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Toxic responses of metabolites produced by *Ostreopsis* cf. *ovata* on a panel of cell types

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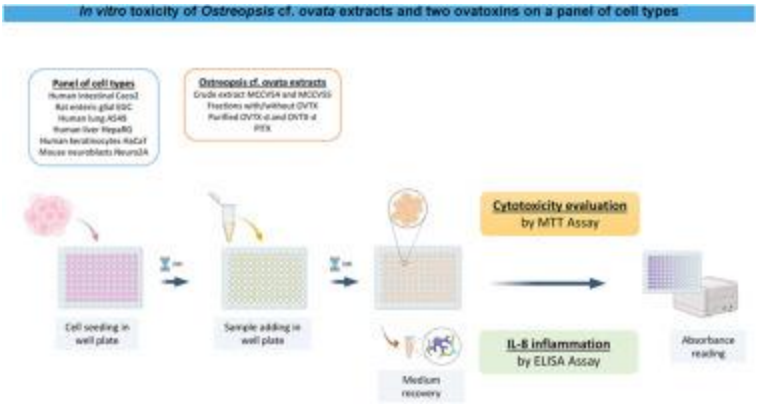
Abstract :

Blooms of the dinoflagellate Ostreopsis cf. ovata are regularly associated with human intoxications that are attributed to ovatoxins (OVTXs), the main toxic compounds produced this organism and close analogs to palytoxin (PlTX). Unlike for PlTX, information on OVTXs'toxicity are scarce due to the absence of commercial standards. Extracts from two cultures of Mediterranean strains of O. cf. ovata (MCCV54 and MCCV55), two fractions containing or not OVTXs (prepared from the MCCV54 extract) and OVTX-a and -d (isolated from the MCCV55 extract) were generated. These chemical samples and PlTX were tested on a panel of cell types from several organs and tissues (skin, intestine, lung, liver and nervous system). The MCCV55 extract, containing a 2-fold higher amount of OVTXs than MCCV54 extract, was shown to be more cytotoxic on all the cell lines and more prone to increase interleukin-8 (IL-8) release in keratinocytes. The fraction containing OVTXs was also cytotoxic on the cell lines tested but induced IL-8 release only in liver cells. Unexpectedly, the cell lines tested showed the same sensitivity to the fraction that does not contain OVTXs. With this fraction, a pro-inflammatory effect was shown both in lung and liver cells. The level of cytotoxicity was similar for OVTX-a and –d, except on intestinal and skin cells where a weak difference of toxicity was observed. Among the 3 toxins, only PlTX induced a pro-inflammatory effect mostly on keratinocytes. These results suggest that the ubiquitous Na+/K+ ATPase target of PlTX is likely shared with OVTX-a and -d, although the differences in pro-inflammatory effect must be explained by other mechanisms.

Graphical abstract



Highlights

► Extracts of *Ostreopsis* cf. *ovata* were cytotoxic to a broad range of cell lines. ► The fraction not containing ovatoxins induced IL-8 release on liver and lung cells. ► PlTX was more cytotoxic than OVTX-a and –d on cells from intestine and the nervous system. ► A similar IC_{50} was observed on lung cells for the three toxins. ► OVTX-a and –d did not induce IL-8 release on intestinal, lung and skin cells.

Keywords : ovatoxins, palytoxin, in vitro, pro-inflammatory effect, cytotoxicity

Introduction

Human intoxications through aerosol inhalation as well as dermal and ocular contacts are recorded regularly along the coasts of the Northwestern Mediterranean Sea including Italy, France and Spain where blooms of the dinoflagellate *Ostreopsis* cf. *ovata* occur (Berdalet et al. 2022). Recently, similar intoxications have been reported in the Bay of Biscay (Atlantic Ocean) (Paradis and Labadie 2022), together with the presence of *O*. cf. *ovata* and *O*. cf. *siamensis* in the same areas (Chomérat et al. 2022). Although the toxic compounds responsible for such effects have not been clearly identified (Berdalet 2020), *O.* cf. *ovata* was shown to produce palytoxin (PlTX)-like compounds named ovatoxins (OVTXs) (Ciminiello et al. 2012). Several OVTX analogs (a to l) have been identified in different species of the genus *Ostreopsis* (Patocka et al. 2018) but their purification from dinoflagellate cultures has always been described as challenging, limiting the toxicological studies required to carry out hazard assessment of OVTXs. As a consequence, only few results with pure OVTX-a have been published so far, while PlTX toxicity has been more largely investigated (Patocka et al. 2015).

PlTX has been shown to inhibit the Na^+/K^+ ATPase (Habermann 1989), located in the plasma membrane of animal cells (Mobasheri et al. 2000). It has also been suggested that the H^+/Na^+ ATPase may be a PlTX target (Scheiner-Bobis et al. 2002; Patocka et al. 2018). Moreover, PlTX disrupts the regulation of signaling pathways through different mechanisms depending on the cell type, as described for the MAP kinases activity (Wattenberg 2011). Consequently, PlTX has been shown to affect a broad range of cells including intestinal cells (Valverde et al. 2008; Pelin et al. 2012; Fernández et al. 2013), neuronal cells (Vale et al. 2007; Louzao et al. 2008; Nicolas et al. 2015), erythrocytes, macrophages and splenocytes (Satoh et al. 2003; Aiba et al. 2005; Crinelli et al. 2012; Pezzolesi et al. 2012), aortic smooth muscle cells (Sheridan et al. 2005), skin cells (Pelin et al. 2013 ; Pelin et al. 2016a) and carcinoma cells (Bellocci et al. 2008; Görögh et al. 2013). Studies are now needed to compare the cell toxicity of OVTXs with PlTX. Up to now, inconsistent results on the *in vitro* toxicity of OVTX-a compared to PlTX have been reported. Indeed, while OVTX-a was first shown to be far less potent than PlTX in inducing cytotoxicity on HaCaT keratinocytes (100 fold) and in the hemolytic test (10 fold) (Pelin et al. 2016b), similar activities were measured with PlTX, OVTX-a or extracts containing OVTXs in the hemolytic test (Pezzolesi et al. 2012; Poli et al. 2018), and in the *Artemia franciscana* bioassay (Pavaux et al. 2020). Importantly, *in vivo* toxicity assessments in rodents after inhalation exposure of OVTX-a and PlTX have

resulted in close values for lethality (50% lethal dose, LD_{50}) as well as similar symptoms and toxic effects (Poli et al. 2018).

In this study, the toxicity of extracts, fractions and pure OVTX-a and -d obtained from cultures of Mediterranean strains of *O.* cf. *ovata* were investigated on a panel of cell types, representative of several organs and tissues: skin, intestine, liver, lung and nervous system. The mitochondrial activity was measured through the MTT assay and the pro-inflammatory effect was detected by the release of interleukin 8 (IL-8). Finally, the results obtained with OVTX-a and -d were compared with the data obtained with PlTX.

Material and methods

Chemicals

Cell culture products were purchased from Gibco (Cergy-Pontoise, France). Bovine serum albumin (BSA), 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT), streptavidin horseradish peroxide (HRP) were supplied by Sigma-Aldrich (Saint Quentin Fallavier, France). Primary IL-8 antibody (M801), biotin-conjugated human IL-8 (M802B), recombinant IL-8 cytokine, SuperBlock blocking buffer, 3,3',5,5'-tetramethylbenzidine (TMB),Tween 20, Tumor Necrosis Factor alpha (TNFα) and sulfuric acid were obtained from Thermofisher Scientific. Monoclonal IL-8, biotinyled monoclonal IL-8 antibodies, SuperBlock blocking buffer, TNFα and TMB were purchased from ThermoFisher Scientific (Waltham, MA). Palytoxin (purity = 85.8%) was purchased from Wako (Pure Chemical Industries, Ltd., Japan) and solubilized in dimethylsulfoxide (DMSO).

