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First eco-toxicological evidence of ivabradine effect on the marine bacterium *Vibrio fischeri*: A chiral view

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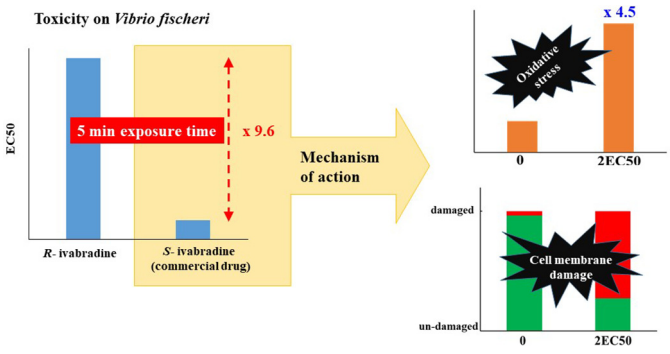
HIGHLIGHTS

- Eco-toxicological effects of ivabradine were investigated in marine bacteria
- Realistic (not nominal) concentrations were employed for EC_{50} calculation
- Enantioselective bioluminescence inhibition was observed
- *S*-ivabradine was more toxic than the *R*-isomer to marine bacteria
- Oxidative stress causing enzyme and cell membrane damage was the toxicity mechanism

GRAPHICAL ABSTRACT



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ABSTRACT

Ivabradine (*S*-ivabradine) is a contemporary antihypertensive drug designed and commercialized for cardiovascular diseases treatment over the world. In this work the enantiomer-specific stability and acute toxicity of ivabradine to the marine bacterium *Vibrio fischeri* as well as the potential mechanism of action were investigated for the first time. With this aim, real concentrations of ivabradine enantiomers under abiotic and biotic conditions were determined by Capillary Electrophoresis (CE) with cyclodextrins (CDs) as chiral selectors. A moderate chiral stability without enantiomeric interconversion was observed for ivabradine. The bioluminescence inhibition method revealed an enantioselective toxicity of ivabradine to marine bacterium. The order of ecotoxicity was R-ivabradine < racemic ivabradine < S-ivabradine with EC50 (t = 5 min) values about 75.98, 11.11 and 7.93 mg/L, respectively. Confocal Live/Dead stained images showed that bacterial envelops cells were seriously damaged after exposure to *S*-ivabradine. *S*-ivabradine also disturbed the esterase activity and significantly increased the ROS level compared with the control. Thus, oxidative stress originating membrane cells damage and enzymatic activity changes was shown to be the primary mechanism of *S*-ivabradine toxicity to marine bacterium. Our results highlight the need for more eco-toxicological evaluations of the cardiovascular drug *S*-ivabradine on other aquatic organisms to establish the risk on the environment.

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1. Introduction

Pharmaceutical pollution represents one of the major issues that society has to face in this century at both economic and social levels (Branchet et al., 2021; Minguez et al., 2016; Chaturvedi et al., 2021; Casado et al., 2019). The elevated consumption, high and "pseudo"-persistence, insufficient removal from traditional wastewater treatment plants or direct discharge of pharmaceuticals lead to the water bodies contamination (Chaturvedi et al., 2021; Saari et al., 2017). Antihypertensive drugs, which include beta-blockers (β-blockers), calcium channel blockers (CCBs), angiotensin-converting-enzyme (ACE) inhibitors, the angiotensin II receptor antagonists (sartans), belong to one of the most frequent therapeutic categories detected in the aquatic environment as a consequence of both the increase and (over)aging effect of population (Branchet et al., 2021; Saari et al., 2017; Godoy et al., 2015). At the same time, such detection reflects the potential threats to aquatic ecosystems (Branchet et al., 2021; Saari et al., 2017; Godoy et al., 2015). Ecotoxicity data on non-target aquatic organisms regarding antihypertensive drugs show that they can pose a potential long-term risk for both fresh and marine water species (Saari et al., 2017; Godoy et al., 2015).

In spite of the fact that many of these drugs are chiral and give rise to two or more stereoisomers, literature on eco-toxicological effects of chiral pharmaceuticals at enantiomeric level are rare. The classic framework for the ecological risk assessment of chiral substances is based on the threshold effects determined for the racemic formulation and employing nominal concentrations, revealing only superficial phenomenon (Wen et al., 2020). For this reason, eco-toxicity evaluation employing enantiomers of chiral contaminants expresses a more complete scientific perspective (Wen et al., 2020). In addition, the determination of the stability and real concentration of chiral compounds at enantiomeric level is fundamental in environmental risk assessment considering that one of the enantiomers may be active from a biological point of view, although the other(s) may be inactive, have a different biological activity or even be more toxic leading to synergism in the racemic mixture (Saz and Marina, 2016).

