Effect of mixture of diazinon and benzo[a]pyrene in Glutathione S-transferase of Nile tilapia#

Efeito da mistura de diazinon e benzo[a]pireno na S-transferase da Glutationa em Tilápias do Nilo

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Abstract

Currently, with the growing contamination of aquatic ecosystems, many compounds, such as pesticides and hydrocarbons, are improperly released into rivers and lakes. Studies about the exposure of single pollutants causing biochemical variations are abundant in the literature. However, there are few studies focused on the biological effects of complex mixtures. The aim of this investigation was to evaluate whether mixture diazinon and benzo[a]pyrene can affect the biochemical activities of classic biomarkers such as acetylcholinesterase (AChE), carboxylesterase (CbE), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) in Nile tilapia and compare the effects on enzymatic systems of the exposure to a mixture of compounds and the effects observed when they are exposed separately. We measured the activity of enzymes in gills and liver of Nile Tilapia (*Oreochromis niloticus*) after 2 and 7 days of exposure to Diazinon (0.5 mg/L) and benzo[a]pyrene (1.0 mg/L) individually and in mixture. The results showed that in the mixture group after 7 days of exposure, the benzo[a]pyrene increased the inhibitory action of Diazinon in GST enzyme activity, in liver tissue. This indicates that the toxicity of the interactions between pesticide and polycyclic aromatic hydrocarbon may show synergistic effect. These results suggest the importance of studies with mixture of compounds, because these data will help to understand the results obtained in field studies, such as those from environmental monitoring.

Keywords: Biomarkers, Pharmacological. Benzo[a]pyrene. Diazinon. Cichlids.

Resumo

Atualmente, com a crescente contaminação dos ecossistemas aquáticos, muitos compostos, como pesticidas e hidrocarbonetos, são lançados de forma indevida em corpos d'água. Estudos relacionando a exposição de poluentes, individualmente, com variações bioquímicas são abundantes na literatura. Entretanto, ainda são poucos os voltados às misturas complexas. Sendo assim, o objetivo do presente estudo foi analisar se a mistura diazinon e benzo[a]pireno pode afetar as atividades bioquímicas de biomarcadores clássicos, tais como acetilcolinesterase (AChE), carboxilesterase (CbE), catalase (CAT), glutationa peroxidase (GPx) e glutationa S-transferase (GST) em Tilápias do Nilo (*Oreochromis niloticus*) e comparar os efeitos nos sistemas enzimáticos quando estão expostos à mistura e aos compostos separadamente. Foi medida a atividade das enzimas em brânquias e fígados de Tilápia do Nilo após 2 e 7 dias de exposição ao Diazinon (0,5 mg/L) e ao benzo[a] pireno (1,0 mg/L) individualmente e em mistura. Os resultados mostraram que no grupo mistura do fígado após 7 dias de exposição, o benzo[a]pireno aumentou a ação inibidora do Diazinon na atividade da enzima GST. Isso indica que a toxicidade das interações ambientais entre pesticida e hidrocarboneto policíclico aromático pode apresentar efeito sinérgico. Desse modo, é importante levar em conta esse fator, pois irá auxiliar na compreensão de resultados obtidos em estudos de campo, como em um monitoramento ambiental.

Palavras-chave: Biomarcadores Farmacológicos. Benzo[a]pireno. Diazinon. Ciclídeos.

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INTRODUCTION

The contamination of aquatic environments is of worldwide concern due the accelerated industrial process and the increase of urban and agricultural activities. Many chemicals generated in these processes are launched improperly directly or indirectly in rivers and lakes. Rarely the organisms present in these environments are exposed to a single compound[1,2]. Among the various compounds released in aquatic ecosystems pesticides, polycyclic aromatic hydrocarbons (PAHs) and metals are of major concern.

These substances, once released to the environment, can cause oxidative stress in the animals, which is the imbalance between the formation and elimination of reactive oxygen species (ROS), such as H_2O_2, O^{+2} and hydroxyl radical (HO^{-1}). These species are produced as a result of interactions of the contaminants with molecular oxygen or the interference of the pollutant on processes of electron transfer in redox reactions, or ROS generation during the processes of detoxification[3]. These ROS can oxidize molecules originating damage to DNA, proteins and lipids, changes in metabolism and even cell death[4].

Organisms protect themselves of ROS by increasing the production of antioxidant enzymatic and non-enzymatic systems, through enzymes such as catalase (CAT), glutathione peroxidase (GPx)[5,6,7] and also glutathione S-transferase (GST)[8,9,10,11] a phase II biotransformation enzyme.