Extracts and fractions from O. cf. ovata and purified OVTX-a and –d

The same extracts prepared from cultures of *O.* cf. *ovata* (MCCV54 and MCCV55) as well as the same purified OVTX-a and –d used in Gémin *et al*. (2022) were tested in the current study. Two fractions prepared from the extract MCCV54 were also tested. They were obtained as described previously: Pavaux *et al*. (2020) for fractions of *O.* cf. *ovata* containing or not OVTXs (named OVTX+ and OVTX- respectively) and Gémin *et al*. (2022) for extracts of the strains MCCV54 and MCCV55, and pure OVTX-a and -d. Briefly, two strains of *O.* cf. *ovata* (MCCV54 and MCCV55) were obtained from the Mediterranean Culture Collection of Villefranche-sur-Mer (France). Cells were cultivated and harvested as described in Gémin *et al*. (2022) to collect 75 g of MCCV54 and 35 g of MCCV55 (wet pellets). Extracts of both strains were prepared by adding MeOH/H_2O (1:1 v/v) on 5 g cell pellets (ratio: 4 mL/g of wet pellet) and the suspension was vortexed and centrifuged at 4000 g during 5 min. The supernatant of each extraction process was concentrated by evaporation under a nitrogen flow (N-evap, Organomation) at 30 °C. Two fractions were then prepared from MCCV54 following size exclusion chromatography: one combining fractions where OVTX-a to -f were detected (OVTX+) by MS; one combining fractions where those metabolites were not detected (OVTX-) (see Pavaux *et al*. (2020) for details). A similar fraction OVTX+ prepared from the MCCV55 extract was further purified by semi-preparative chromatography to obtain 223 and 10.5 µg of OVTX-a and -d respectively (see Gémin *et al*. (2022) for details). The fraction OVTX- was dried by rotary evaporation (Büchi rotavapor R-200), weighted and then dissolved into ultra-pure water (at 50 mg/mL). The fraction OVTX+ was not evaporated completely and therefore could not be expressed in ng/mL. However, the different OVTXs were quantified (see below) and the total quantity of OVTXs present in the fraction was estimated in

PlTX equivalent, considering an equivalent factor of 1 for each variant. Extracts from MCCV54 and MCCV55, OVTX+ fraction of MCCV54 as well as the purified OVTX-a and -d were concentrated in DMSO prior to toxicity investigations. The quantification of OVTXs was performed by LC-MS/MS as described in Gémin *et al*. (2022).

Cell cultures

Human intestinal Caco2 cells (HTB-37), obtained from the American Type Culture Collection (ATCC) (Manasas, VA), were maintained in Minimum Essential Medium containing 5.5 mM D-glucose, Earle's salts and 2 mM L-alanyl-glutamine (MEM GlutaMAX™) supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids, 50 IU/mL penicillin and 50 µg/mL streptomycin. Non-differentiated cells were used at passages 29 to 42.

Rat enteric glial cell line (EGC) (CRL2690) was obtained from the American Type Culture Collection (ATCC, Manasas, VA). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 25 mM D-Glucose and 4 mM L-glutamine, supplemented with 10% heat-inactivated fetal calf serum (FCS), 50 IU/mL penicillin and 50 µg/mL streptomycin. Cells were used at passages 45 to 65.

Human lung A549 were obtained from the American Type Culture Collection (CRM-CCL-185), and were cultured in DMEM containing 5.5 mM D-glucose and 4 mM L-glutamine supplemented with 10% heat-inactivated fetal calf serum (FCS), 50 U/mL penicilin, and 50 µg/mL streptomycin. Cells were used at passages 10 to 30.

Human liver HepaRG cells were purchased from Biopredic international (Rennes, France) and were cultured in Williams E medium supplemented with 10% FCS, 50 U/mL penicillin, 50 µg/mL streptomycin, 5 µg/mL insulin, 2 mM glutamine and 50 µM hydrocortisone hemisuccinate. Non-differentiated cells were used at passages 14 to 20. HepaRG cells were only used for testing the extracts containing or not OVTXs to investigate if any specific effect on hepatocytes can be induced, at least for the extract not containing OVTXs (OVTX-).

Human keratinocytes HaCaT were obtained from the American Type Culture Collection (CL-00090). They were cultured in DMEM containing 5.5 mM D-glucose and 4 mM L-glutamine supplemented with 10% heat-inactivated fetal calf serum (FCS), 50 U/mL penicillin and 50 µg/mL streptomycin. Cells were used at passages 32 to 40.

Cell treatment

Cells were seeded in 96 well plates for cytotoxicity and IL-8 release at 10,000 cells per well for A549, EGC, HepaRG and HaCaT, at 20,000 cells per well for Caco2 and at 45,000 cells per well for Neuro2a.

All cells were grown at 37 °C and 5% CO_2 and passaged once or twice a week depending on the cell line. The day after seeding, cells were exposed to the extracts, the fractions or the pure OVTX-a and –d for 24 hours, in the corresponding medium for each cell line but without FCS. For each cell line and experiment, a vehicle control (2% DMSO) was included. At least 3 independent experiments were performed.

Cytotoxicity (MTT assay)

After treatment, cells were incubated at 37 °C for 2 h with 100 µL of 500 µg/mL MTT solution prepared in medium, prior to the addition of 100 µL/well of DMSO to solubilize the mitochondrial dye. The plates were gently shaken for 5 min and the absorbance was recorded with a FLUOstar Optima microplate reader (BMG Labtek, Champigny sur Marne, France) at 570 nm. Cytotoxicity was evaluated as cell viability reduction and expressed as % cell viability compared to cells exposed to the solvent alone.

Pro-inflammatory effect (IL-8 release)

The medium from HaCaT, Caco2, HepaRG and A549 cell cultures was collected after 24h treatment and frozen at -20 °C until analysis. The release of IL-8 was measured using an enzyme-linked immunosorbent assay (ELISA). TNFα (100 ng/mL) was used as positive control. 96-well microplates (Nunc maxisorp) were coated with human recombinant IL-8 primary antibodies at 1 µg/mL and incubated overnight at 4 °C. Between each step, the wells were washed with PBS-Tween 20 (0.05%). After saturation with SuperBlock for 1 h, samples and standards (IL-8 recombinant) were added into the wells and incubated at room temperature for 1.5 h. Biotin-conjugated human IL-8 antibodies (0.1 µg/mL) were then added followed by 100 µL of HRP 1:1000 labeling. Finally, 50 µL of the chromogenic substrate TMB was added and the reaction was stopped with 50 µL sulfuric acid (1 M). Plates were read with a FLUOstar Optima microplate reader (BMG Labtek, Champigny sur Marne, France) at 405 nm. The concentrations of IL-8 expressed in pg/mL were calculated against a standard curve prepared in duplicate. Three independent experiments with two technical replicates per experiment were performed.

Statistics

GraphPad Prism software (version 9.5.1) was used for IC_{50} calculation using the non-linear regression, sigmoïdal, 4PL model as well as for statistical analyses of IL-8 release. An analysis of the variance (ANOVA) was performed, and, when the effect of concentration was significant (*p-value* < 0.05), the values were compared to the control using the Dunnett's test.

Results

A) Extracts of *O.* cf. *ovata*

The two extracts differ in the number of OVTX analogs detected: 5 (OVTX-a, -b, -c, -d and -e) in MCCV54 extract and only 3 (OVTX-a, -d and -e) in MCCV55 extract (Gémin et al., 2022). Nevertheless, OVTX-a was the major analog (around 58 and 89% of the total OVTX for MCCV54 and MCCV55, respectively).

The two Mediterranean strains MCCV54 and MCCV55 of *O.* cf. *ovata* showed a different profile of OVTXs with OVTX-a being the main OVTX for both (Gémin et al. 2022). The cytotoxicity assays showed a concentration-dependent effect of both extracts towards the different cell types, with a concentration inhibiting cell viability by 50% (IC_{50}) ranging from 17.3 and 91.9 ng PlTX eq/mL and 2.5 to 31.7 ng PlTX eq/mL, for MCCV54 and MCCV55 respectively (Figure 1 and Table 1).

