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Tamoxifen Affects the Toxicokinetics of *o,p′*-DDT in Male Nile Tilapia (*Oreochromis niloticus*)

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Abstract The goal of the present study was to evaluate the role of Tamoxifen (TAM), in the distribution and/or elimination kinetics of *o,p′*-DDT, in male tilapias. A non-compartmental analysis was chosen to describe the time course of *o,p′*-DDT plasma concentrations. Mean plasma concentration of *o,p′*-DDT following IP administration indicates a very complex kinetic profile. Tamoxifen decreased the *o,p′*-DDT mean half-life (t½) from 20.38 to 16.11 days, the Mean Residence Time (MRT) from 28.7 to 23.23 days, and clearance (CL) from 0.0031 to 0.001 mL/min. The distribution pattern of *o,p′*-DDT in tissues and the clearance in plasma suggest that storage points mediated through the membrane-receptor lipophilicity can be involved.

Keywords *o,p′*-DDT · Tamoxifen · Tilapia · Toxicokinetic · Non-compartmental analysis

The Dichlorodiphenyl-trichloroethane (DDT), its analogs and other xenobiotics act as endocrine disruptors (EDs) (Robison et al. 1985; Colborn et al. 1993). There is increasing evidence showing that many xenobiotics cause reproductive dysfunctions by altering the reproductive endocrine system. Several studies in vivo have shown that the effects of the EDs are mainly related with their ability to interact with the oestrogen receptor (Jensen et al. 1995). An important mechanism of exposure to xenobiotics for fish during early life stages is through the maternal transfer of hydrophobic contaminants sequestered in adult lipid reserves. Sequestration of xenobiotics in tissues is related with their affinity to a particular macromolecule (proteins, nucleic acids, etc.) but is also related to its lipid solubility (octanol–water partition coefficient, K_{ow} (Mühelbach et al. 1985). In particular teleost fish have several major storage sites for lipophilic sustances, such as the liver, red muscle and lipid deposits (largely triacylglycerols). Lipid deposits can be found subcutaneously and in the abdominal cavity, often associated with the intestine and the pyloric ceca (Evans 1997).

Tamoxifen (TAM) is a non-steroidal modulator at the oestrogen receptor level. It has mixed oestrogenic and antioestrogen properties, which depend on the oestrogen receptor (ER) receptor subtype (ERα, ERβ) present in the cellular environment. The recent study of Leaños-Castañeda et al. (2004) showed that the concomitant *o,p′*-DDT + Tamoxifen treatment was associated with a decrease in the blood levels of *o,p′*-DDT in male tilapias. It is possible that this effect could be related with TAM-induced changes in the properties of the lipidic membranes of the *o,p′*-DDT-storage tissues, and also that the inhibition by TAM of the oestrogenic effect of *o,p′*-DDT is at least partially due to the eduction in *o,p′*-DDT levels. Interactions at this level may be reflected in the time course of the compound in the organisms with consequent changes in distribution and elimination patterns. Therefore the aim of this study was to compare the disposition kinetics of *o,p′*-DDT with and without Tamoxifen.

Materials and Methods

In this experiment were used Tamoxifen citrate salt (95% pure) and heparin sodium salt were purchased from Sigma

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(Mexico). *o,p′*-DDT (1-(2-Chlorophenyl)-1-(4-phenyl)-2,2,2 trichloroethane) was purchased from Lancaster Synthesis Inc., (97% pure) (Windham, NH). Chromatography standards of *o,p′*-DDT (1,1,1-Trichloro-2-(p-chlorophenyl)-2-(o-chlorophenyl) ethane) and Endosulfan II were purchased from AccuStandard (New Haven, CT). All chemicals were used as received. The experimental unit was Male Nile Tilapia (*Oreochromis niloticus*) were bred and raised from broodstock kept at CINVESTAV in Merida, Yucatan, Mexico. Sexually mature male tilapias weighing between 200 and 250 g were randomly divided into three experimental groups of 60 males each. One group of fish was injected intraperitoneally with a single dose of *o,p′*-DDT (20 mg/kg), while another group was injected with the same dose of *o,p′*-DDT plus TAM citrate (5 mg/kg, one dose). A third group was injected only with corn oil (vehicle). For all specimens, blood samples were taken from the caudal vein. Later of *o,p′*-DDT was extracted from plasma samples as described by Ungerer and Thomas (1996). Samples were analyzed by gas chromatography using a Hewlett Packard Model 5890 Series II gas chromatograph with electron capture detection. The separation was performed isothermally on a 30 m × 0.25 mm (0.25 mm film) column (Supelco PTE-5). Helium was used as a carrier gas at a flow rate of 1 ml min^{-1}. The injector temperature was set at 275°C, the oven temperature at 100°C and the detector temperature at 325°C. Pure *o,p′*-DDT and Endosulfan II were used as standards to prepare calibration curves for each compound. Samples (1 g) of intestine, brain, muscle and liver at 12, 24 and 168 h after treatment application were extracted according to procedures described by Sericano et al. (1990) and Wade et al. (1993). Organic compounds were separated and quantified by gas chromatography using a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a DB-5 30 m column and an electron capture detector.