Eco-toxicity evaluation at enantiomeric level requires the development of chiral analytical methodologies enabling the individual determination of enantiomers of chiral pollutants. Among the analytical techniques able to determine enantiomers, Capillary electrophoresis (CE) is a very attractive option due to its simplicity, great enantioresolution power, high separation efficiency and versatility derived from the use of chiral selectors in the background electrolyte. Moreover, CE needs small sample volume and low reagent consumption, thus it is considered an environmentally friendly technique (Bernardo-Bermejo et al., 2020).

Ivabradine is a unique antiarrhythmic compound that promotes only HCN_4 channel inhibitor (Mazzola, 2015; Osei et al., 2020). Ivabradine was first authorized by the European Medicines Agency (EMA) in 2005 for stable angina treatment and since 2015, it was approved by the Food and Drug Administration (FDA). It is extensively used in the contemporary medical therapy for heart failure in cardiovascular treatment (including angina pectoris, arterial hypertension, and supraventricular arrhythmias) of patients with a contraindication to β-blockers or in combination with a β-blocker as well as a CCB when the disease is not controlled by the β-blockers or CCB alone (Osei et al., 2020; Zachariah et al., 2017; Roth et al., 2017). To our knowledge, ivabradine enantiomers have only been separated in two works by employing CE (Casado et al., 2019) or High Performance Liquid Chromatographic (HPLC) (Ferencz et al., 2020). Ivabradine enantiomers were separated by CE in 6 min using sulfated-γ-CD as chiral selector (Casado et al., 2019). A cellulose tris(3-chloro-4-methylphenylcarbamate) column and 0.06% (v/v) diethylamine in methanol/acetonitrile 98/2 (v/v) as mobile phase were used in the HPLC method obtaining the chiral separation in 20 min (Ferencz et al., 2020). Both methods were applied to the analysis of enantiomerically pure pharmaceutical formulations.

As the use of ivabradine is expanded worldwide, cases of environmental issues related to this drug are expected to emerge. Ivabradine is commercialized as a pure soluble *S*-enantiomer under different brand names, with no *in vivo* conversion (European Medicines Agency EMEA, 2005). The existing data related to environmental stability and impacts of ivabradine were determined on standard soil compartment or sewage treatment plants, and were performed only before commercialization approval by EMA (European Medicines Agency EMEA, 2005). On the other hand, the environmental risk assessment for the aquatic compartment could not be completed at that time due to missing data, being imposed post-marketing follow-up evaluations in order to address ivabradine environmental contamination issue. Besides this context and the fact that ivabradine has been on the market for longer than a decade in Europe and over the world, to our knowledge there are no published studies focused on ivabradine stability, eco-toxicological effects, and mechanistic framework for aquatic environments.

The aim of this work was to assess the eco-toxicity of the contemporary cardiovascular ivabradine drug on aquatic organisms through a systematic evaluation of eco-toxicological profiles of individual *S*- and *R*-enantiomers as well as the racemic mixture (*Rac*-ivabradine). Toxic effects were determined on marine bacterium *V. fischeri* by acute bioluminescence inhibition assay. Chiral stability under abiotic and biotic conditions and consequently real concentrations must be determined, and for this purpose, CE with CDs as chiral selectors was used. To establish the mechanistic pathways, this work aimed to examine the oxidative stress, membrane cells damage, viability, and enzymatic activity. To the best of our knowledge, this is the first work reporting the effects of ivabradine at enantiomeric and racemic levels on aquatic organisms. The results of this study will contribute to gain knowledge regarding eco-toxicological risk of ivabradine on aquatic organisms.

2. Materials and methods

2.1. Chemicals and reagents

Methanol was acquired from Merck (Darmstadt, Germany). Sulfated-γ-CD (DS ≈ 14, MW 2726.20 g mol^{-1}) was obtained from Cyclolab (Budapest, Hungary). Formic acid, sodium hydroxide, *S*-ivabradine, fluorescein diacetate (FDA) and 2′,7′-dichlorofluorescin diacetate (H_2DCFH-DA) were procured from Sigma-Aldrich (St. Louis, MO, USA). *R*-ivabradine was obtained from Toronto Research Chemicals Canada (North York, ON, Canada). Live/dead BacLight bacterial viability dye was acquired from Invitrogen (Thermo-Fisher, Waltham, MA, USA). Freeze-dried marine bacterium *V. fischeri* (NRRL-B 11177), culture medium, and standard reagents were supplied by MicroBio Tests Inc. (Ghent, Belgium). The ultrapure water (Millipore Milli-Q-System, Bedford, MA, USA) was used.