There are many studies in the literature that discuss the effects of PAHs and organophosphate pesticides alone on biochemical enzymatic systems of fish[8,12,13,14]. But aquatic animals are continuously exposed to mixture wide range of mixed compounds in their natural habitat and studies focused in understanding how these compounds behave when they interact with other contaminants in the environment are scarce[15,16]. These enzymatic systems may serve as biomarkers for exposure and/or effect of these pollutants.

Thus, studies concerning to the effects on aquatic organisms of complex mixtures are required, since this situation is easily found in aquatic environments, however, there are few studies about it with mixtures of contaminants in the same category.

So, this research tried to clarify the hypothesis that if increasing the concentration of the pesticide diazinon in the presence of benzo[a]pyrene, there will be and inhibition of the known induction effect of B[a]P in GST activity. Previous studies performed in our laboratory showed a small effect on GST enzyme activity in liver of Nile tilapia after 7 days of exposure to the compounds mixed in sub-lethal concentrations[16].

METHOD

Exposure experiments

The Nile tilapia (*Oreochromis niloticus*) were used in the experiment are sexually inverted, length 14.12 ± 0.84 cm and weight 81.75 ± 6.12 g, kept under constant aeration and fed daily with commercial feeds during the research. They were obtained from the "Tilápia do Brasil" fisheries located in Buritama, São Paulo, Brazil.

The study was conducted in eight 40 x 60 x 50 cm aquarium, each containing 120 L of water and six animals per aquarium. The fish were acclimated for one week, before beginning the experiment, and fed daily. The aquariums were kept under constant aeration and photoperiod of 12 h. Two aquarium without contaminants (control), two with B[a]P (0.5 mg/L), two with Dz (1.0 mg/L), and two with B[a]P (0.5 mg/L) + Dz (1.0 mg/L).

B[a]P was added to four of the eight aquariums for a pre-exposure period of three days. After the pre-exposure period, the six fish from one of the B[a]P-exposed aquariums and six fish from one non-exposed aquarium were collected. They were then anesthetized with 2 mL/L of 2-phenoxyethanol, and had their livers, gills and brains collected and frozen at -80 ºC. Afterwards, that Dz was added to the other aquariums. The remaining aquariums in each group were left for another 5 days. This left us with the exposure periods of 2 days (for the first set of aquariums) and 7 days (for the second set of aquariums). Livers and gills were collected of animals and frozen at -80 °C for analysis of biomarkers.

In the end this experiment, before being discharged into a public sewer, water was treated through activated charcoal filters in order to retain toxic waste and the remains of the animals were collected by a company specialized in waste treatment biological.

Sample preparation and enzymatic analysis

The activities of esterases (AChE e CbE) were measured and determined in supernatant. The sample were homogenized in a 0.1 M Tris buffer (1:4, w:vol), pH 8.0. Acetylthiocholine and phenylthioacetate were used as substrates for the activities of AChE and CbE, respectively and the sample was centrifuged at 30,000 g for 30 min at 4 ºC[17]. The supernatant fraction was collected and frozen at -80 ºC.

For CAT, GPx and GST analysis, the samples were weigh and homogenized (1:4, w:vol) in a phosphate 0.1 M butter and 1 mM protease inhibitor (PMSF). The samples were centrifuged at 9,000 g for 20 min at 4 °C. The supernatant portion was collected and centrifuged at 50,000 g for one additional hour at 4 ºC, to obtain the microsomal and cytosolic fractions. The enzymatic activities of CAT, GPx and GST were analyzed in the cytosolic fraction.

The activity of CAT was determined at 240 nm at 30 ºC by the H_2O_2 decomposition velocity. The liver sample were diluted (1:9, vol:vol) in Tris-HCl 20 mM butter pH 7.5[18]. GPx activity was assayed at 340 nm by the oxidation of NADPH at 30 ºC[19]. GST activities was measured at 340 nm, using 1-chloro-2.4--dinithrobenzene (CDNB) and reduced glutathione as substrates[20]. Protein levels in AChE, CbE, CAT, GPx and GST extract were measured using bovine serum albumin (BSA) as standard at 595 nm[21].

Statistical Analysis

As the data were all non-parametric, the Mann Whitney test was used to identify significant differences between control groups, pre exposed and 7-day exposed groups. For the 2-day exposed groups the Kruskal Wallis test was employed, followed by the Tukey's multiple comparison, for comparing all treatments. The analyses were made with the aid of Statistica 7.0 software. Only values $p < 0.05$ was accepted as significant for indicating statistical differences.

