Original Article

INFLUENCE OF DEXAMETHASONE AND LEVAMISOLE ON MACROPHAGE RECRUITMENT, GIANT CELL FORMATION AND BLOOD PARAMETERS IN THE TROPICAL FISH *Piaractus mesopotamicus*

INFLUÊNCIA DA DEXAMETASONA E LEVAMISOL NO RECRUTAMENTO DE MACRÓFAGOS, FORMAÇÃO DE CÉLULAS GIGANTES E PARÂMETROS HEMATOLÓGICOS NO PEIXE TROPICAL Piaractus mesopotamicus

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ABSTRACT: The aim of this study was to evaluate the effect of parenteral administration of dexamethasone and levamisole on the accumulation of macrophages and formation of giant cells in chronic inflammation by foreign body and blood parameters in pacu (*Piaractus mesopotamicus*). It was used 50 mg/kg of levamisole and 2.0 mg/kg of dexamethasone and the combination of both drugs. Coverslips were implanted under the skin. After 2, 7, and 15 days post-implantation (DPI), fish were anesthetized for removal of coverslips and the number of macrophages and giant cells were count. It was also collected blood from the caudal vessel to evaluate: red blood cell count, hematocrit, hemoglobin concentration, leukocytes count, thrombocytes count, MCV, and MCHC. We observed that dexamethasone affects negatively the formation of giant cells in the chronic inflammation for foreign body. Levamisole, despite being immunostimulatory in several species, showed limited action. However, it was enough to counteract the effect of dexamethasone; the association of the drugs did not interfere significantly in erythrocyte and leukocyte number in most of the treatments and times studied. In dexamethasone group there was a reduction in the number of erythrocytes and hemoglobin associated with increased mean corpuscular volume, suggesting slight macrocytic anemia. At 15 DPI, most groups showed the recovery of hematologic response. As in mammals, dexamethasone affects negatively the inflammatory response. Levamisole showed little effect by itself. However, in some parameters the association of both drugs causes similar response to control and naïve groups, showing the antagonistic effect of these drugs.

KEYWORDS: Glucocorticoid. Coverslips. Inflammation. Pacu.

INTRODUCTION

Inflammatory cells migration to injured areas is likely the main event of the inflammation process to protect the body against foreign elements. In this process, multinucleated giant cells (MGCs) play an important role in cellular defense mechanisms to eliminate foreign bodies (WADA et al. 1996, KUMAR et al., 2004). These cell formations involve the extraneous matter attempting to isolate it, creating a physical barrier. This stage is known as foreign body-type multinucleated giant cell. After, nuclei are arranged in the periphery of the cytoplasm forming Langhans cells (MACLAUCHLAN et al., 2009).

This cell type has been described in *Francisella* infection in saltwater fish (OLSEN, et al., 2006), oomycete infection in freshwater fish (OIDTMANN et al., 2008), *in vitro* model (COUSO et al., 2002), and *in vivo* model (BELO et al., 2005, 2012). Petric et al. (2003a) showed that the implantation of glass coverslips in the subcutaneous tissue of *Piaractus mesopotamicus* induces the formation of MGCs. However, the ability of MGCs to eliminate foreign bodies seems to vary between fish conditions and the effects of common drugs in this process is unknown (JOHNSON et al. 2004, BELO et al., 2012).

Dexamethasone (DEX), a synthetic glucocorticoid, is one of the drugs most commonly used in humans and veterinary medicine as anti-inflammatory. It inhibits phagocytosis, vasodilatation and chemotaxis (RYAN et al., 2011) due to the synthesis of lipocortin-1, which blocks the action of fosfolipase A2 (CHATTOPADHYAY

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et al., 2010), TNF-α, and interleukins 1, 2, and 6 (SONG et al., 2005).