2.2. Bioassays

Bioluminescence inhibition of *V. fischeri* (VFBI) was established by using the commercial BioTox™ kit. This is a well-established and extensively employed test for marine environments (Abbas et al., 2018). In brief, the targeted pollutant produces changes in the metabolism and/or structure of marine bacteria cells resulting in a reduction of their natural bioluminescence. The inhibitory effect due to the substance is calculated in contrast to the bacterial response generated by a saline control solution, which mimics natural decrease in light. Subsequently, the substance's concentrations that induce 50% inhibition of bioluminescence (EC_{50}, in mg/L) can be established.

The toxicity measurements were performed in 96-well microplates using Luminoscan Ascent luminometer (Thermo) as described below. Stock solutions of individual enantiomers, *R*-ivabradine and *S*-ivabradine (2000 mg/L in methanol) were prepared and used immediately. Working solutions for each individual enantiomer and the racemate (50:50 enantiomeric ratio) of 0.78, 1.5, 3.75, 6.25, 12.5, 25, 50, 100, and 200 mg/L were prepared by diluting the stock solutions with saline water (20% sodium chloride, NaCl, w/v, pH 7.0 ± 0.2) in order maintain the allowed percentage of 2% salinity. Working dilutions, control solution (2% NaCl, w/v), and microplates were temperate at 15 °C before exposure test. Prior to testing,

freeze-dried bacterium *V. fischeri* was reactivated in 2% NaCl solution as indicated in BioTox™ kit instructions. The microplate containing 100 μL of sample aliquots (previously prepared working dilutions of each individual enantiomer or racemate) and 100 μL of the reactivated bacterial inoculum per well was incubated in the luminometer, at 15 °C, during 60 min. The luminescence was recorded at each 1 min exposure time point. Final concentrations tested of *S*-ivabradine, *R*-ivabradine and *Rac*-ivabradine (considering dilution with bacterial inoculum) were 0.39, 0.78, 1.5, 3.75, 6.25, 12.5, 25, 50, and 100 mg/L. The bioluminescence inhibition produced along the tested range of concentrations was calculated as percentage of control's luminescence level. The concentrations of *R*-ivabradine, *S*-ivabradine and *Rac*-ivabradine that induced 50% inhibition of bioluminescence (EC_{50}, in mg/L) were subsequently calculated with the median-effect model (Chou and Talalay, 1984) implemented on CompuSyn Software (ComboSyn, Inc., Paramus, NJ, USA) (Chou and Martin, 2005).

Biochemical endpoints including esterase enzyme, oxidative stress and membrane cell integrity were investigated in order to establish the toxicity pathways of ivabradine. The esterase activity of bacterial cells was checked using FDA. 195 μL ivabradine treated and un-treated (control) bacterial sample aliquots were incubated with 5 μL dye (0.02 FDA% w/w in DMSO) in 96-black well microplate in a fluorescence reader (Fluoroskan Ascent FL, Thermo Fisher Scientific, Waldham, MA, USA), at room temperature. Fluorescence signal was recorded at $\lambda_{ex/em}$ of 485–528 nm every 5 min during 30 min. Intracellular oxidative stress was investigated using specific reactive oxygen species (ROS) staining H_2DCFDA. Fluorescence ($\lambda_{ex/em}$ = 485–528 nm) measurements of black 96-well plate with 50 μL of 10 mM H_2DCFDA and 150 μL ivabradine treated and un-treated (control) bacterial sample aliquots per well were recorded at each 5 min during 25 min of incubation at 25 °C using the same Fluoroskan Ascent FL Fluorometer. All readings were performed by triplicates, and the ivabradine treated samples were compared with the control. Cell membrane damage was determined through Live/Dead BacLight Bacterial Viability staining and laser confocal microscopy (CLSM, Leica TCS-SP5, Wetzlar, Germany). 10 μL of SYTO9/propidium iodide (PI) mixture in DMSO (1:1:10 v:v ratio) were incubated with the ivabradine treated and un-treated (control) bacterial samples, as indicated by the manufacturer. Viable cells were marked in green by SYTO9 ($\lambda_{ex/em}$ = 488–499/540 nm), while membrane-injured cells were marked in red by PI ($\lambda_{ex/em}$ = 561–577/645 nm). The cells number distribution was quantified with Image J program (National Institute of Health, Rasband, WS, USA). All results were provided as means values and standard deviation (±SD).