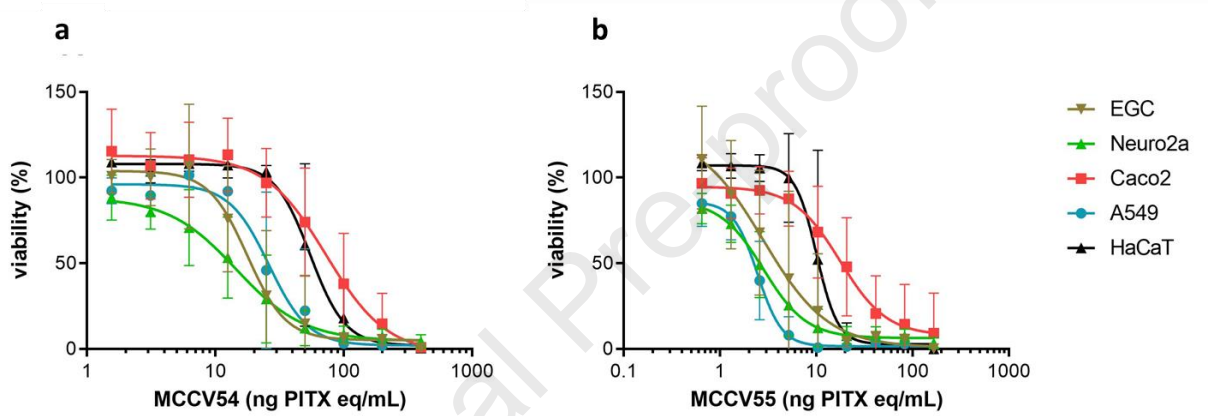


Figure 1: Cytotoxicity of MCCV54 (a) and MCCV55 (b) extracts on a panel of cell lines after 24 h treatment determined by the MTT assay. Mean ± standard deviation of 3 to 6 independent experiments

Except the lung A549 cells that showed the highest sensitivity to the MCCV55 extract, the cells from the nervous system EGC and Neuro2A cells were very sensitive to both extracts with a similar level of toxicity for each cell line. However, the MCCV54 extract was 4 to 5 times less potent than the MCCV55 extract on these two cell lines (IC_{50} around 20 and 4 ng PlTX eq/mL respectively, Table 1).

Table 1: IC_{50} (ng PlTX eq/mL) of MCCV54 and MCCV55 extracts on a panel of cell lines after 24 h treatment using the MTT assay. Mean ± standard deviation of 3, 5 (*) or 6 (**) independent experiments

Cell type	**IC_{50} (ng PlTX eq/mL)**	
	MCCV54	**MCCV55**
A549 (lung)	33.2 ± 19.4	2.5 ± 1
Caco2 (colon)	91.9 ± 60.4 *	31.7 ± 37 *

EGC (enteric nervous system)	22.7 ± 21.6 **	5.2 ± 5 **
Neuro2A (nervous system)	17.3 ± 10.8 *	3.3 ± 1.9 *
HaCaT (skin)	62.2 ± 29.8	11.8 ± 7.1

The sensitivity of the A549 lung cells to both extracts was also very close to the ones obtained with the nervous cells (Table 1) with IC_{50} (2.5 ng PlTX eq/mL) slightly lower for the MCCV55 extract and slightly higher for the MCCV54 extract (33.2 ng PlTX eq/mL). Among the cell types tested, the highest IC_{50} was obtained with the intestinal Caco2 cell line for both extracts (IC_{50} = 91.9 and 31.7 ng PlTX eq/mL for MCCV54 and MCCV55 extracts respectively, Table 1) while the skin model gave an intermediate response (IC_{50} = 62.2 and 11.8 ng PlTX eq/mL for MCCV54 and MCCV55 extracts respectively, Table 1).

The pro-inflammatory potential was investigated by IL-8 release after 24 h treatment with the *O*. cf. *ovata* extracts on A549, Caco2 and HaCaT cells. In contrast to the positive control (TNFα 100 µg/mL), no increase of IL-8 release was observed with both extracts at concentrations up to 400 µg PlTX eq/mL in the A549 and Caco2 cell lines (Figure 2). Compared to the solvent control, a slight but non-significant increase of IL-8 was also observed on HaCaT cells with the MCCV55 and MCCV54 extracts (Figures 2a and 2b). However, with the MCCV54 extract, a significant increase of IL-8 was observed only at 100 ng PlTX eq/mL on HaCaT cells due to the low cell viability (<5%) observed for the highest concentrations as shown in Figure 1a.

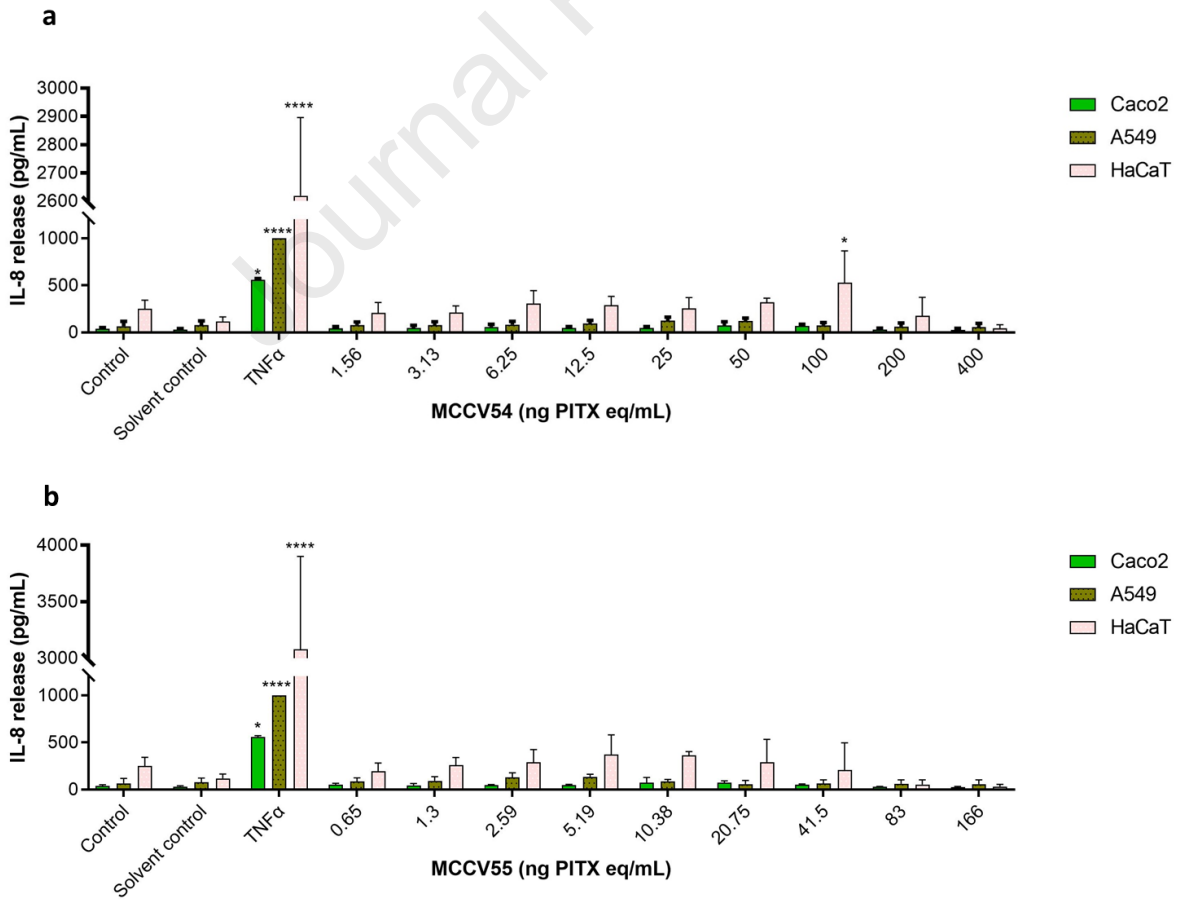


Figure 2: IL-8 release from Caco2, A549 and HaCaT cells following a 24 h treatment with MCCV54 (a) and MCCV55 (b) extracts. Mean ± standard deviation of 3 independent experiments. Statistical significance is indicated by * p≤0.05 and **** p≤0.0001.Positive control = TNFα 100 ng/mL

B) Fractions prepared from MCCV55 extract

Two fractions were prepared and analysed from the MCCV54 extract: one containing the different identified OVTXs as determined by LC-MS/MS called OVTX+ and one called OVTX- formed by the remaining fractions. The OVTX+ fraction (7.73 mg/mL) contain a mixture of OVTX-a (58.6%), OVTX-b (26.7%), OVTX-c (3.4%), OVTX-d (6.0%), OVTX-e (3.5%) and OVTX-f (1.8%) based on LC–MS/MS quantification (Gémin et al., 2020) and was concentrated in DMSO (Pavaux et al., 2020). The fraction OVTX+ was cytotoxic to all cell types tested (Figure 3a and Table 2). However, it could be pointed out that an increased production of formazan formation at the lowest concentrations tested was reported with the two fractions on EGC and HepaRG cells. A similar IC_{50} was observed for the OVTX+ fraction on EGC and A549 cells (IC_{50} = 4.4 and 7.3 ng PlTX eq /mL respectively, Table 2), while the Neuro2A cells (IC_{50} = 11.1 ng PlTX eq/mL) were slightly less sensitive (2.5 fold) compared to the other nervous cell line tested EGC. Non-differentiated liver HepaRG cells were also highly sensitive to the OVTX+ fraction (IC_{50} around 5 ng PlTX eq/mL, Table 2). Moreover, as observed with the extracts, the non-differentiated Caco2 cells showed the highest IC_{50} (15.7 ng PlTX eq/mL); nevertheless, the IC_{50} was in the same order of magnitude as for the other cell lines (Table 2).