Levamisole (LEV), an antihelminthic and immunomodulator drug, is widely used in veterinary medicine as an antiparasitic. In fish, Li et al. (2006) observed that oral administration of LEV stimulates nonspecific defense mechanisms and the specific immune response against *Acinetobacter iwoffii* in *Clarias fuscus*. Findlay et al. (2000) showed that Atlantic salmon treated with LEV bath enhances the resistance to amoebic gill disease and stimulate the nonspecific immune system. Bich Hang et al. (2014) suggested that LEV increase the immune response in striped catfish (*Pangasianodon hypophthalmus*) against infection by *Edwardsiella ictaluri*. Gopalakannan & Arul (2006) claimed that 250 $mg.kg^{-1}$ of diet for 90 days enhances the nonspecific immunity, considering lysozyme, white blood cell count and NBT assay, of *Cyprinus carpio* acting as an immunostimulant, which appear to improve the immune status of the fish.

The aim of the current study was to evaluate the effects of DEX and LEV on the kinetics of the accumulation of macrophages, formation of MGCs and blood parameters in the chronic inflammation by foreign body in the farmed fish pacu (*Piaractus mesopotamicus*).

MATERIAL AND METHODS

This experiment was carried out with 105 pacus (*Piaractus mesopotamicus*) (200.71 ± 26.7 g weight and 18.27 ± 1.6 cm long) in 15 tanks (200L) with water flow of 1 L/min and continuous aeration randomly distributed according Table 1 (n=7). Water quality was measured weekly and remained at the comfort zone of this species (DO = 5.03 mg/L, T^o = 29.1°C, pH = 6.8, and Ce = 117.9 μS/cm) (Boyd, 1990). It was used a multi-parameter probe (YSI Model MPS 556, Chicago, US).

Fish were acclimated during two weeks prior the evaluation. It was used commercial feed for pacu (28% CP and 4.000 kcal/ kg DE). Fish fed twice daily (3% of biomass). This study was approved by the local ethics committee (Universidade Estadual Paulista - Unesp, FCAV Ethics committee: 023025/11).

All fish were pre-anesthetized in aqueous solution of benzocaine (1: 20.000) and then anesthetized at concentration of 1:10.000 (WEDEMEYER, 1970) to implant the glass coverslip and administration of the drugs. The implant was performed according to the methodology of Petric et al. (2003). Briefly, it was made a 1 cm skin incision in the dorsal-lateral region of the fish, behind the operculum and close to the insertion of the dorsal fin. A rounded glass coverslip (9 mm diameter) was placed in the subcutaneous tissue and then the skin was sutured to close the incision. Finally, intramuscular injections of dexamethasone and/or levamisole were applied according Table 1.

Table 1. Distribution of fish in the treatments

Experimental groups	Days post-implantation (DPI)		
	2	7	15
C: Control	n=7	n=7	n=7
NV: Naïve	n=7	n=7	n=7
LEV	n=7	n=7	n=7
DEX	n=7	n=7	n=7
DEX+LEV	n=7	n=7	n=7

LEV: Levamisole (Ripercol®, Fort Dodge) at dose of 50 mg/Kg; DEX: Dexamethasone 0.2% (Azium®, Intervet Schering-Plough) at dose of 2 mg/Kg.

Dexamethasone dose (2.0 mg/kg) used in this experiment was previously tested in this species in our laboratory (CLAUDIANO et al., 2013). To determinate the dose of levamisole it was performed an essay with four doses (25, 50, 100, and 200 mg of levamisole/kg of fish) and was evaluated the total number of leukocytes in blood. Hence, it was determinate that 50 mg/Kg cause

leukocytosis two hours after application. Thus, this dose was used in the experiment. Control group (CG) had subcutaneous implant of a coverslip but was not injected with the drugs. Naïve group (NV) was not injected with any drugs and had no glass implantation.

Two, seven and fifteen days after implantation of the coverslips, seven fish per group and time were euthanized by immersion in an aqueous solution of benzocaine (1:500). Blood samples were collected from the caudal vessel in tubes containing EDTA (10%) to obtain erythrocyte count, hematocrit, and hemoglobin concentration (YUNIS-AGUINAGA et al., 2016). Subsequently, we calculated the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) (YUNIS et al., 2015)

Total and differential leukocyte counts were performed on blood smears stained with May-Grünwald-Giemsa-Wright (TAVARES-DIAS; MORAES, 2003). The identification and nomenclature were performed according to Tavares-Dias et al. (1999). The values for total number of leukocytes and thrombocytes were calculated by indirect method counting 2000 red blood cells, according by the equation: Total number of leukocytes or thrombocytes (μL) = [(number of leukocytes or thrombocytes counted in the smear) x (erythrocyte global count per μL)]. 2000 / number of erythrocytes counted in the blood smear.