2.3. Chiral stability

To study the possible inter-conversion and stability of ivabradine enantiomers, individually and in the racemic mixture, 20 mg/L of the individual enantiomers *S*-ivabradine and *R*-ivabradine as well as 40 mg/L *Rac*-ivabradine solutions were incubated under both biotic (with bacteria) and abiotic (without bacteria) conditions. Incubation was carried out in 2% NaCl solution (pH 7.0) and at a temperature of 15 °C as in the standard biossays previously described.

The concentrations of *S*-ivabradine and *R*-ivabradine as well as *Rac*-ivabradine were measured at the starting (0 min) and at the end of the exposure times 5, 10, 15, and 60 min by chiral CE. The runs were performed by triplicate.

2.4. Chiral analysis

CE measurements were performed with an Agilent 7100 CE system (Agilent Technologies, Waldbronn, Germany), equipped with HP^{3D} CE ChemStation software by employing an uncoated fused silica capillary of 50 μm I.D. (375 μm O.D.) with a total length of 58.5 cm (50 cm effective length) (Polymicro Technologies, Phoenix, AZ, USA). The separation and determination of ivabradine enantiomers in *V. fischeri* culture media were achieved using the chiral method described by Casado et al. (50 mM formate buffer (pH 2.0) − 4 mM of sulfated-γ-CD, temperature 25 °C, voltage −30 kV and hydrodynamic injection of 50 mbar for 5 s) with 6 min of analysis time and 2.7 resolution (Casado et al., 2019).

2.5. Statistical analysis

Tukey's HSD (honestly significant difference) post-hoc analysis was completed using IBM SPSS Statistics software. Statistically significant differences were recognized at p-value <0.05.

A scheme of the workflow followed in this work is included in the Supplementary Information (Fig. S1).

3. Results and discussion

3.1. Chiral stability profile

The stability of ivabradine's individual enantiomers and racemic mixture (*Rac*-ivabradine) was investigated under both abiotic (in absence of bacteria) and biotic (in presence of bacteria) conditions at 5, 10, 15, and 60 min of exposure time, using CE with CDs as chiral selectors. Good performance was verified for the analytical chiral method with adequate precision, absence of matrix interferences, recovery values >96% and LODs values of 0.11 mg/L for both *R*- and *S*-enantiomers (see details of the analytical characteristics in Table S1). The stability profiles are presented in Fig. 1.

In general, the real concentration under both abiotic and biotic experimental conditions was lower than the nominal concentration, indicating a moderate stability of ivabradine in marine environments. However, the stability profile was not affected by the exposure time up to 60 min (data not shown). Significant differences (in contrast to the nominal concentration) were observed for both individual enantiomers (light blue) and racemic mixture (dark blue), with higher variation for individual enantiomers. When enantiomers were in the racemic mixture, the variations were lesser for both abiotic and biotic conditions. The commercial drug *S*-ivabradine enantiomer presented the highest decay under both biotic and abiotic runs (about 14%). No interconversion between enantiomers was observed at any exposure time. Similarly, the nonsteroidal anti-inflammatory *RS*-naproxen formed a more stable racemic compound than the therapeutically used enantiopure form *S*-naproxen (Braun et al., 2011). All concentrations were readjusted and the toxicity was calculated applying the mean values of biotic variation as presented in Fig. 1.

S-ivabradine was found to be stable under neutral hydrolysis (H_2O, 80 °C, 48 h), oxidative (30% H_2O_2, 25 °C, 7 days), photolytic (0.1 N HCl, 0.1 N NaOH and H_2O, 200 W h/m^2), and thermal (100 °C, 7 days) conditions whereas a total of five degradation products were observed under