Pharmacokinetic parameters were calculated from plasma concentration–time data using non-compartmental analysis. All kinetic analyses were performed using the nonlinear interactive program PkCalc (Shumaker and PkCalc 1986).

Results and Discussion

The intestine and the liver showed the highest concentrations of *o,p′*-DDT at all the times studied (Fig. 1a), with a reduction in the levels of *o,p′*-DDT at the seventh day following the *o,p′*-DDT + TAM treatment, with respect to the group injected only with *o,p′-DDT*, with the exception of the liver (Fig. 1b). At 12 h after treatment there were no statistically significant differences in *o,p′*-DDT concentrations in the tissues with respect to the TAM treatment, except for intestine ($p < 0.0019$); however, after seven days (168 h) there

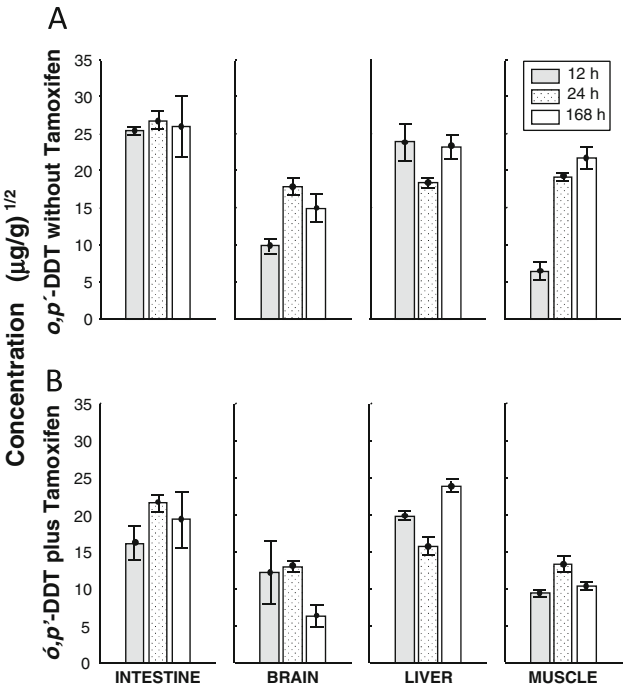

Fig. 1 **a** Concentration of *o,p′-DDT* in tissues of male tilapia (*O. niloticus*) of the *o,p′*-DDT group without TAM, at 12, 24 and 168 h after treatment. **b** Concentration of *o,p′-DDT* in tissues of male tilapia (*O. niloticus*) of the *o,p′*-DDT plus TAM group, at 12, 24 and 168 h after treatment

were statistically significant differences in *o,p′*-DDT tissue concentrations in the intestine, brain and muscle ($p < 0.023$, 0.0035, and 0.00019, respectively), but not for the liver. The present data suggest a preferential accumulation of DDT in intestine and liver. The high lipid content of these tissues, together with its high blood irrigation could explain the preferential accumulation of *o,p′*-DDT observed in this study.

A decrease in the concentration of *o,p′*-DDT in tissues was generally observed in the *o,p′*-DDT + TAM group. This decrease was more evident at the end of the experiment (168 h post-treatment.) The opposite effect was observed in *o,p′*-DDT concentrations in blood in the presence of Tamoxifen. This could be explained by an increase in blood lipid levels due to Tamoxifen's oestrogenic effect and increase in triglyceride levels from a decrease in lipolytic enzymes (Marcos et al. 2005). The increase found in this work is contrary to the results found by Leaños-Castañeda et al. (2007), who found a decrease in plasma *o,p′*-DDT when co-administered with Tamoxifen.

Variance equality was tested by Bartlett's test, and they were found to be statistically different ($p < 0.05$), thus a noncompartmental analysis was chosen to describe the time course of *o,p′*-DDT plasma concentrations. It can be used to estimate basic pharmacokinetic parameters, even when the data are highly variable (Kleinow et al. 2008).