RESULTS

Table 1 show activities of AChE, CbE, CAT, GPx and GST in gills of *O. niloticus* exposed to B[a]P, Dz and Dz+B[a]P after 2 and 7 days. AChE activity was decreased after 2 and 7 days to Dz and Dz+B[a]P when compared to the respective control. After 2 days, we also observed decreasing in enzymatic activity in fish group exposed to mixture when compared to B[a]P alone. Moreover, the mixture Dz+B[a]P caused significant decrease in CbE activity after 2 days when compared to the group control and B[a]P alone. No differences were observed in activities of oxidative stress enzymes throughout the experiment for any chemical tested and any time, however, after 7 days, exposure to Dz alone caused decreasing in CAT activity levels were decreased to Dz exposure when compared to the respective control. GST activity increased in fish exposure to B[a]P after all exposure time, as well as to Dz+B[a]P in the 2 periods, when compared control and the enzymatic activity to Dz+B[a]P was also increased when compared to the Dz exposure.

Table 1. Enzymatic activities measured in the gills of *O. niloticus* exposed to B[a]P and Dz

Enzyme	Group	pre-exposure	2 days after	7 days after
AChE	Control	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01
	B[a]P	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00
	Dz	-	0.01 ± 0.00[a]	0.01 ± 0.00[a]
	Dz+B[a]P	-	0.01 ± 0.00[a,b]	0.01 ± 0.01[a]
CbE	Control	0.09 ± 0.03	0.10 ± 0.05	0.09 ± 0.04
	B[a]P	0.09 ± 0.01	0.10 ± 0.03	0.09 ± 0.02
	Dz	-	0.06 ± 0.03	0.07 ± 0.01
	Dz+B[a]P	-	0.05 ± 0.01[a,b]	0.09 ± 0.04
CAT	Control	4.64 ± 1.58	5.34 ± 0.72	6.53 ± 1.55
	B[a]P	4.93 ± 2.11	5.96 ± 2.63	4.42 ± 1.03
	Dz	-	6.17 ± 1.61	4.16 ± 1.17[a]
	Dz+B[a]P	-	5.37 ± 1.80	5.20 ± 1.63
GPx	Control	0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.00
	B[a]P	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.00
	Dz	-	0.01 ± 0.00	0.02 ± 0.01
	Dz+B[a]P	-	0.01 ± 0.00	0.01 ± 0.00
GST	Control	0.07 ± 0.02	0.09 ± 0.04	0.13 ± 0.05
	B[a]P	0.21 ± 0.08[a]	0.20 ± 0.07[a]	0.25 ± 0.08[a]
	Dz	-	0.08 ± 0.03	0.09 ± 0.02
	Dz+B[a]P	-	0.20 ± 0.05[a,c]	0.23 ± 0.01[a,c]

Activity expressed in U/mg protein (AChE, CbE, CAT, GPx and GST), data expressed in means ± standard deviation. a. Significant difference compared to the respective control; b. Significant difference between B[a]P and Dz+B[a]P at the same exposure time; c. Significant difference between Dz and Dz+B[a]P at the same exposure time.

Enzymatic activities measured in the liver are presented in Table 2. In this tissue, Dz was able to decrease AChE activity when alone and when in combination with B[a]P only after 2 and 7 days of exposure when compared to control. However, to Dz+B[a]P the activity was also decreased compared to B[a]P. After 2 days, CbE activity showed inhibition in all group when compared to control. Antioxidant enzymes activities were not affected for any chemical tested and any time. The exposure to Dz+B[a]P for 7 days caused decrease in GST activity when compared to control.

Table 2. Enzymatic activities measured in the liver of *O. niloticus* exposed to B[a]P and Dz