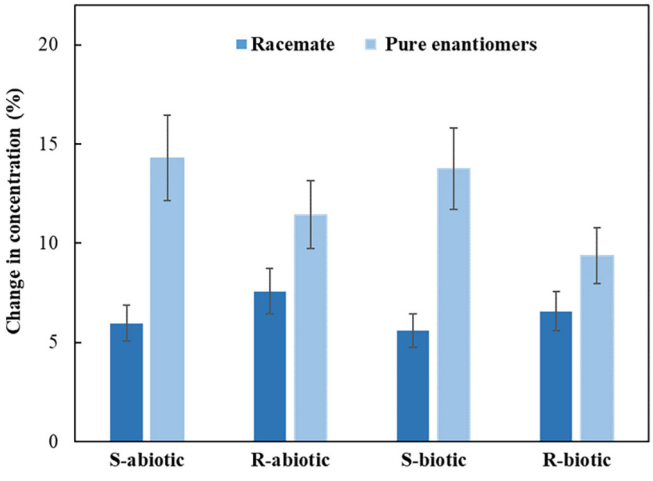

Fig. 1. Stability profiles of 20 mg/L individual *S*-ivabradine and *R*-ivabradine enantiomers (light) and 40 mg/L of their racemic mixture (dark) under abiotic (in absence of bacteria) and biotic (in presence of bacteria) conditions, at 60 min exposure time.

Table 1
Calculated EC_{50} and EC_{20} values (±SD) of individual *S*-ivabradine and *R*-ivabradine enantiomers as well as their racemic mixture in the *Vibrio fischeri* assay.

Exposure time (min)	EC_{50} (mg/L)	EC_{20} (mg/L)	r^2
S-ivabradine (commercial drug)			
5 min	7.93 ± 0.01	1.34 ± 0.02	0.98
10 min	11.58 ± 0.02	2.03 ± 0.01	0.95
15 min	11.82 ± 0.01	2.28 ± 0.02	0.96
60 min	13.81 ± 0.03	3.25 ± 0.01	0.99
R-ivabradine			
5 min	76.0 ± 0.1	1.02 ± 0.01	0.95
10 min	82.82 ± 0.05	1.74 ± 0.02	0.95
15 min	110.28 ± 0.02	2.95 ± 0.01	0.94
60 min	216.9 ± 0.1	25.90 ± 0.05	0.95
Rac-ivabradine			
5 min	11.11 ± 0.05	0.81 ± 0.01	0.95
10 min	15.6 ± 0.1	1.30 ± 0.05	0.93
15 min	18.37 ± 0.02	1.86 ± 0.01	0.92
60 min	41.1 ± 0.1	8.59 ± 0.03	0.93

acid (1 N HCl and 1 N H_2SO_4, 80 °C, 1 h) and basic (3 N NaOH:ACN, 60:40 v/v, 80 °C, 48 h) hydrolytic conditions (Patel et al., 2015). An increased time (24 h – 120 h), temperature (80 °C), acid (2 M HCl, 80 °C, 24 h), basic (1 M NaOH, 80 °C, 24 h), oxidation (3 – 15% H_2O_2, 80 °C, 24 h) reagents and light (H_2O, 24 – 48 – 120 h, 500 W/m^2) were also found to cause its degradation (Pikul et al., 2016). However, these are forced and un-realistic conditions of exposure. Up to our knowledge, no enantiomeric stability or biodegradability data of ivabradine in aquatic environments were previously reported for comparison, being this study the first one carried out with this aim.

3.2. Eco-toxicological profile

The toxicity of ivabradine's enantiomers and the racemic mixture on marine bacterium *V. fischeri* were determined using bioluminescence inhibition endpoint, and the related EC_{50} and EC_{20} (effective concentration that causes 20% of inhibition, considered also as the first concentration producing a significant effect on organisms) parameters are presented in Table 1. Real enantiomeric concentrations determined in Section 3.1 were employed for the calculation of those parameters. The toxicity values were established for 5, 10, 15, and 60 min time exposure.

Considering bioluminescence inhibition of *V. fischeri*, our results show that the commercial active drug *S*-ivabradine is more toxic compared to *R*-ivabradine and *Rac*-ivabradine for marine organisms, at any of the exposure times tested (5, 10, 15 and 60 min). The order of toxicity was *R*-ivabradine < *Rac*-ivabradine < *S*-ivabradine with EC_{50} (t = 5 – 60 min) value ranges about 76.00 – 216.90 mg/L, 11.11 – 41.10 mg/L, and 7.93 – 13.81 mg/L, respectively. The enantioselective ecotoxicity of ivabradine can be attributed to the specific receptors-binding ability of enantiomers (Wen et al., 2020). Incubations between 5 and 15 min are considered as short-time exposure and are usually used to report acute toxicity for the marine bacterium. Exposure about 60 min can be considered as long-term exposure, data that are rare to find; however, are highly valued for the risk assessment. Our results confirm that marine bacterium *V. fisheri* is sensitive to the commercial ivabradine pharmaceutical formulation, namely *S*-ivabradine. Based on the obtained EC_{50} (t = 5 min) values and the European Regulation, EC1272/2008, the commercial drug *S*-ivabradine can be ranked as harmful for marine ecosystem, while *Rac*-ivabradine and *R*-ivabradine present moderate concern.