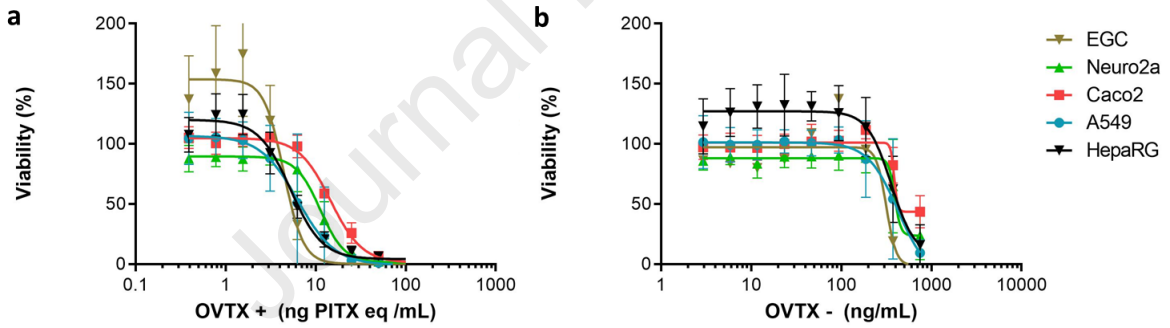


Figure 3: Cytotoxicity of the fraction containing OVTX (OVTX+) (a) or not (OVTX-) (b) on a panel of cell lines after 24 h treatment using the MTT assay. Mean ± standard deviation of 3 to 4 independent experiments

With the fraction OVTX-, cytotoxicity was observed on the various cell lines tested (Figure 3b and Table 2). The EGC were the most sensitive with an IC_{50} of 241.6 ± 71.1 ng/mL (Table 2) and a steep response between 125 and 250 ng/mL (Figure 3b). Higher and very close IC_{50} values were obtained on both lung and liver cells (334 ± 128 and 346 ± 42 ng/mL respectively) (Table 2).

Table 2: IC_{50} of OVTX+ and OVTX- fractions on a panel of cell lines after 24h treatment using the MTT assay. Mean ± standard deviation of 3 or 4(*) independent experiments

Cell type	**IC_{50}**	
	OVTX+ (ng PlTX eq/mL)	**OVTX- (ng/mL)**

A549 (lung)	7.3 ± 5.3	334 ± 128
Caco2 (colon)	15.7 ± 2.1	387.9 ± 11.9 *
HepaRG (liver)	5.2 ± 0.4	346 ± 42.4
EGC (enteric nervous system)	4.4 ± 0.5	241.6 ± 71.1
Neuro2A (nervous system)	11.1 ± 1.8	498.1 ± 241

The fraction OVTX+ did not induce the release of IL-8 from lung and colon cell lines up to 100 ng PlTX eq/mL (Figure 4a). However, a pro-inflammatory effect was obtained on liver HepaRG cells with a significant increase only at 6.25 ng/mL, the highest concentrations being too cytotoxic to induce pro-inflammatory effect (Figure 3a). The fraction OVTX- did not induce any pro-inflammatory effect on the intestinal Caco2 cells at any concentration tested, whereas a significant increase of IL-8 was observed at the highest concentrations (375 and 750 ng/mL) for lung A549 and hepatic HepaRG cells (Figure 4b).

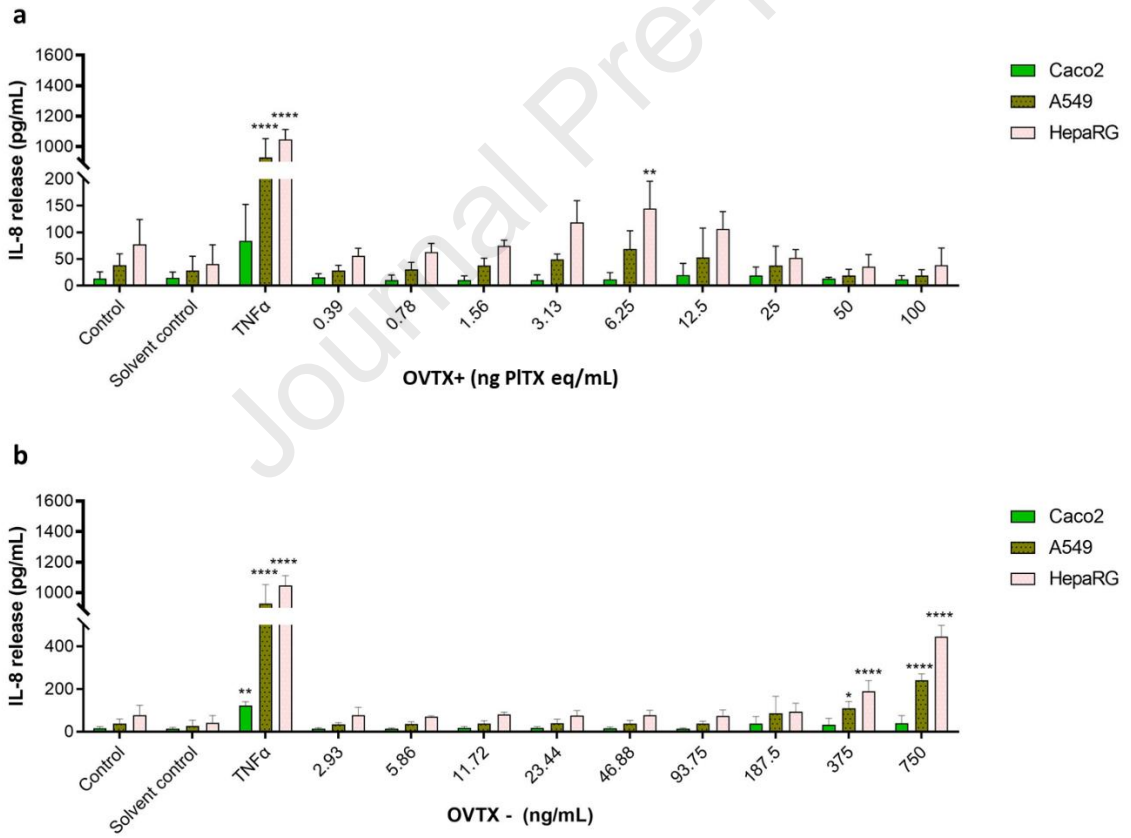


Figure 4: IL-8 release from Caco2, A549 and HepaRG cells following a 24 h treatment with OVTX+ (a) and OVTX- (b) fractions. Mean ± standard deviation of 3 independent experiments. Statistical significance is indicated by * p≤0.05, ** p p≤0.01 and **** p≤0.0001.Positive control = TNFα 100 ng/mL

C) Pure OVTX-a and d and PlTX

OVTX-a and –d (Figure 5a and 5b respectively) as well as PlTX (Figure 6) showed a concentration-response curve for cytotoxicity on each cell type tested. However, some differences between the responses were noticed. Globally, the IC_{50} obtained with the 3 toxins on the different cell types ranged between 0.39 and 18.16 ng/mL (Table 3) with fold changes between 1 (for A549 cells) and 5 (for EGC).