Finally, coverslips were carefully removed and washed with 0.9% of saline solution, fixed in Bouin solution for 1 minute and stained with hematoxylin-eosin (HE). The number of macrophages and nuclei of MGCs were counted using an image analyzer Videoplan KS100, (Carl Zeiss, Sao Paulo, Brazil), in forty randomly chosen fields, using 400X of magnification. All data were transformed [log (x+1)] and subjected to analysis of variance (ANOVA). Tukey test was used to compare means ($p<0.05$). Data transformations were used according to pertinence.

RESULTS AND DISCUSSION

Multinucleated giant cells (MGCs) formation depends of monocytes recruitment from blood. After diapedesis and transformation into macrophages, these cells reach inflammation focus helped by chemotaxis, mainly IL-4 and IL-13, inducing macrophage fusion (Brodbeck; Anderson, 2009).

In this study, macrophage accumulation and MGCs formation showed that the number of cells increased significantly over the time. In almost all groups and times, the number of mononuclear and multinuclear cells were higher at 7 and 15 days post-implantation (DPI) compared to 2 DPI ($p<0.05$) (Table 2). These findings are in accordance with results described by Petric et al. (2003a) and Belo et al. (2005, 2012). See Figure 1.

Table 2. Number of mononuclear and multinuclear macrophages in *Piaractus mesopotamicus* (Mean ± SE)

DPI	Treatments	Number of nuclei											
		1		2		3 to 5		6 to 10		11 to 20		> 20	
2	C	3.19	Ab	0.98	Bb	0.35	Bb	0.31	Ab	0.21	Ab	0.00	Aa
	LEV	3.26	Ab	0.85	Bb	0.39	Bb	0.00	Ab	0.00	Ab	0.00	Aa
	DEX	3.38	Ab	0.96	Bb	0.63	Bb	0.16	Ab	0.21	Ab	0.00	Aa
	DEX + LEV	3.53	Ab	2.00	Ab	1.64	Ab	0.85	Aa	0.00	Ab	0.00	Ab
7	C	4.27	Aa	2.92	Aa	2.57	Aa	1.26	Aa	1.11	Aa	0.58	ABa
	LEV	4.30	Aa	2.57	Aa	2.47	Aa	1.72	Aa	1.24	ABa	0.64	Aba
	DEX	4.42	Aa	2.96	Aa	2.51	Aa	1.49	Aa	1.05	ABa	0.00	Ba
	DEX + LEV	4.24	Aa	2.91	Aa	2.61	Aa	1.56	Aa	0.71	Ba	0.46	Aa
15	C	4.20	Aa	2.75	Aa	2.33	Aa	1.44	Aa	0.78	Aab	0.31	ABa
	LEV	4.10	Aba	2.73	Aa	2.19	Aa	1.21	Aa	0.25	Ab	0.25	Aba
	DEX	3.88	Bb	2.72	Aa	2.33	Aa	1.05	Aa	0.50	Aab	0.00	Ba
	DEX + LEV	4.06	Aba	2.82	Aa	2.55	Aa	1.81	Aa	1.16	Aa	0.92	Aa
	F value	16.76		11.70		19.80		5.35		3.85		1.98	
	Pr > F[4]	<.0001		<.0001		<.0001		<.0001		<.0001		0.0206	
	CV (%)[4]	7.28		28.67		28.91		63.32		92.90		196.74	

[1]Mean values (n = 7) transformed log (x + 1). [2]Lowercase letters compare evaluation times within treatments; and capital letters compare treatments within evaluation time. [3]Means followed by equal letters, in the columns, do not differ, by Tukey's test (p> 0.05). C: Control group. LEV: Levamisole at dose of 50 mg/Kg. DEX: Dexamethasone 0.2% at dose of 2 mg/Kg.