As no eco-toxicological data were previously reported for ivabradine, the values of EC_{50} obtained in this work were compared with the data reported for diltiazem and metoprolol, the most detected CCB and β-blocker, respectively. Grabarrczyk et al. revealed that the metoprolol β-blocker posed a low risk to *V. fischeri*, with a 3% inhibition of luminescence at a concentration of 100 mg/L (Grabarczyk et al., 2020).The toxicity data available for diltiazem on *V. fischeri* indicate EC_{50} values about 407.0, 263.7 and 152.0 mg/L at exposure times of 5 min, 15 min, and 24 h, respectively (Saari et al., 2017). The results obtained in this study show that the toxicity of *S*-ivabradine drug is >10 times higher than that of diltiazem CCB compound on marine bacterium.

Moreover, considering the increased usage of *S*-ivabradine drug, its expected raised detection in aquatic environments, and the toxicity values obtained in this work, the newest cardiovascular *S*-ivabradine drug can produce a high ecological risk for marine organisms.

3.3. Mechanism of toxicity

The possible mechanism of toxicity for *S*-ivabradine towards marine bacterium *V. fischeri* was investigated *via* multilevel responses including

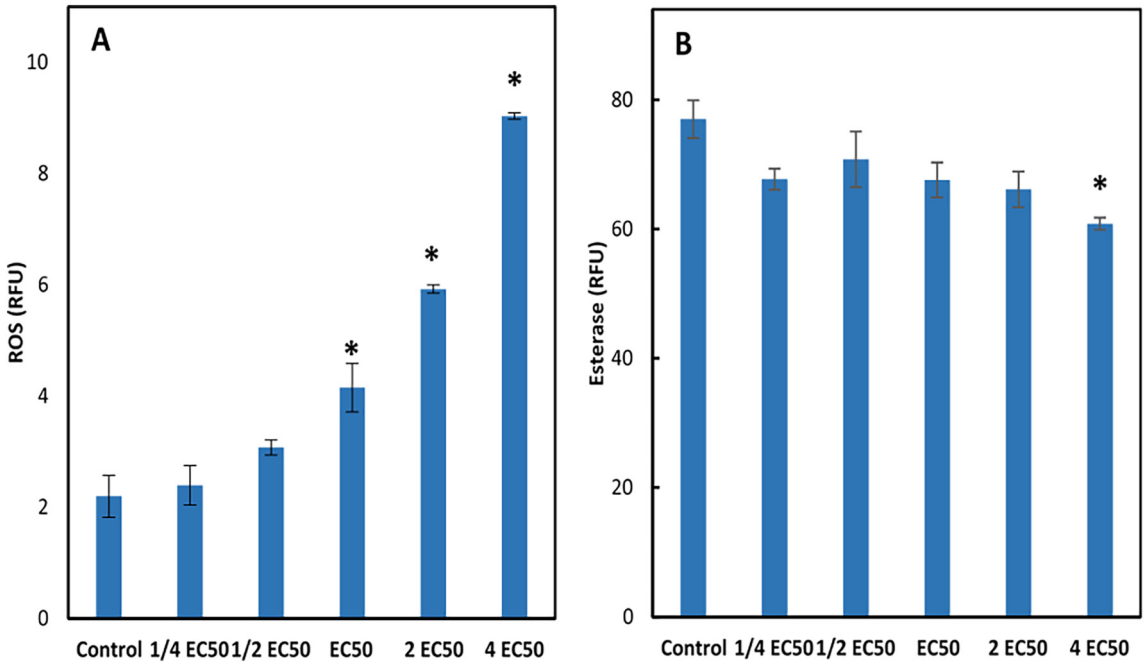

Fig. 2. Effects of *S*-ivabradine on biochemical endpoints: reactive oxygen species, ROS (A) and esterase (B) (*$p < 0.05$).

esterase activity, oxidative reactive species (ROS) and membrane cellular damage (Live/Dead distribution).