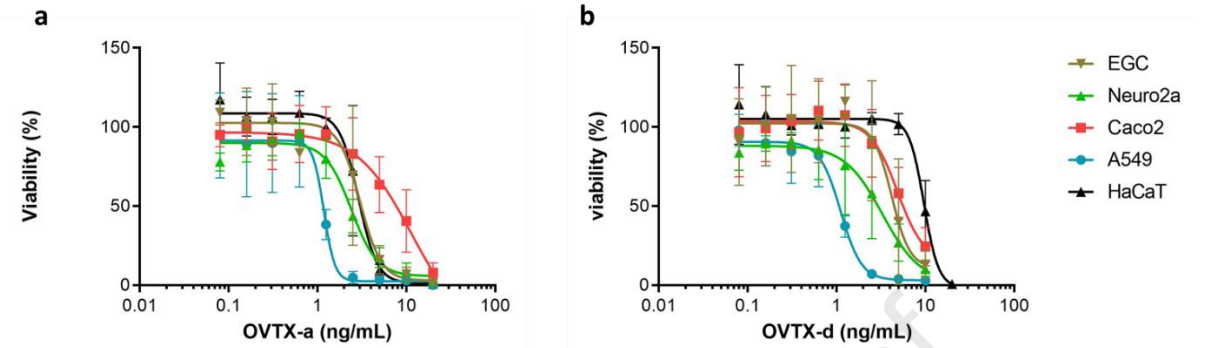


Figure 5: Cytotoxicity of the purified OVTX-a (a) and OVTX-d (b) on a panel of cell lines after 24 h treatment using the MTT assay. Mean ± standard deviation of 3 to 5 independent experiments

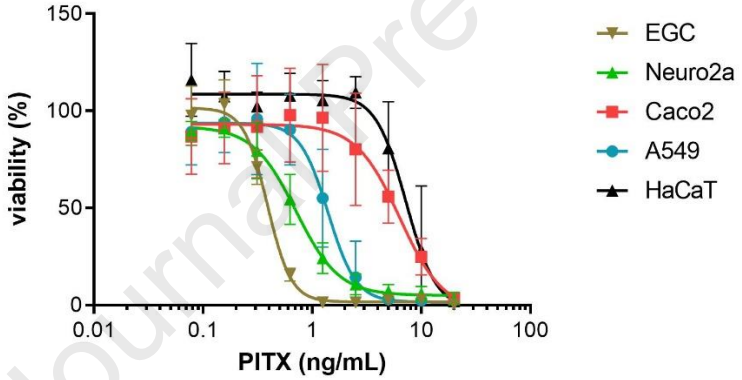


Figure 6: Cytotoxicity of PlTX on a panel of cell lines after 24 h treatment using the MTT assay. Mean ± standard deviation of 3 independent experiments

The cytotoxicity assay with OVTX-a provided an IC_{50} ranking over the panel of cell types as follows: A549<Neuro2A<EGC<HaCaT<Caco2. The IC_{50} with OVTX-a ranged from 1.78 ng/mL for A549 cells to 18.16 ng/mL for Caco2 cells and IC_{50} values around 2.5 ng/mL for the 3 other cell lines (Table 3). The IC_{50} rankings for OVTX-d and PlTX were slightly different: A549<Neuro2A <EGC< Caco2<HaCaT and EGC<Neuro2A< A549< Caco2<HaCaT respectively. The IC_{50} with OVTX-d ranged from 1.24 ng/mL for A549 cells to 10.73 ng/mL for HaCaT cells and the ones for PlTX ranged from 0.39 ng/mL for EGC to 7.71 ng/mL for HaCat cells (Table 3). The A549 cells were again the most sensitive to OVTX-a and –d. The 2 nervous cell lines (i.e. EGC and Neuro2A) showed a very close IC_{50} (around 2.5 ng/mL) for these two OVTXs (Table 3). Interestingly, the pattern was different with HaCaT keratinocytes that were more sensitive cells to OVTX-d compared to OVTX-a, while the opposite was observed for Caco-2 cells (Table 3). About PlTX, the two nervous cell lines EGC and Neuro2A gave the lower IC_{50} with very similar values (0.39 and 0.69 ng/mL respectively) while the HaCaT and Caco2 cells were less affected (IC_{50} of 7.71 and

283 6.70 ng/mL respectively (Table 3). The lung cells were rather sensitive to the 3 toxins, OVTX-a and –d
284 showing a similar IC_{50} to PlTX, around 1.5 ng/mL.

285

286 Table 3: IC_{50} (ng/mL) of OVTX-a, OVTX-d and PlTX on a panel of cell lines after 24h treatment using the
287 MTT assay. Mean ± standard deviation of 3, 4(*) or 5(**) independent experiments

Cell type	**IC_{50} (ng/mL and *nM*)**		
	OVTX-a	**OVTX-d**	**PlTX**
A549 (lung)	1.78 ± 0.97 *0.67 ± 0.37*	1.24 ± 0.03 *0.47 ± 0.01*	1.48 ± 0.44 *0.55 ± 0.21*
Caco2 (colon)	18.16 ± 12.20 *6.86 ± 4.60*	5.94 ± 3.53 ** *2.23 ± 1.33*	6.70 ± 2.40 *2.50 ± 0.90*
EGC (enteric nervous system)	2.89 ± 1.70 * *1.09 ± 0.64*	2.51 ± 1.88 * *0.94 ± 0.71*	0.39 ± 0.06 *0.15 ± 0.02*
Neuro2A (nervous system)	2.33 ± 0.31 *0.88 ± 0.12*	2.95 ± 1.72 * *1.11 ± 0.65*	0.69 ± 0.26 *0.26 ± 0.1*
HaCaT (skin)	2.99 ± 1.19 *1.13 ± 0.45*	10.73 ± 3.43 *4.03 ± 1.29*	7.71 ± 3.41 *2.88 ± 1.27*

288

289 Concentrations up to 20 ng/mL of the two ovatoxins OVTX-a and -d did not induce an increase of IL-8
290 release after a 24 h treatment on any of the three cell lines HaCaT, Caco2 and A549 (Figure 7a and 7b).
291 No pro-inflammatory effect was induced by PlTX on Caco2 cells. Nevertheless, a slight, but not
292 statistically significant, increase of IL-8 release was observed on A549 cells with PlTX (Figure 7c). The
293 only significant increase of IL-8 release following PlTX treatment was detected at 5 and 10 ng/mL on
294 HaCaT keratinocytes.