Belo et al. (2005) found that *Piaractus mesopotamicus* with high serum cortisol levels – stressed fish – presented lesser MGCs, due likely to the anti-inflammatory action of this corticosteroid in the initial phase of the process. This response is due to the inhibition of the formation of eicosanoids and pro-inflammatory cytokines, blocking chemotaxis (CLAUDIANO et al. 2013). Dexamethasone in conjunction with cortisol –from handling stress– may inhibit the action of IL-4 and IL-13: important cytokines to macrophages fusion (BRODBECK; ANDERSON, 2009).

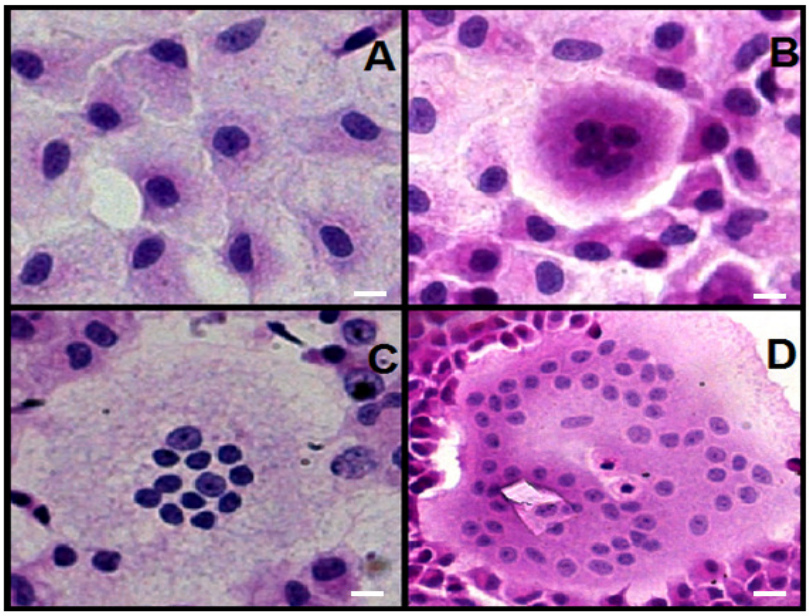

Figure 1. Photomicrograph of macrophages (A), MGC 3 to 5 nuclei (B), MGC 11 to 20 nuclei (C), and MGC >20 nuclei (D). Seven days after the implantation of glass coverslips in the subcutaneous tissue of the pacu (*Piaracus mesopotamicus*). Hematoxylin-eosin stained. Scale bar = 20 μm.

Belo et al. (2005) also observed the beneficial effects of dietary supplementation with vitamin E on the formation of macrophages polykaryons in *P. mesopotamicus*. Reduction in plasma cortisol favored the accumulation of macrophages and the formation of giant cells. Similar results were observed in *P. mesopotamicus* fed diet supplemented with vitamin C (PETRIC et al., 2003b) and kept at high stocking density (BELO et al., 2012).

In our study, there was no difference between fish collected at 7 and 15 DPI. Except dexamethasone group (DEX) at 15 DPI that presented lesser macrophages compared to the others groups (Table 2). At 7 and 15 DPI, fish from DEX group were the only one that presented no giant cells (more than 20 nuclei). Which support the findings of Belo et al. (2005, 2012).

Fish treated with dexamethasone (DEX) + levamisole (LEV) showed higher number of macrophage polykaryons ($P < 0.05$) up to 5 nuclei at 2 DPI. No other difference was observed in other groups at this time of evaluation ($p > 0.05$) (Table 2). However, these results suggest that the association of the dexamethasone with levamisole did not affect the kinetics of the evolution of the response. In most cases, this association presents similar response that the observed in control group.

The half-life of dexamethasone in fish has not been reported. But its half-life in humans is 72 h (HAYNES, 1990) and rats 36 h (MELGERT et al., 2001). However, there seems to be ongoing drug effects for a significant period after drug clearance from the plasma (RAEDER, 2010). In our study, treatment with dexamethasone inhibited the formation of polykaryons with more than 20 nuclei 15 DPI compared to the control group ($p<0.05$) (Table 2). This effect may reflect the inhibition of the early stages of the reaction. In contrast, the association of the dexamethasone with

levamisole blocked the inhibition of the first, as observed at 15 DPI, suggesting the compensatory effect of levamisole in this case.