Enzyme	Group	pre-exposure	2 days after	7 days after
AChE	Control	0.71 ± 0.43	0.66 ± 0.34	0.54 ± 0.20
	B[a]P	1.27 ± 0.72	0.60 ± 0.22	0.46 ± 0.18
	Dz	-	0.08 ± 0.02[a]	0.11 ± 0.03[a]
	Dz+B[a]P	-	0.06 ± 0.02[a,b]	0.12 ± 0.08[a,b]
CbE	Control	2.16 ± 0.67	3.11 ± 1.41	2.39 ± 0.99
	B[a]P	2.37 ± 0.53	1.66 ± 0.68[a]	1.58 ± 0.63
	Dz	-	1.01 ± 0.31[a]	1.37 ± 0.42
	Dz+B[a]P	-	0.82 ± 0.22[a]	1.75 ± 0.75
CAT	Control	62.43 ± 11.40	65.80 ± 17.87	65.92 ± 9.51
	B[a]P	68.79 ± 20.17	70.28 ± 20.88	75.36 ± 29.24
	Dz	-	68.81 ± 16.44	59.71 ± 9.12
	Dz+B[a]P	-	65.45 ± 16.00	42.92 ± 2.48
GPx	Control	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00
	B[a]P	0.02 ± 0.02	0.01 ± 0.01	0.02 ± 0.00
	Dz	-	0.01 ± 0.00	0.02 ± 0.00
	Dz+B[a]P	-	0.01 ± 0.01	0.01 ± 0.01
GST	Control	1.83 ± 1.12	2.01 ± 0.41	1.63 ± 0.68
	B[a]P	1.34 ± 0.53	1.44 ± 0.79	1.29 ± 0.35
	Dz	-	1.94 ± 1.10	1.34 ± 0.36
	Dz+B[a]P	-	1.55 ± 0.37	0.60 ± 0.08[a]

Activity expressed in U/mg protein (AChE, CbE, CAT, GPx and GST), data expressed in means ± standard deviation. a. Significant difference compared to the respective control; b. Significant difference between B[a]P and Dz+B[a]P at the same exposure time; c. Significant difference between Dz and Dz+B[a]P at the same exposure time.

DISCUSSION

In general, in tilapias, the B[a]P and Dz alone have typical responses[16]. Dz exposure causes strong inhibition of AChE activity in gills and liver after 2 and 7 days and reduction of CbE activity in both tissues. Dz and B[a]P, when exposed separately, do not cause any significant effect in antioxidant stress enzymes CAT and GP_x. B[a]P caused a decrease in GST activity in liver and stronger increase in the gills, however, in tilapia exposed to the mixture, we observed an inhibitory effect of Dz in the activation of the enzyme by B[a]P in the gills.

Regarding to GST activity, we noticed that after 2 days, B[a]P caused an induction of GST activity in gills when compared to the control. When Dz was added to B[a]P the enzyme was inhibited in comparison with the control, and in the mixture the enzyme activity also decreased compared to Dz exposure in gills. In liver, this occurred only after 7 days of exposure. These results clearly indicate that Dz was able to interfere on the induction of GST promoted by B[a]P. The increase in concentration of Dz in the mixture shows an increasing of the inhibitory effect of this pesticide in the GST activity even with the presence of B[a]P, corroborating our hypothesis.

The methyl parathion, an organophosphate insecticide, induced GST activity in muscle and gills of the freshwater fish characid (*Brycon cephalus*) after 96 h of exposure, indicating the activation of defense mechanism that neutralized the effects of methyl parathion and suggested that GST could be a possible biomarker to organophosphate[22]. Moreover, GST activity varied in tasmanian lacewing (*Micromus tasmaniae*) after exposure to chlorpyrifos and Dz, indicating that this biomarker could be problematic for this type of exposure[23]. In the present study, we can suggest that, even when the activity of GST doesn't change, it does not indicate that pollutants are not present, because there is a possibility that a mixture of then can cause interference in induction and inhibition of this enzyme.

B[a]P alone did not cause effect in esterases activity. However, for AChE activity after 2 and 7 days, in both gills and liver, as well as for CbE activity after 2 days, the inhibition was the same to Dz and Dz + B[a]P exposure. Probably the B[a]P helped in the inhibitory effect of Dz on AChE and CbE enzymes. One study reported a decrease in esterase activity after exposure to a PAH (naphthalene), and suggested that the reduction in the activity of esterase enzymes could be due to changes in lipid metabolism, generated to exposure[24,25]. The esterase enzymes also aid the breakdown of lipids in the fatty acid chain, generating energy for other porpuses[26,27]. Lipid activity was not measured in this study, such suggestions could be responsible to inhibition the enzyme activities in the mixture.

CONCLUSION

Due the variety of compounds in aquatic environments is important to study the interaction of these substances in biochemical parameters. As can be observed, our research supports a better understanding of enzymatic responses to intoxication in tilapia by a mixture of organophosphate pesticide and a PAH when compared to these compounds separately.

This study identified the effect in GST activity after 7 days of exposure to Dz+B[a]P. Dz was able to decrease GST activity in the liver of tilapia when in combination with B[a]P, commonly, GST is induced by B[a]P alone in fish. These results have great relevance in environmental monitoring studies contaminated by organophosphate pesticides and PAHs.

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