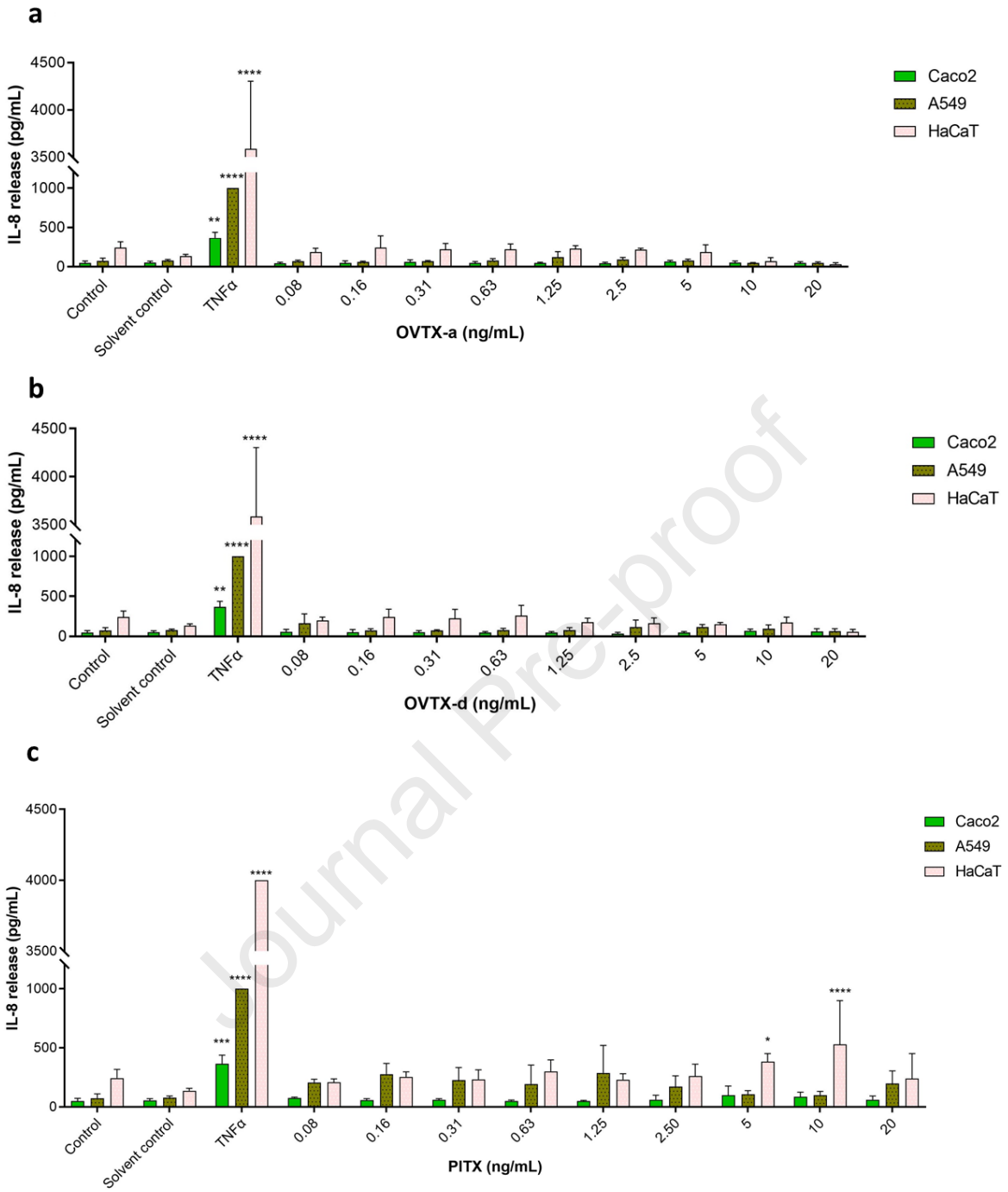


Figure 7: IL-8 release from Caco2, A549 and HaCaT cells following a 24 h treatment with OVTX-a (a) OVTX-d (b) and PlTX (c). Mean ± standard deviation of 3 independent experiments. Statistical significance is indicated by * p≤0.05, ** p≤0.01, *** p≤0.001 and **** p≤0.001. Positive control = TNFα 100 ng/mL

Discussion

In order to assess the level of toxicity of the metabolites produced by *O*. cf. *ovata*, we investigated the toxicity of extracts, fractions as well as purified OVTX-a and –d from two strains of *O.* cf. *ovata* on a panel of cell types. Due to challenges faced during the purification of OVTXs (Gémin 2020), the amount

of purified OVTX-a and –d only allowed performing *in vitro* bioassays. The responses of the different tested chemical samples on cell viability and pro-inflammatory effect using a range of cell lines were compared to the ones obtained with PlTX. The biological assays on the extracts from two strains of *O.* cf. *ovata*, MCCV54 and MCCV55, showed that, irrespective of the cell line, the IC_{50} was always lower for the MCCV55 extract. In fact, the MCCV54 extract contains around twice less OVTX (in PlTX equivalent) than the MCCV55 extract and has also a different toxin profile including a higher percentage of OVTX-a (89 and 58% respectively) in MCCV55 compared to MCCV54 (Gémin et al. 2022). Accordingly, differences from 3 to 5 fold between the IC_{50} of the two extracts were roughly found, except for the A549 cells where the MCCV55 extract showed an even lower IC_{50} (2.5 ng PlTX eq/mL), around 13 fold less that of the MCCV54 extract. This suggests that some metabolites more concentrated in the MCCV55 extract are strongly active on lung cells. These results are in line with a two-fold lower LD_{50} observed for the crustacean *Artemia franciscana* exposed to the same MCCV54 and MCCV 55 strains in another study (Pavaux et al. 2020).

The toxic response observed with the fraction devoid of OVTX (OVTX-) can be explained by the presence of other toxic compounds or OVTXs at concentrations lower than the LOD. A similar conclusion was raised with the same extracts and fractions of *O.* cf. *ovata* tested on *Artemia* (Pavaux et al. 2020). Indeed, besides OVTXs, the strain MCCV54 studied here has been recently shown to produce toxic metabolites of unidentified structure, named liguriatoxin and rivieratoxins (Ternon et al. 2022). Based on their HRMS data, none of these new compounds appear to be close PlTX analogues and, on human NCI-H460 lung cells, these molecules exhibit a far lower toxicity (IC_{50} of 680 and 3120 ng/mL respectively) compared to PlTX (IC_{50} of 0.0446 ng/mL) using the MTT assay (Ternon et al. 2022). A zwitterionic compound, ovataline, was also recently isolated from *O.*cf. *ovata* cultures (Lee et al. 2022) but no information on its cytotoxicity has been published so far. Ostreol A and B, isolated from a South Korean strain of *O.* cf. *ovata*, have been reported to induce respectively toxicity in brine shrimp (Hwang et al. 2013) and cytotoxicity on liver, neuronal and intestinal cells lines (Hwang et al. 2017). However, the *m/z* of these toxins was not detected in the MCCV54 and MCCV55 extracts by LC-MS/MS. The presence of other compounds in the two fractions OVTX- and OVTX+ could explain why there has been an increase in the metabolic activity measured by the MTT assay at the lowest concentrations tested in EGC mostly and, to some point, in HepaRG cells also (see figure 3 a and b). In fact, it has been shown with different toxic agents that formazan formation could be enhanced by increased membrane permeability, mitochondria number and activity (Ghasemi et al., 2021). Such effect can depend on the cell type as well as culture conditions (serum, medium,…), consistent with its observation only on two cell types among the 5 tested in this study.

In this study, OVTX-a and -d exhibit similar IC_{50} values on the different cell types after a 24 h treatment, except on HaCaT cells where OVTX-d was 3.5 fold more potent than OVTX-a. The same IC_{50} was obtained with OVTX-a and -d and with PlTX on lung cells. However, PlTX was slightly more potent on the two nervous cell lines (EGC and Neuro2A cells) while OVTX-a showed 2-times lower IC_{50} on HaCaT cells after 24 h treatment. This is not in agreement with the toxicity reported previously on HaCaT human keratinocytes where a 100-fold difference between OVTX-a and PlTX was described (Pelin et al. 2016b). In order to verify that this discrepancy was not due to a different protocol for cytotoxicity testing in both studies, we compared the toxicity of the 3 toxins with the same design proposed by Pelin et al (2016) that differs for the duration of the treatment (only 4 h) and a higher number of cells seeded. In this case, we did not observe any significant difference in either the response curves or IC_{50} values for OVTX-a, OVTX-d and PlTX (supplementary data, Figure S1 and Table S1). In support with our findings, the *in vivo* study of Poli et al (2018) concluded also to a close toxicity equivalence by inhalation between OVTX-a and PlTX (LD_{50} of 0.031 and 0.041 µg/kg respectively). Indeed, the isomer "AC" (for Adachi Culture) isolated by Poli et al (2018) could not be confirmed to be a derivative of OVTX-a, since no standard is currently available. Although OVTX-a and -d only differ by an oxygen in the fragment B

(Ciminiello et al., 2010), we observed some discrepancies in their response on the different cell types that could be due to differences in affinity for the same cellular target(s) and/or differences in the mechanisms of action. Indeed, OVTX- a and -d may not act with the same affinity on the Na^+/K^+ ATPase, shown as the main target for PlTX (Scheiner-Bobis et al. 2002; Takeuchi et al. 2008). Although OVTX-a differs by only two oxygens to PlTX (Ciminiello et al. 2012), it has been shown that binding affinity of OVTX-a to HaCaT cells was twice lower than PlTX (Pelin et al. 2016b). However, this result may be taken with caution as the same authors reported a 100-fold difference in toxicity on HaCaT cells that we could not confirm. Moreover, the toxins may act through other targets. For example, toxicity of PlTX has been shown to affect intracellular Ca^{2+} and conductive pathway for H^+ (Scheiner-Bobis et al. 2002; Wattenberg 2007). Nevertheless, additional studies are required to elucidate these hypotheses. The absence of difference of IC_{50} values between PlTX and OVTX-a and –d observed on A549 cells is supported by the results of acute toxicity in rats showing a very similar LD_{50} for OVTX-a and PlTX by inhalation (Poli et al. 2018).