The use of levamisole as immunostimulant has been proved in several animals, including fish. However, Li et al. (2006) showed that *Clarias fuscus* supplemented with levamisole for a week only had effect at high doses. When they used similar doses to this study, there were no differences with control group. Mulero et al. (1998) found no differences in the phagocyte functions *in vitro* of seabream treated with levamisole.

In this study, we observed no effect of levamisole comparing with control group. However, the results show that when it was associated with dexamethasone neutralized the effect of the latter. It probably happened due to the low concentration of levamisole used in this experiment and the low response of the pacu defense system to this drug.

Macrophage polykaryons with nuclei randomly distributed in the cell cytoplasm characterize foreign body-type multinucleated giant cell. Gradually, the nuclei mobilize to the periphery of the cytoplasm forming Langhans cells (PETRIC et al, 2003a,b; Belo et al, 2005; 2012). Sakabe et al. (2013) observed these cells 8 DPI in Nile tilapia supplemented with high levels of linseed oil. In our study, it was no possible observed them. It may because the species; according of data of our laboratory, Nile tilapia presents more aggressive response to pathogens than *P. mesopotamicus* (CASTRO et al., 2014; LOURENÇO et al., 2014, MANRIQUE et al., 2014, MARCUSSO et al., 2014, 2015, SILVA et al., 2015).

In the hematological assay, we observed at 2 DPI a decrease in the number of erythrocytes ($p < 0.05$) in the blood associated with increased mean corpuscular volume (MCV) of fish in treated groups compared with NV group (Table 3). However, the hematocrit value did not change. On day 7 after induction of inflammation, fish treated with levamisole and/or dexamethasone showed decreased ($p<0.05$) erythrocyte number and increase of MCV ($p<0.05$) (Table 3). We also observed an increased in the number of erythrocytes and decreased MCV in almost all groups at 15 DPI; except DEX + LEV group that presented low counts of erythrocytes and high values of MCV ($p<0.05$) compared to control and NV groups.

Red blood cells parameters suggests slight macrocytic anemia, characterized by reduction of erythrocytes on the rate of hemoglobin in treated groups compared to C and NV groups. The reduction of erythrocytes and hemoglobin may be stimulated the hematopoietic tissue and released larger red blood cells, in order of carrying a regular amount of hemoglobin. This situation is common in cases of hemolysis (JANNINI, 1978). Hemoconcentration is a strategy to increase the oxygen-carrying capacity in situations of high energy demand and mostly under stress (MONTERO et al, 1999; CARVALHO; FERNANDES, 2006; TRENZATO et al., 2006).

Chronic exposure to stress increased concentrations of cortisol and glucose and also enhances hematological changes (YADA; NAKANISHI, 2002). The absorption of iron could be affected and induced malformation or produce hemolysis, inhibiting the synthesis of hemoglobin and competing for the binding site of oxygen, causing anemia or hemodilution and reducing the capacity of carry blood oxygen (TRENZATO et al., 2006). This situation was observed in treated groups with dexamethasone but not with levamisole.

The high number of erythrocytes is associated with decreased of VCM in control, LEV and DEX groups at 15 DPI. These showed the recovery of hematologic response. The animals treated with dexamethasone and levamisole (DEX + LEV) showed no recovery of the response and maintained slight macrocytic hyperchromic anemia. Probably, in this group there was no interference of levamisole. Dexamethasone maintained his effects and produced anemia. It is important to note that the pharmacological effects of these drugs are little known in teleost fish (SMITH et al., 2007).

It was also observed in this study that the amount of total leukocytes varied in relation of treatments and the times studied. After two days of the coverslip implantation, there was an increase of total leukocytes in all implanted groups, treated or not, suggesting leukocytosis at the beginning of the inflammation process. The highest counts were observed in the groups that received levamisole and/or dexamethasone (Table 4).