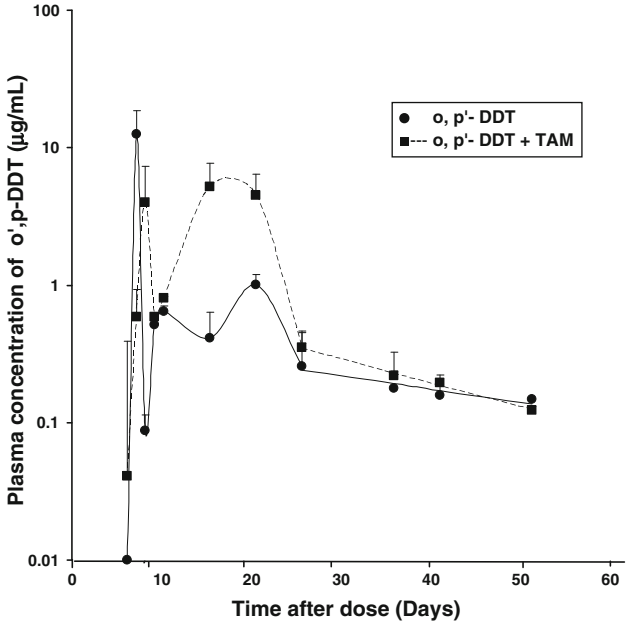

Fig. 2 Mean plasma concentration time-profiles of *o,p′-DDT* after a single intraperitoneal dose of 20 mg/kg with and without Tamoxifen (5 mg/kg) to male tilapias (*Oreochromis niloticus*). Each point represents the mean of three fish. (±SE)

Figure 2 shows the time-course of the mean plasma *o,p′*-DDT concentration obtained from a single IP injection to male tilapias. In the same figure, the time-course of the test compound following the concomitant administration of Tamoxifen is also shown. Each time point is the mean of three fish. The main kinetic parameters estimated by a non-compartmental kinetic analysis are shown in Table 1.

Visual examination of the mean plasma concentration of *o,p′*-DDT curve following IP administration to tilapias indicates a very complex kinetic profile. A lag time of six days is manifest. At the sixth day after treatment, a very sharp increase in the plasma concentration of *o, p′*-DDT, followed by a rapid decline was observed (first peak $t_{max} = 7$ days, $C_{max} = 12.61$ µg/ml; second peak: $t_{max} = 20$ days, $C_{max} = 1.26$ µg/ml). Afterward, fluctuations in the form of several minor peaks were displayed. In the *o,p′*-DDT + TAM treatment, a similar kinetic behavior was observed. However, differences respect to the *o,p′*-DDT treatment alone were evident in the t_{max} and in the magnitude of the peaks (first peak: $t_{max} = 8$ days, $C_{max} = 4.01$ µg/ml; second peak: $t_{max} = 15$ days, $C_{max} = 3.68$ µg/ml). The first peak in both treatments after a prolonged lag-time may indicate a rapid initial uptake of *o,p′-DDT* in the tissues followed by a sudden release of the compound from some depot site.

The secondary peaks may be associated with a slower desorption process from the storage tissues mainly from intestine or liver. In the mixed treatment this second process is very remarkable, which suggests a probable mobilization of DDT from the adipose storages induced by TAM. The results from the non-compartmental modeling for *o,p′*-DDT treatment showed a rate constant of terminal elimination (k_{el}) of 0.034 d^{-1}, with a corresponding terminal elimination half-life of 20.38 days. The observed volume of distribution (Vd_{ss}) were 0.139 L, and the systemic clearance (Cl) was 0.0031 ml/min; the $AUC_{0-\infty}$ Was 6.66 (µg d/ml) and the mean time of residence in the body (MRT) was 28.7 days. For the *o,p′*-DDT + TAM treatment, the terminal elimination constant (k_el) was 0.043 d^{-1}, and the corresponding terminal elimination half-life was 16.11 days. The volume of distribution (Vd_{ss}) was 0.0471 L the systemic clearance (Cl) 0.001 ml/min, the $AUC_{0-\infty}$ was 14.21 (µg. d/ml) and the MRT was 23.23 days.

The kinetic parameters of *o,p′*-DDT in fishes obtained in the present study revealed that this compound shares some characteristics observed with other DDT analogs. A rapid

Table 1 Toxicokinetic parameters of *o,p′-DDT* following a single i.p. dose of 20 mg/kg, with or without Tamoxifen (5 mg/kg) to male tilapias (*O. niloticus*)

Toxicokinetic parameter	Treatments	
	o,p′-DDT	*o,p′*-DDT + TAM
Elimination constant (k_{el}, d^{-1})	0.034	0.043
Mean half life ($t_{1/2el}$)	20.38	16.11
Area under the curve ($AUC_{0-\infty}$, µg. d/mL)	6.66	14.21
Area under the curve-moment ($AUMC_{0-\infty}$, µg. d^2 mL)	309.86	475.44
Mean residence time (MRT, d)	28.7	23.23
Apparent volume (Vd_{ss}, L)	0.139	0.0471
Clearance (Cl, mL/min)	0.0031	0.001
Time for maximum (T_{max}, 1st peak, d)	7	8
Maximum concentration (C_{max} (1st peak, µg/mL)	12.61	4.01
Time for maximum (T_{max}, 2nd peak, d)	20	15
Maximum concentration C_{max} (2nd peak, µg/mL)	1.26	3.68

Each parameter was calculated from the mean plasma concentration time profile of three fish

uptake by tissues is demonstrated because the first day after administration, no measurable amounts of *o,p′-DDT* were found in blood, while significant quantities were observed in all other tissues analyzed (Figs. 1, 2). In addition to the high lipophilicity of this compound and the high affinity by adipose tissues, some anatomical and physiological peculiarities in fishes could also explain this particular distribution. It has been demonstrated that the distribution of xenobiotics at early time points may be related with the route of administration. Thus, xenobiotics absorbed from the gastrointestinal tract or administered intraperitoneally are transported first to the liver by the hepatic portal vein and then to the gills via the ventral aorta before distributing into the general circulation (Kleinow et al. 2008). Consequently, elimination pathways functioning in the gut, liver and gills could have affected the initial disposition of *o,p′*-DDT in this experiment, since it was administered intraperitoneally.

The *o,p′*-DDT long terminal half-lives (t½), low body clearance (Cl), and long mean residence time (MRT) in the body were consistent with its lipophilic characteristics. It was postulated that this characteristic is determinant for the sequestration of DDT by the lipidic portion of the tissue membranes, limiting its movement. Since the metabolism of DDT in fish is very limited (Pritchard et al. 1973), this sequestration is the main factor that could explain the prolonged stay of *o,p′*-DDT in this study. The wide distribution of *o,p′-DDT* in the total body of the fish and the high sequestration by several tissues was also reflected in the high value of the volume of distribution (Vd_{ss}).

In the present study the co-administration of Tamoxifen notably changes the kinetic profile of *o,p′*-DDT with respect to the treatment without TAM. The terminal half-life (t½) and the mean residence time (MRT) are noticeably shortened. In addition, the presence of a marked double-peak in the plasma concentration–time curve is observed, which is corroborated with a higher area under curve ($AUC_{0-\infty}$) value. All of these changes are showing an important interaction of TAM at the *o,p′*-DDT storage sites. Although further studies are needed to elucidate the real mechanism of this interaction, the possibility that TAM might facilitate the mobilization of *o,p′*-DDT from the deposit sites deserves special consideration. The influence of TAM in several aspects of lipid metabolism in cell and tissues has been recently demonstrated (Clarke et al. 2001).

The same as DDT, Tamoxifen is also a lipophilic compound and it is sequestered within the bilayer of lipid membranes (Clarke et al. 1990). A competition by lipid sites within the lipidic membranes, in addition to a secondary alteration in the membrane structure induced by TAM, all may contribute to displacement of *o,p′-DDT* from its adipose stores.

The side effects of Tamoxifen are related to differential oestrogenic antoestrogenic properties at various target tissues (Jordan 1993). Amongst the antioestrogenic effects of Tamoxifen is the modification of the kinetics of *o,p′*-DDT in male tilapia tissues. This has been explained via the inhibition of the oestrogenic effect of *o,p′*-DDT by the antioestrogenic effect of Tamoxifen via the oestrogen receptor (Leaños-Castañeda et al. 2007).

The distribution pattern of *o,p′*-DDT in tissues and the clearance in plasma suggest that storage points mediated through the membrane-receptor lipophilicity can be involved, because the results obtained here for the non-compartmental kinetics, such as mean half-life (t½), mean residence time (MRT) and clearance (CL) correspond to the lipophilic characteristics of both Tamoxifen and *o,p′*-DDT.

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