toxicity differences between antidepressants on lysosomal membranes should be related to the capacity of the derivatives to catalyze or not the acidic hydrolysis of the ester function of PC through optimum cation-π interactions. The distance between the protonated amine group of Paroxetine and the ester function is in agreement with a catalytic action of Paroxetine. Moreover, this catalytic action is reinforced by a strong stabilization of the complex. In contrast, Venlafaxine with only one aromatic ring is unable to form a stable cation-π interaction with the ammonium group (choline) which would explain its non-toxicity compared to other tested CADs. The analysis of the complex associated to Citalopram showed that the distances for cation-π interactions are higher than the reference for this kind of interaction. Moreover, the angle between the two aromatic rings is 110.5°, far from the optimum value of 70°. So, these geometrical values translate a lower interaction between PC and Citalopram. From these data and by generalizing to CADs, cation-π interactions associated to at least two aromatic rings and hydrogen bond represent the main interaction to explain the catalytic hydrolysis activity of this drug on membrane and particularly on PC.

5. Conclusion

The objective of our study was to establish more comprehensive data on the acute ecotoxicity of antidepressants on non-target aquatic organisms. We highlighted relevant ecotoxicological data for eight antidepressant drugs. Nevertheless, the drug concentrations leading to significant acute toxic effects were at several orders of magnitude higher than actual environmental concentrations (Fent et al., 2006). However, adverse effects may result from lower exposure concentrations into chronic toxicity and the putative hazardous effects of pharmaceutical mixture cannot be excluded. By *in vitro* and *in silico* approaches, our study suggested,

for the first time, some mechanistic hypothesis about the difference observed in the toxicity of antidepressants. A narcotic MOA is expected for *D. magna* but it is likely that the mechanism of toxicity goes beyond the simple narcosis (basal toxicity). Indeed narcosis results from a general and reversible disruption of cell membrane functioning (van Wezel and Opperhuizen, 1995). However, the observed effects on lysosomal membranes were inconsistent with the definition of narcosis as a reversible membrane disruption, since the alteration of the lysosomal membrane can lead to the release of lysosomal contents to the cytoplasm and to the irreversible cell lysis. This alteration would involve a more specific phenomenon related to molecular interaction between CAD and membrane phospholipids. Recent data concerning lysosomotropism and phospholipidosis properties of CADs are in agreement with complementary structures of the two partners (i.e. CAD and PC).

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