Only few toxicity data have been reported on OVTX-a using *in vitro* assays (Pelin et al. 2012; Poli et al. 2018; Gémin et al. 2022). As observed on HaCaT cells, inconsistent results have been obtained when comparing OVTX-a and PlTX in the hemolytic test. Indeed, while Pelin et al (2016b) concluded that PlTX was 10 times more active than OVTX-a, the recent study of Poli et al. (2018) did not confirm this result but described a similar IC_{50} for the two toxins (0.43 ng/mL for PlTX and 0.56 ng/mL for OVTX-a isomer AC). In contrast to OVTX-a, several in vitro studies have been performed with PlTX on a broad range of cell types. A difference in sensitivity to PlTX between the cell lines was reported, with HaCaT keratinocytes and intestinal Caco2 being the most sensitive (Pelin et al. 2018). However, we observed that Caco2 cells were less sensitive to PlTX and OVTX-a and -d, among the cell types included in our study. Nevertheless, it must be emphasized that no cell lines from lung and the nervous system were tested in the previous report (Pelin et al. 2018). PlTX showed an IC_{50} for cell metabolism (Alamar blue) of 1 nM (2.6 ng/mL) after 24h exposure on rat hepatocytes clone 9 (Cagide et al. 2009), a value very close to the IC_{50} we determined on A549 cells. In our study, the IC_{50} for PlTX on Neuro2A cells (0.87 ng/mL) was higher than the ones reported in the literature ranging from 5 pM to 170 pM (0.013 to 0.442 ng/mL) (Ledreux et al. 2009; Kerbrat et al. 2011; Nicolas et al. 2015), which is certainly due to discrepancies in the protocol (number of cells seeded, use of ouabain and veratridine).
In this study, no pro-inflammatory effects were induced by either OVTX-a or –d, although an pro-inflammatory effect with the same two toxins was observed on differentiated Caco2 monolayers at 5 and 1.25 ng/mL respectively (Gémin et al. 2022). The discrepancy of response observed between non-differentiated and differentiated Caco2 cells can be due to a lower number of cells and a lower IL-8 production per cell using non-differentiated cells. Indeed, the release of IL-8 obtained with the positive control (TNFa 100 ng/mL) was twice higher with differentiated Caco2 cells (Gémin et al. 2022) compared to the non-differentiated ones (this study). Nevertheless, the absence of inflammatory response following OVTX-a and -d exposure observed in this study was consistent with the absence of response on non-differentiated Caco2 cells observed with the two *O.* cf. *ovata* extracts as well as with the $OVTX^+$ fraction. This was not the case on differentiated Caco-2 cells as MCCV54 and MCCV55 extracts induced IL-8 release (Gémin et al. 2022). Interestingly, PlTX significantly increased IL-8 release in HaCaT cells as already described (Pelin et al. 2016a). Surprisingly, in A549 cells, OVTX-a and –d did not induce any pro-inflammatory effect, while PlTX and the OVTX+ fraction induced a slight but non-significant response. This is inconsistent with the neutrophilic inflammation reported in nasal epithelium and lungs of rats exposed to PlTX aerosols (Poli et al. 2018), although some discrepancy with the inflammation was reported *in vivo* in lung and liver after aerosol administration of OVTX-a (Poli et al. 2018).

Conclusion

This study provides for the first time a comparison of *in vitro* toxicity for two pure ovatoxins, OVTX-a and –d, as well as *O.* cf. *ovata* extracts and fractions containing or not OVTXs over a broad panel of cell types. The extracts and fractions were shown to be cytotoxic in all the cell lines tested. The fraction containing OVTXs induced IL-8 release only in liver cells, while the fraction not containing OVTXs induced a pro-inflammatory response both in lung and liver cells, suggesting the presence of toxic compounds other than OVTXs. The pure OVTX-a and –d showed a very similar toxic potency compared to PlTX over several cell lines using the MTT assay. Moreover, OVTX-a and -d did not induce any pro-inflammatory effect on the skin, lung and intestinal cells while PlTX increased IL-8 release in keratinocytes, and, to a lesser extent, on lung cells. The A549 cell model showed a rather high sensitivity to OVTX-a and -d in agreement with the toxicity reported by inhalation in rats. The overall results suggest that the targets of OVTX-a and -d are rather ubiquitous. If the Na^+/K^+ ATPase target of PlTX is likely shared with OVTX-a and -d, the differences in pro-inflammatory effect must be explained by other mechanisms. Additional studies are required to investigate the affinity of OVTXs towards the main cellular targets already described for PlTX and to confirm or not their involvement in human intoxications.

References

Aiba T, Ishida K, Yoshinaga M, et al (2005) Pharmacokinetic Characterization of Transcellular Transport and Drug Interaction of Digoxin in Caco-2 Cell Monolayers. Biological and Pharmaceutical Bulletin 28:114–119. https://doi.org/10.1248/bpb.28.114

Bellocci M, Ronzitti G, Milandri A, et al (2008) A cytolytic assay for the measurement of palytoxin based on a cultured monolayer cell line. Analytical Biochemistry 374:48–55. https://doi.org/10.1016/j.ab.2007.10.033

Berdalet E, Pavaux AS, Abós-Herràndiz R, et al (2022) Environmental, human health and socioeconomic impacts of Ostreopsis spp. Blooms in the NW Mediterranean. Harmful algae 119:102320. https://doi.org/10.1016/j.hal.2022.102320

Cagide E, Louzao MC, Espiña B, et al (2009) Production of Functionally Active Palytoxin-like Compounds by Mediterranean Ostreopsis cf. siamensis. Cellular Physiology and Biochemistry 23:431–440. https://doi.org/10.1159/000218190

Chomérat N, Antajan E, Auby I, et al (2022) First Characterization of Ostreopsis cf. ovata (Dinophyceae) and Detection of Ovatoxins during a Multispecific and Toxic Ostreopsis Bloom on French Atlantic Coast. Marine Drugs 20:. https://doi.org/10.3390/md20070461

Ciminiello P, Dell'Aversano C, Dello Iacovo E, et al (2012) Isolation and Structure Elucidation of Ovatoxin-a, the Major Toxin Produced by Ostreopsis ovata. Journal of the American Chemical Society 134:1869–1875. https://doi.org/10.1021/ja210784u

Crinelli R, Carloni E, Giacomini E, et al (2012) Palytoxin and an Ostreopsis toxin extract increase the levels of mRNAs encoding inflammation-related proteins in human macrophages via p38 MAPK and NF-κB. PLoS One 7:e38139. https://doi.org/10.1371/journal.pone.0038139

439 Fernández DA, Louzao MC, Vilariño N, et al (2013) The kinetic, mechanistic and cytomorphological
440 effects of palytoxin in human intestinal cells (Caco-2) explain its lower-than-parenteral oral
441 toxicity. The FEBS Journal 280:3906–3919. https://doi.org/10.1111/febs.12390

442 Gémin M-P (2020) Effets in situ et in vitro des paramètres environnementaux sur l'abondance, le
443 métabolome et le contenu toxinique de l'Ostreopsis d. ovata et purification des ovatoxines

444 Gémin MP, Lanceleur R, Meslier L, et al (2022) Toxicity of palytoxin, purified ovatoxin-a, ovatoxin-d
445 and extracts of Ostreopsis cf. ovata on the Caco-2 intestinal barrier model. Environmental
446 toxicology and pharmacology 94:103909. https://doi.org/10.1016/j.etap.2022.103909

447 Ghasemi M, Turnbull T, Sebastian S, et al (2021) The MTT Assay: Utility, Limitations, Pitfalls, and
448 Interpretation in Bulk and Single-Cell Analysis. Int J Mol Sci. 22(23):12827
449 https://doi.org/10.3390/ijms222312827.