As described in Fig. 2A, the signal of ROS increased with the increase of *S*-ivabradine concentration. The ROS level for bacteria exposed at doses over EC_{50} of *S*-ivabradine was significantly higher than that of the control ($p < 0.05$). The increased ROS level demonstrated that *S*-ivabradine induced an oxidative stress response on marine bacteria. Antihypertensive compounds have been linked to oxidative stress (Saari et al., 2017; Ajima et al., 2017; Li et al., 2011), but to the best of our knowledge, the ability of *S*-ivabradine to produce oxidative stress in aquatic organisms has not been reported.

The activity of the esterase enzyme, mainly involved in the oxidative stress defence, was measured after exposure to *S*-ivabradine (Fig. 2B). The esterase level of the marine bacteria presented dose-dependent pattern in contact with *S*-ivabradine (Fig. 2B). The activity of esterase on *V. fischeri* was inhibited over 20% compared to the control at 4 EC_{50} of *S*-ivabradine

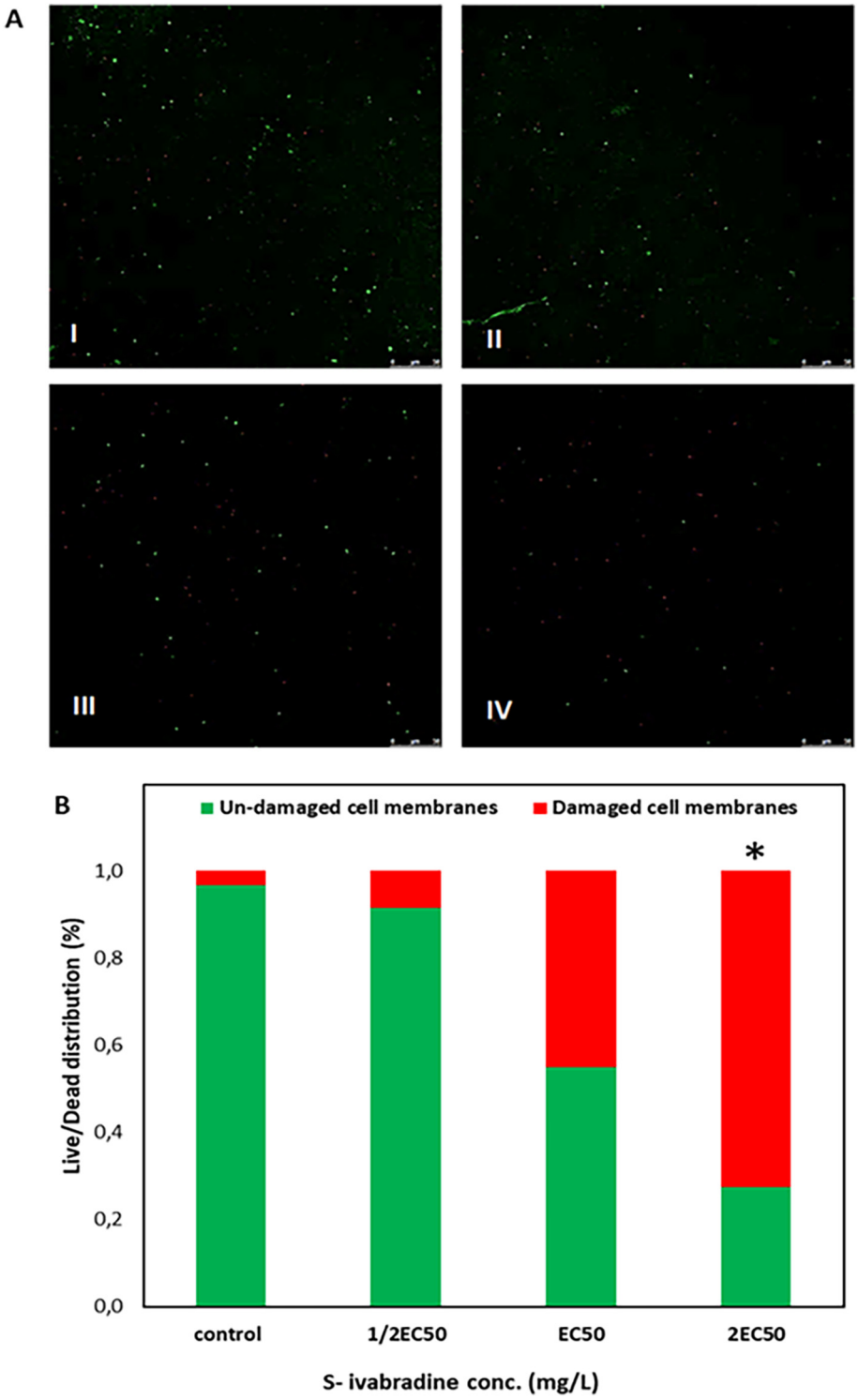

Fig. 3. Membrane cell damage caused by *S*-ivabradine: (A) Confocal merged micrographs of the Live/Dead staining (I, control; II, ½ EC_{50}; III, EC_{50}; IV, $2EC_{50}$). Scale bar of 50 μm; (B) Membrane cells damage degree (%), live/dead cells number distribution (*$p < 0.05$).

dose. The change trend of esterase activity showed to decline with the increasing of the concentration, which was opposite to ROS pattern, indicating an irreversible damage in the metabolic defences of bacteria upon overproduction of ROS. When ROS levels surpassed the scavenging ability of antioxidant enzymes (like glutathione *S*-transferases, superoxide dismutase, and catalase), they become enzymatic overall inhibitors (Amariei et al., 2020).

Membrane cell damage in bacteria exposed to *S*-ivabradine was studied using Live/Dead assay, as illustrated in Fig. 3A. Control shows green-stained cell bodies, indicating viable cell with un-damaged envelops. When increasing *S*-ivabradine concentration, a lower number of green-stained bacterial cells and an increasing number of red-stained cells were observed, demonstrating the membrane cells impairment. The membrane cell damage degree was then categorized by the live-green/dead-red stained cells distribution, which is depicted in Fig. 3B. The percentage of damaged cells (red) (Fig. 3B) was greater in the *S*-ivabradine treated samples (8.5, 45.1, and 72.6% for ½ EC_{50}, EC_{50}, and 2 EC_{50}, respectively) than in controls (3.4%). The non-reversible damage on bacterial cell membranes can be associated to the increase of oxidative stress through ROS overproduction.

Overall, these data demonstrate that *S*-ivabradine induced up-regulation on ROS level in marine bacterium *V. fischeri*. The oxidative species react with principal cellular components, which are extremely sensitive to oxidation, damaging the esterase activity and membrane cells integrity. So, the injurious effect on enzymatic activity and membrane cells because of oxidative stress induction describes the main pathways for toxicity mechanism of *S*-ivabradine in *V. fischeri*. Similar mode of action has been reported in freshwater aerobic bacteria after exposure to ibuprofen or triclosan (Amariei et al., 2020). However, no studies were reported on the ecotoxicity of the newest cardiovascular drug ivabradine (*S*-ivabradine) on aquatic organisms, being our work the first one providing information regarding the mechanism of toxicity of *S*-ivabradine on marine organisms.

4. Conclusions

This is the first time that the stability and eco-toxicological effects of ivabradine's individual enantiomers and their racemic mixture on the marine bacterium *V. fischeri* are investigated. Real (not nominal) concentrations were determined by CE method based on the use of a 50 mM formate buffer (pH 2.0) in presence of 4 mM sulfated-γ-CD as chiral selector. The *S*-ivabradine enantiomer presented the highest decay under both biotic and abiotic conditions, indicating moderate stability in saline environment. Our results clearly demonstrate enantioselectivity in bioluminescence inhibition induced by ivabradine on *V. fischeri*, being the commercial drug *S*-ivabradine the most toxic with an EC_{50} of 7.93 mg/L. The acute toxicity order was *R*-ivabradine < *Rac*-ivabradine <*S*-ivabradine. Biochemical measurements indicated that the oxidative stress originating enzymatic and membrane cell injuries can be the main mechanistic pathway of the cardiovascular drug *S*-ivabradine toxicity in marine bacterium *V. fischeri*. The results obtained in this work are limited to this marine bacterium and future work using organisms from different trophic levels will have to be performed for an accurate environmental risk assessment. Based on the described enantioselective toxic effects of ivabradine, this work settles the base for the evaluation of the ecological impact of the contemporary cardiovascular *S*-ivabradine drug in marine environments.

Credit authorship contribution statement

G.A: Conceptualization, Investigation, Data Curation, Methodology, Validation, Formal analysis, Visualization, Writing- Original draft preparation; **S.J.J**: Investigation, validation, formal analysis; **M.A.G**: Conceptualization, Methodology, Data Curation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition; **M.L.M**: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Project administration, Resources, Funding acquisition; **K.B**: Conceptualization, Methodology, Validation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Ethical approval

This article does not contain any study with human participants or animals performed by any of the authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156617.

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