Table 3. Red blood count (RBC), hematocrit (Ht), hemoglobin (Hb), Mean corpuscular volume (MCV), and Mean corpuscular hemoglobin concentration (MCHC) of blood of *Piaractus mesopotamicus* (Mean ± SE)

DPI	Treatments	RBC (10^6 µL^{-1})				Ht (%)				MCV (fL)				Hb (g dL^{-1})				MCHC (g dL^{-1})			
2	NV	1.84	±	0.09	Aa	42.14	±	1.59	ABa	232.42	±	13.03	Ca	9.60	±	0.47	Aa	22.85	±	2.28	Aa
	C	1.34	±	0.03	Cb	39.21	±	0.89	Ba	294.14	±	11.93	Aa	9.27	±	0.55	Aa	23.71	±	1.45	Aa
	LEV	1.59	±	0.04	Ba	40.28	±	1.77	ABa	252.85	±	7.02	ABCb	9.21	±	0.53	Aa	22.71	±	0.68	Aa
	DEX	1.87	±	0.15	Aa	43.33	±	1.68	Aa	239.33	±	20.78	BCb	9.04	±	0.26	Aa	21.00	±	1.34	Aa
	DEX + LEV	1.37	±	0.04	BCab	40.14	±	0.98	ABa	287.83	±	5.61	ABab	9.50	±	0.70	Aa	23.85	±	2.14	Aa
7	NV	1.84	±	0.09	Aa	42.14	±	1.59	Aa	232.42	±	13.03	Ca	9.60	±	0.47	Aa	22.85	±	2.28	Aa
	C	1.55	±	0.05	Bb	40.00	±	1.12	Aa	258.28	±	5.77	Ca	8.59	±	0.38	Aa	21.57	±	0.61	Aa
	LEV	1.12	±	0.04	Cb	42.87	±	2.40	Aa	377.00	±	19.75	ABa	8.89	±	0.32	Aa	20.50	±	0.64	Aa
	DEX	1.03	±	0.08	Cb	41.10	±	1.03	Aab	402.25	±	40.95	Aa	8.32	±	0.32	Aa	19.75	±	1.10	Aa
	DEX + LEV	1.13	±	0.10	Cb	37.50	±	1.45	Ba	337.25	±	25.45	Ba	8.67	±	0.36	Aa	23.20	±	0.20	Aa
15	NV	1.84	±	0.09	Ba	42.14	±	1.59	Aa	232.42	±	13.03	Ba	9.60	±	0.47	Aa	22.85	±	2.28	Aa
	C	2.17	±	0.08	Aa	39.14	±	1.10	ABa	179.83	±	9.50	Cb	9.21	±	0.24	ABa	23.71	±	1.12	Aa
	LEV	1.79	±	0.03	Ba	39.07	±	1.15	ABa	218.14	±	5.50	BCb	8.06	±	0.30	Ba	20.71	±	0.52	Aa
	DEX	1.72	±	0.10	BCa	38.07	±	0.45	Bb	225.71	±	15.01	BCb	8.39	±	0.23	ABa	22.14	±	0.50	Aa
	DEX + LEV	1.53	±	0.11	Ca	40.78	±	0.89	Aa	277.28	±	24.42	Ab	9.21	±	0.25	ABa	22.57	±	0.64	Aa
	F Value	11.05				1.53				10.52				1.95				1.79			
	Pr >F[4]	< .0001				0.08				< .0001				0.0146				0.0291			
	CV (%)[4]	14.40				8.07				16.36				14.70				14.83			

[1]Lowercase letters compare evaluation times within treatments; and capital letters compare treatments within evaluation time. [2]Means followed by equal letters in the columns do not differ by Tukey's test (p> 0.05). C: Control group. NV: Naïve. LEV: Levamisole (50 mg/Kg). DEX: Dexamethasone (2 mg/Kg)

Table 4. Total leukocytes, thrombocytes, neutrophils, monocytes and lymphocytes of blood of *Piaractus mesopotamicus* (Mean ± SE)

DPI	Treatments	Total leukocytes (10^3. μL^{-1})	Thrombocytes (10^3. μL^{-1})	Neutrophils (10^3. μL^{-1})	Monocytes (10^3. μL^{-1})	Lymphocytes (10^3. μL^{-1})
2	NV	7.19 ± 1.83 Da	19.23 ± 2.02 Aa	0.32 ± 0.07 Aa	0.18 ± 0.03 Ba	6.66 ± 1.77 Ca
	C	8.16 ± 1.91 CDb	21.44 ± 5.63 Aa	0.37 ± 0.05 Aa	0.24 ± 0.07 Bb	7.95 ± 2.22 BCb
	LEV	13.98 ± 1.28 ABa	21.50 ± 3.82 Aa	0.64 ± 0.12 Ab	0.39 ± 0.11 ABa	12.74 ± 1.23 Aba
	DEX	19.44 ± 2.69 Aa	21.14 ± 2.68 Aa	0.51 ± 0.20 Aa	0.64 ± 0.32 Aa	18.06 ± 2.19 Aa
	DEX + LEV	11.26 ± 1.86 BCDa	19.01 ± 2.83 Aa	0.18 ± 0.04 Aa	0.15 ± 0.06 Ba	10.85 ± 1.81 Ba
7	NV	7.19 ± 1.83 ABa	19.23 ± 2.02 Aa	0.32 ± 0.07 Aa	0.18 ± 0.03 Ba	6.66 ± 1.77 ABa
	C	10.72 ± 2.75 Aab	14.04 ± 3.46 ABa	0.77 ± 0.20 Aa	0.52 ± 0.15 Aab	9.36 ± 2.50 Aab
	LEV	3.87 ± 0.97 Bb	10.79 ± 2.14 ABb	0.30 ± 0.10 Ab	0.34 ± 0.16 ABa	3.19 ± 0.77 Bb
	DEX	3.77 ± 0.96 Bb	6.34 ± 1.44 Ba	0.25 ± 0.10 Aa	0.28 ± 0.04 ABa	3.19 ± 1.00 ABb
	DEX + LEV	6.49 ± 1.14 ABa	12.98 ± 1.42 ABab	0.24 ± 0.09 Aa	0.42 ± 0.11 ABa	5.74 ± 1.04 ABa
15	NV	7.19 ± 1.83 BCa	19.23 ± 2.02 Aa	0.32 ± 0.07 Ba	0.18 ± 0.03 Ba	6.66 ± 1.77 Ba
	C	15.23 ± 2.26 Aa	14.26 ± 2.24 ABa	0.39 ± 0.09 Ba	0.58 ± 0.16 Aa	14.26 ± 2.08 Aa
	LEV	12.33 ± 2.04 ABa	19.82 ± 3.40 Aa	2.20 ± 1.89 Aa	0.17 ± 0.09 Ba	9.93 ± 2.64 ABa
	DEX	15.88 ± 1.80 Aa	15.56 ± 2.33 ABCa	0.14 ± 0.09 Ba	0.39 ± 0.19 ABa	15.30 ± 1.65 Aa
	DEX + LEV	5.57 ± 1.19 Ca	8.15 ± 1.16 Cb	0.12 ± 0.03 Ba	0.16 ± 0.05 Ba	5.24 ± 1.17 Ba
	F Value	3.71	2.74	0.86	1.81	3.46
	Pr >F[4]	< .0001	0.0005	0.63	0.0283	< .0001
	CV (%)[4]	52.14	45.7	288.72	96.87	55.63

[1]Mean values (n = 7). [2]Lowercase letters compare evaluation times within treatments; and capital letters compare treatments within evaluation time. [3]Means followed by equal letters, in the columns, do not differ, by Tukey's test (p> 0.05). C: Control group. NV: Naïve. LEV: Levamisole at dose of 50 mg/Kg. DEX: Dexamethasone 0.2% at dose of 2 mg/Kg.

Several studies concluded that diets supplemented with immunostimulants can increase the number of leukocytes, as observed in matrinxa (*Brycon amazonicus*) supplemented with high doses of vitamin C (AFFONSO et al, 2007); pacu (*Piaractus mesopotamicus*) supplemented with vitamins C and E (SAFI et al., 2006;. ANDRADE et al., 2007; GARCIA et al., 2007) and *Saccharomyces cerevisiae* (REQUE et al, 2010; SALVADOR et al., 2012).

Martins et al. (2002) observed an increase in total number of leukocytes in hybrid tambacu (*P. mesopotamicus* male x *Colossoma macropomum* female) subjected to repeated capture stress. In our study, fish treated with dexamethasone and with the association of the drugs resembling fish from the first study at the beginning of inflammation. Results also showed an increase in the number of thrombocytes, neutrophils, monocytes and lymphocytes. In situations of acute inflammation in fish experimentally or naturally stimulated occur neutrophilia and monocytosis in the blood, besides the accumulation of neutrophils and monocytes in the affected area (ROBERTS, 1989; SUZUKI; LIDA, 1992).

Seven days after implantation DEX, LEV and DEX + LEV groups showed a decrease in the number of leukocytes, thrombocytes, neutrophils, and lymphocytes; especially in DEX group. The decrease in the number of blood monocytes can be explained due to the migration of these cells to the site of inflammation (REQUE et al 2010). Monocytes are important due to they secrete several biologically active substances and also processed and removed antigens such as senescent cells, debris, bacteria and toxins from the blood (MEYER et al, 1995).

High doses of levamisole may be toxic and cause immunosuppression, and underdosing may not be efficient (LI et al, 2006). *Fuscus clarias* supplemented with 600 mg of levamisole/kg diet showed immune stimulation after 7 days of treatment activity. However, this effect was decreased until immunosuppression 8 weeks post-treatment (LI et al. 2006).

DEX, LEV and control groups showed an increase in the number of total leukocytes 15 DPI with the same profile of 2 DPI. The leukocytosis is explained due to an increase in one or more cell types (JANNINI, 1978). In DEX + LEV group at 15 DPI, we observed less total leukocytes, which reinforce the above findings that in the combination of drugs, the former may induce reduction of the number of cells that the second one can not counteract.

CONCLUSION

Dexamethasone affects negatively the chronic inflammatory response of macrophages while levamisole, at 50 mg/Kg, showed little effect by itself. Interestingly, DEX + LEV group presented similar response to control group showing the antagonistic effect of these.

RESUMO: O objetivo deste estudo foi avaliar o efeito da administração parenteral de dexametasona e levamisol na acumulação de macrófagos e formação de células gigantes na inflamação crônica por corpo estranho e avaliação de parâmetros sanguíneos em pacu (*Piaractus mesopotamicus*). Utilizou-se 50 mg/kg de levamisol e 2,0 mg / kg de dexametasona e a combinação de ambos fármacos. As lamínulas foram implantadas sob a pele. Depois de 2, 7 e 15 dias pós-implantação (DPI), os peixes foram anestesiados para a remoção das lamínulas e contagem do número de macrófagos e células gigantes. Foi coletado sangue da veia caudal para realizar contagem de células vermelhas, leucócitos, trombócitos, concentração de hemoglobina, MCV e MCHC. Observou-se que a dexametasona afeta negativamente a formação de células gigantes na inflamação crónica por corpo estranho. O levamisol, apesar de ser considerado imunoestimulante em várias espécies, mostrou ação limitada. No entanto, foi suficiente para neutralizar o efeito da dexametasona; a associação das drogas não interferiu significativamente no número de eritrócitos e de leucócitos na maioria dos tratamentos e períodos estudados. No grupo da dexametasona, houve redução do número de eritrócitos e concentração de hemoglobina associado ao aumento do volume corpuscular médio sugerindo leve anemia macrocítica. Aos 15 DPI, a maioria dos grupos mostrou recuperação na resposta hematológica. Como em mamíferos, a dexametasona afeta negativamente a resposta inflamatória. O levamisol mostrou pouco efeito por si só. No entanto, em alguns parâmetros a associação das duas drogas provoca resposta similar ao grupo controle e naive, mostrando o efeito antagonista de estas drogas.

PALAVRAS-CHAVE: Glicocorticoides. Lamínulas. Inflamação. Pacu.

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