450 Görögh T, Bèress L, Quabius ES, et al (2013) Head and neck cancer cells and xenografts are very
451 sensitive to palytoxin: decrease of c-jun n-terminale kinase-3 expression enhances palytoxin
452 toxicity. Molecular Cancer 12:12. https://doi.org/10.1186/1476-4598-12-12

453 Habermann E (1989) Palytoxin acts through Na+,K+-ATPase. Toxicon : official journal of the
454 International Society on Toxinology 27:1171–87. https://doi.org/10.1016/0041-
455 0101(89)90026-3

456 Hwang BS, Yoon EY, Jeong EJ, et al (2017) Determination of the Absolute Configuration of
457 Polyhydroxy Compound Ostreol B Isolated from the Dinoflagellate Ostreopsis cf. ovata. The
458 Journal of Organic Chemistry 83:194–202. https://doi.org/10.1021/acs.joc.7b02569

459 Hwang BS, Yoon EY, Kim HS, et al (2013) Ostreol A: A new cytotoxic compound isolated from the
460 epiphytic dinoflagellate Ostreopsis cf. ovata from the coastal waters of Jeju Island, Korea.
461 Bioorganic & Medicinal Chemistry Letters 23:3023–3027.
462 https://doi.org/10.1016/j.bmcl.2013.03.020

463 Kerbrat AS, Amzil Z, Pawlowiez R, et al (2011) First evidence of palytoxin and 42-hydroxy-palytoxin in
464 the marine cyanobacterium Trichodesmium. Marine drugs 9:543–60.
465 https://doi.org/10.3390/md9040543

466 Ledreux A, Krys S, Bernard C (2009) Suitability of the Neuro-2a cell line for the detection of palytoxin
467 and analogues (neurotoxic phycotoxins). Toxicon 53:300–308.
468 https://doi.org/10.1016/j.toxicon.2008.12.005

469 Lee S, Moon SJ, Yoo YD, et al (2022) Ovataline: A Polyketide Isolated from the Benthic Dinoflagellate
470 Ostreopsis cf. ovata with 5α-Reductase Inhibitory Activity in RWPE-1 Prostatic Cells. Organic
471 Letters 24:4182–4186. https://doi.org/10.1021/acs.orglett.2c01443

472 Louzao MC, Ares IR, Cagide E (2008) Marine toxins and the cytoskeleton: a new view of palytoxin
473 toxicity. The FEBS Journal 275:6067–6074. https://doi.org/10.1111/j.1742-
474 4658.2008.06712.x

475 Mobasheri A, Avila J, Cózar-Castellano I, et al (2000) Na+, K+-ATPase Isozyme Diversity; Comparative
476 Biochemistry and Physiological Implications of Novel Functional Interactions. Bioscience
477 Reports 20:51–91. https://doi.org/10.1023/A:1005580332144

Nicolas J, Bovee TFH, Kamelia L, et al (2015) Exploration of new functional endpoints in neuro-2a cells for the detection of the marine biotoxins saxitoxin, palytoxin and tetrodotoxin. Toxicology in Vitro 30:341–347. https://doi.org/10.1016/j.tiv.2015.10.001

Paradis C, Labadie M (2022) Ostreopsis : un nouvel envahisseur des plages basques ? Vigil'Anses 17:6–8

Patocka J, Gupta RC, Wu Q, Kuca K (2015) Toxic potential of palytoxin. Journal of Huazhong University of Science and Technology [Medical Sciences] 35:773–780. https://doi.org/10.1007/s11596-015-1506-3

Patocka J, Nepovimova E, Wu Q, Kuca K (2018) Palytoxin congeners. Archives of Toxicology 92:143–156. https://doi.org/10.1007/s00204-017-2105-8

Pavaux A-S, Ternon E, Dufour L, et al (2020) Efficient, fast and inexpensive bioassay to monitor benthic microalgae toxicity: Application to Ostreopsis species. Aquatic Toxicology 223:105485. https://doi.org/10.1016/j.aquatox.2020.105485

Pelin M, Florio C, Ponti C, et al (2016a) Pro-inflammatory effects of palytoxin: an in vitro study on human keratinocytes and inflammatory cells. Toxicology Research 5:1172–1181. https://doi.org/10.1039/C6TX00084C

Pelin M, Forino M, Brovedani V, et al (2016b) Ovatoxin-a, A Palytoxin Analogue Isolated from Ostreopsis cf. ovata Fukuyo: Cytotoxic Activity and ELISA Detection. Environmental Science & Technology 50:1544–1551. https://doi.org/10.1021/acs.est.5b04749

Pelin M, Ponti C, Sosa S, et al (2013) Oxidative stress induced by palytoxin in human keratinocytes is mediated by a H+-dependent mitochondrial pathway. Toxicology and Applied Pharmacology 266: 1-8 https://doi.org/10.1016/j.taap.2012.10.023

Pelin M, Sosa S, Brovedani V, et al (2018) A Novel Sensitive Cell-Based Immunoenzymatic Assay for Palytoxin Quantitation in Mussels. Toxins 10:. https://doi.org/10.3390/toxins10080329

Pelin M, Sosa S, Della Loggia R, et al (2012) The cytotoxic effect of palytoxin on Caco-2 cells hinders their use for in vitro absorption studies. Food and Chemical Toxicology 50:206–211. https://doi.org/10.1016/j.fct.2011.10.032

Pezzolesi L, Guerrini F, Ciminiello P, et al (2012) Influence of temperature and salinity on Ostreopsis cf. ovata growth and evaluation of toxin content through HR LC-MS and biological assays. Water research 46:82–92. https://doi.org/10.1016/j.watres.2011.10.029

Poli M, Ruiz-Olvera P, Nalca A, et al (2018) Toxicity and pathophysiology of palytoxin congeners after intraperitoneal and aerosol administration in rats. Toxicon : official journal of the International Society on Toxinology 150:235–250. https://doi.org/10.1016/j.toxicon.2018.06.067

Satoh E, Ishii T, Nishimura M (2003) Palytoxin-induced increase in cytosolic-free Ca(2+) in mouse spleen cells. European journal of pharmacology 465:9–13. https://doi.org/10.1016/s0014-2999(03)01459-6

Scheiner-Bobis G, Hübschle T, Diener M (2002) Action of palytoxin on apical H+/K+-ATPase in rat colon. European journal of biochemistry 269:3905–11. https://doi.org/10.1046/j.1432-1033.2002.03056.x

Sheridan RE, Deshpande SS, Adler M (2005) Cytotoxic actions of palytoxin on aortic smooth muscle cells in culture. Journal of Applied Toxicology 25:365–373. https://doi.org/10.1002/jat.1080

Takeuchi A, Reyes N, Artigas P, Gadsby DC (2008) The ion pathway through the opened Na(+),K(+)-ATPase pump. Nature 456:413–6. https://doi.org/10.1038/nature07350

Ternon E, Glukhov E, Trytten E, et al (2022) On the Hunt for New Toxin Families Produced by a Mediterranean Strain of the Benthic Dinoflagellate Ostreopsis cf. ovata. Toxins 14:

Vale C, Gómez-Limia B, Vieytes MR, Botana LM (2007) Mitogen-activated protein kinases regulate palytoxin-induced calcium influx and cytotoxicity in cultured neurons. British Journal of Pharmacology 152:256–266. https://doi.org/10.1038/sj.bjp.0707389

Valverde I, Lago J, Vieites JM, Cabado AG (2008) In vitro approaches to evaluate palytoxin-induced toxicity and cell death in intestinal cells. Journal of Applied Toxicology 28:294–302. https://doi.org/10.1002/jat.1278

Wattenberg EV (2011) Modulation of protein kinase signaling cascades by palytoxin. Toxicon 57:440–448. https://doi.org/10.1016/j.toxicon.2010.11.003

Wattenberg EV (2007) Palytoxin: exploiting a novel skin tumor promoter to explore signal transduction and carcinogenesis. Am J Physiol Cell Physiol 292:C24–C32. https://doi.org/10.1152/ajpcell.00254.2006

Statements & Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Conceived and designed the experiments: R.L. and V.F. Performed the experiments: R.L., ALB, L.M and M-P. G. Analyzed the data: R.L. and V.F. Wrote the paper: R.L., M-P. G., D. R. and V.F. Paper corrections: Z.A., E.T. and O.T. Funding: Z.A., O. T., E.T. and V.F.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

Not applicable

Consent to Participate

All authors have participated to the project Ocean15 and consented to integrate the consortium and to work on the collaborative studies that were performed during the project.

559
560 **Consent to Publish**

561 All authors have approved the manuscript before submission and agree to publication.
562

Highlights:

- Extracts of *Ostreopsis* cf. *ovata* were cytotoxic to a broad range of cell lines.
- The fraction not containing ovatoxins induced IL-8 release on liver and lung cells.
- PlTX was more cytotoxic than OVTX-a and –d on cells from intestine and the nervous system.
- A similar IC_{50} was observed on lung cells for the three toxins.
- OVTX-a and –d did not induce IL-8 release on intestinal, lung and skin cells.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: