Influence of indomethacin on the regenerative process of the tail fin of teleost: morphometric and ultrastructural analysis

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(With 6 figures)

Abstract

When partially amputated or severely injured, teleost fins suffer a regenerative process called epimorphic regeneration characterised by the following stages: the formation of a multistratified epidermal layer, the disorganisation and distal migration of multipotent mesenchymal cells, the proliferation of these cells in order to form the blastema, continuous proliferation of distal blastema to facilitate the growth, and differentiation of the proximal blastema in order to restore its lost structure. The regeneration of the fin is extremely sensitive to the action of some drugs that can interfere in its structure restoration. For this reason, and also based on papers relating that indomethacin can interfere somehow in the tissue restoration of many different organisms, the aim of this work is to evaluate the possible effects of this drug in three different doses in the regeneration of the teleost fish tail fin, taking into consideration the synthesis, the disposition and organisation of lepidotrichial matrix components, the restoration of actinotrichia, as well as the fin area itself. Therefore, histochemical, ultrastructural and morphometric analysis were done and it was observed that indomethacin in doses of 20 and 30 mg.L^{-1} caused a delay in the regenerative process of the dermal skeleton (lepidotrichia and actinotrichia) of the tail fins. These doses could have interfered, momentarily, in the process of blastemal cell differentiation in the cells responsible for the synthesis and disposition of actinotrichia and lepidotrichia or, even interfered in the signalling necessary for the recent differentiated cells to begin synthesising the components of the dermal skeleton.
Keywords: indomethacin, regeneration, fin, fish.

Influência da indometacina no processo regenerativo da nadadeira caudal de teleósteo: análise morfométrica e ultraestrutural

Resumo

Quando parcialmente amputadas ou severamente injuriadas, as nadadeiras de teleósteos sofrem um processo de regeneração chamado de regeneração epimórfica, caracterizado pelas seguintes fases: formação de uma capa epidermal multiestratificada, desorganização e migração distal de células mesenquimais multipotentes, proliferação dessas células para formar o blastema, proliferação contínua do blastema distal para facilitar o crescimento e diferenciação do blastema proximal para restaurar as estruturas perdidas. A regeneração que a nadadeira sofre é extremamente sensível à ação de algumas drogas que podem interferir na reparação de suas estruturas. Em vista disso, e também pelo fato de que há relatos na literatura de que a indometacina pode interferir de alguma maneira na restauração tecidual de diversos organismos, o objetivo deste trabalho foi o de avaliar os possíveis efeitos desta droga, em três doses diferentes, na regeneração da nadadeira caudal de peixe teleósteo, considerando a síntese, deposição e organização dos componentes da matriz lepidotriquial, a regeneração da actinotriquia, bem como a área da nadadeira como um todo. Para isso, foram feitas análises histoquímica, ultraestrutural e morfométrica, e foi observado que a indometacina, nas doses de 20 e 30 mg.L^{-1}, apresentou um atraso no processo de regeneração das unidades esqueléticas (lepidotriquias e actinotriquias) das nadadeiras caudais. Essas doses podem ter interferido, momentaneamente, no processo de diferenciação das células blastemais nas células responsáveis pela síntese e deposição das actinotriquias e das lepidotriquias ou, até mesmo, na sinalização necessária para que as células recém-diferenciadas dessem início à síntese dos componentes das unidades esqueléticas.

Palavras-chave: indometacina, regeneração, nadadeira, peixe.

1. Introduction

Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) commonly used for pain, arthritis, cardiovascular prophylaxis and prevention of colonic polyps and cancers (Pai et al., 2001). Guez et al. (2001) analysed the action of indomethacin in a model of bone remodelling in rats and showed that the drug affected various stages of this remodelling sequence like bone resorption and formation. Several studies have shown the influence of indomethacin in the synthesis of the extracellular matrix of connective tissue (Arumugham and Bose, 1981; Riley et al., 2001). Indomethacin used in wounded gastric epithelial cell monolayer model of rat inhibited the reepithelialisation of gastric mucosa (Pai et al., 2001).

When partially amputated or severely injured, fins are able to complete self-restoration through a process of epimorphic regeneration (Géraudie and Singer, 1992). Fin regeneration is characterised by: formation of a multilayered wound epidermis, disorganisation and distal migration of mesenchymal cells proximal to the amputation plane, proliferation of these mesenchymal cells to form the regeneration blastema, continued distal blastemal proliferation to facilitate outgrowth and proximal blastemal differentiation to replace missing structures (Goss and Stagg, 1957; Santamaria and Becerra, 1991; Johnson and Weston, 1995; Poss et al., 2000a, 2000b).

The tail fin of teleost is composed of structural units named rays or lepidotrichia surrounded by a multilayered epidermis (Montes et al., 1982; Becerra et al., 1983). Each lepidotrichium consists of a pair of concave hemirrays formed by multiple segments joined end to end by ligaments (Becerra et al., 1983). The space between two hemirrays is filled with connective tissue and contains nerves, blood vessels, pigment cells and fibroblasts. Each ray ends distally with a row of small, rigid, fusiform spicules, known as actinotrichia (Marí-Beffa et al., 1989).

The lepidotrichia are filled with extracellular matrix containing type II collagen fibrils of different orientations surrounded by a mineralised ground substance rich in chondroitin sulfate (Montes et al., 1982). The actinotrichia are formed from hyperpolymerised macrofibrils of elastoidin, a protein with characteristics similar to those of collagen (Gross and Dumsha, 1958; Marí-Beffa et al., 1989).

Fin regeneration in teleost fish is extremely sensitive to the action of some drugs, for example beta-aminopropionitrile, penicillamine, dexamethasone and acetylsalicylic acid, which may interfere on the regenerative capacity of fin of teleost fish (Bechara et al., 2000; 2003).

Thus, the objective of the present study was to observe the possible effects of this drug on tail fin regeneration of the teleost fish, considering the synthesis, deposition and organisation of the lepidotrichial extracellular matrix components, the actinotrichia regeneration and the total area of regenerating fins.

2. Materials and Methods

Carp alevins, *Cyprinus carpio* (Linnaeus, 1758), obtained from a fish farm, weighing on average 1.4 g and measuring on average 4.7 cm in length, were divided aleatorily into 4 glass aquaria (n = 40). The water was clean and dechlorinated, the temperature maintained at 26 °C, the pH at 8.0 and aeration was constant. Indomethacin (Merck Sharp and Dohme) was dissolved in the water of the aquariums, where each aquarium received a different concentration, i.e., 10, 20 and 30 $mg.L^{-1}$, and in the other aquarium, containing only water, served as the control.

Next, the fishes were anaesthetised with benzocaine (SYNTH) (1:10000) and their tail fins were amputated transversally (in the dorso-ventral direction) at a distance of 3 mm from the tail muscle peduncle using a sharp razor, according to Becerra et al. (1996). The fish were left in the aquaria until the occurrence of regeneration. In a daily basis, until the end of the experiment, the water of the aquaria was replaced with clean and dechlorinated water and the drug dissolved according to the doses established for each container.

The animals were then anaesthetised, sacrificed and the regenerating fins excised and fixed at intervals of 1, 2, 4, 5, 6, 8, 10 and 12 days after amputation, using 5 specimens for each time interval.

For each time interval, fragments of regenerating fins from control and indomethacin treated fish were processed for light microscopy and for transmission electron microscopy.

The collected samples for light microscopy were fixed in Bouin's solution for 6 hours, embedded in paraffin and sectioned 6 µm thick. Longitudinal and transversal sections were stained with Picrosirius-Hematoxylin method and observed and photographed under microscopes using conventional light and polarised light.

The regenerating fins of fishes of both aquaria, collected at 4, 6 and 8 days intervals after amputation and processed for conventional light microscopy had theirs images analysed using an image analyser software (Image Pro-Plus, version 4.1.12, USA). For each fish, both from control and treated aquarium, 25 longitudinal fin sections were randomly selected. In addition, the areas of regenerating fins were measured with a 2× objective lens.

The means of the areas of each time interval (mean ± S.E.M) were compared using one-way analysis of variance (ANOVA) followed by Tukey's mean comparison test. The level of significance was set at 5% ($p < 0.05$). The statistical analyses were done using the statistical program INSTAT v 2.01 (GraphPad, San Diego, CA. USA).

For transmission electron microscopy, small fragments of regenerating fins were fixed in Karnovsky fixative for 4 hours at 4 °C, washed with a 0.1 M phosphate buffer solution (pH 7.4) containing 7.5% saccharose and subsequently postfixed with 1% osmium solution in 0.2 M phosphate buffer (pH 7.4) for 1 hour at 4 °C. The samples were then washed with glycosated saline (0.6 g of sodium chloride + 7.3 g of sucrose + 100 mL of distillate

water), dehydrated with increasing acetone concentrations, pre-embedded in a mixture of acetone and epon (1:1) for 3 hours and embedded in pure epon for 24 hours. The tissue fragments embedded, longitudinally and transversally to the rays, were polymerised in an incubator at 60 °C for 48 hours. Semithin sections (1 μm) were obtained using a LEICA ultramicrotome and stained in hot Toluidine Blue. Further ultrathin sections (60-70 nm) were subsequently obtained, contrasted with uranyl acetate and lead citrate, and observed and micrographed in a transmission electronic microscope (LEO 906).

3. Results

The control group fish and the fish treated with a dose of 10 mg.L^{-1} of indomethacin showed a similar regenerative process of the tail fin. One day after partial amputation of the tail fin, epidermal cells migrated and completely covered the cut edge, achieving its complete formation on the second day of regeneration (Figure 1). On the fourth day of the regenerative process, it was observed blastema formation, a mass of cells of homogeneous aspects right underneath the regenerative epidermis (Figure 2). Some blastema cells formed a row of cells, one next to the other, and immediately beneath the epidermis, in strong association with the basal layer in both sides of the fin. These cells, known as scleroblasts, are responsible for the synthesis and deposition of the lepidotrichial extracellular matrix in the region turned to the basal layer, and therefore between the row of scleroblasts and the basal layer of the epidermis.

Actinotrichia was initially observed around the fourth day after the excision of the fin (four days of regeneration), next to the connective tissue cells, similar to fibroblasts, indicating that probably they were the cells involved in the synthesis of elastoidin. They initiated their regeneration in the fin distal region, inside the connective tissue matrix, in adjacent position to the epidermis, laid in bilateral rows (Figure 5a).

With six days of regeneration, scleroblasts migrated to the other side of the hemisegment of the regenerating lepidotrichia and were interposed between the epidermis and the hemisegment, maintaining the disposition of a single layer of cells involving, this time, both sides of the lepidotrichial hemisegment and started to secrete extracellular matrix to the hemisegment direction. This action of the scleroblasts is responsible for the growth in

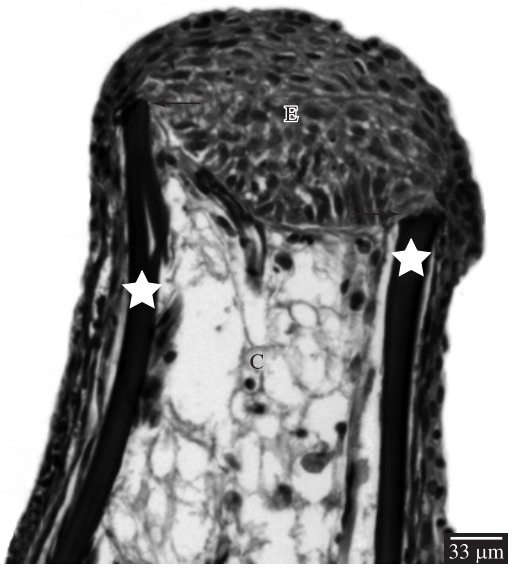

Figure 1. Longitudinal section through the distal end of a regenerating tail fin of a fish of control group, 24 hours after amputation stained with picrosirius-hematoxylin. Observe that the regenerating epidermis (E) has already fully covered the amputated region of the tail fin, forming the epidermal cap. Note the loose connective tissue (C) between the old lepidotrichial hemisegments (stars). The arrows indicate the site of amputation. Bar = 33 μm. For the group treated with indomethacin in the doses of 10, 20 and 30 mg.L^{-1}, there are no differences with the control group.

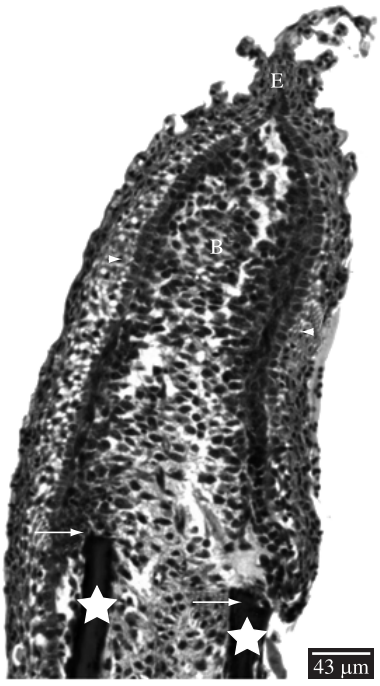

Figure 2. Longitudinal section of the tail fin of a fish of control group after 4 days of regeneration stained with picrosirius-hematoxylin. Observe the epidermal cap (E), the blastema (B) and the lepidotrichial hemisegment (stars). The arrows indicate the site of amputation. Note basal epidermal layer composed of cuboidal cells, adjacent to blastemal tissue (arrowheads). Bar = 43 μm. For the group treated with indomethacin in the doses of 10, 20 and 30 mg.L^{-1}, there are no differences with the control group.

width of the hemisegment through appositional growth (central lepidotrichia layers are older that the further external ones). On days 8, 10 and 12 of regeneration, the fin was thoroughly grown, that is, the epidermis, the connective tissue, the actinotrichia and the lepidotrichia were completing their regenerative process (Figure 4a and Figure 6a). Due to the deposition of extracellular matrix on the hemisegment side, two light regions (less electron dense) were observed, one turned to the basal layer and the other to the hemisegment and they corresponded to the extracellular matrix newly synthesised by the scleroblasts, where collagen fibrils are easily observed, because there was no deposition of calcium salts. There was also a dark central region (more electron dense) called nucleation centre, that showed a ground mineralised substance that obscured the collagen fibrils, indicating that this region was under a more advanced level of regeneration than both the lighter areas surrounding it (Figure 3a).

Similarly to the control group (Figure 1 and Figure 2) and to the group treated with a dose of 10 mg.L^{-1} of indomethacin, the treated group with doses of 20 and 30 mg.L^{-1} also presented complete epidermal cap formation, blastema formation and regeneration of the connective tissue laid in between one lepidotrichia and the other and between two lepidotrichial hemisegments. However, the fish that belonged to these groups presented a delay in the dermal skeleton regenerative process (lepidotrichia and actinotrichia) of the tail fins (Figure 3b, Figure 4b, Figure 5b and Figure 6b). Both lepidotrichia and actinotrichia initiated their regenerative process around the eighth day after amputation (8 days of regeneration) showing a delay of approximately 4 days in their regeneration. The migration of scleroblasts to the other side of the hemisegment of the regenerating lepidotrichia, responsible for the growth in width of the hemisegment, initiated only on the tenth day after amputation and the nucleation centre formation, that is, the beginning of calcium salts deposition in the lepidotriquial matrix happened around the twelfth day of regeneration for the treated group with doses of 20 and 30 mg.L^{-1}, showing a delay of approximately 4 days when compared to the control group.

Although there was a delay of approximately 4 days in the regeneration of the tail fin dermal skeleton of the fish treated with doses of 20 and 30 mg.L^{-1} of indomethacin, the morphometric analysis of the regenerating areas done in the control group fish as well as the treated fish with 3 different doses of indomethacin on days 4, 6 and 8 after amputation (days chosen due to their representability in the total process of regeneration of tail fins) when compared did not indicate any significant alteration ($p > 0.05$) (Table 1).

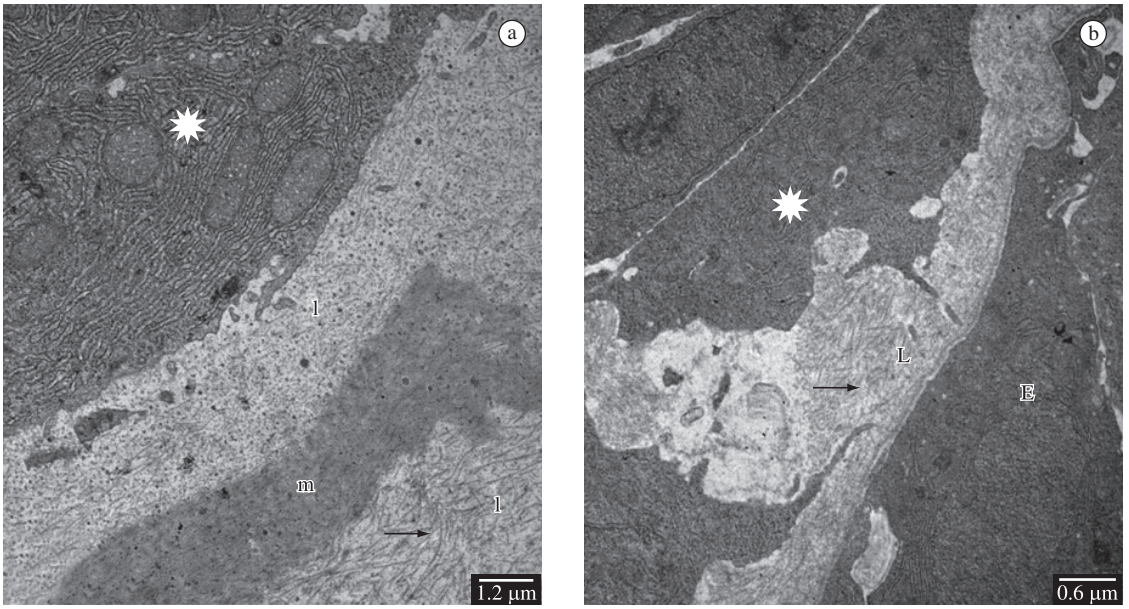

Figure 3. a) Electron micrograph of a longitudinal section of a regenerating lepidotrichia after 8 days of regeneration of a fish from control group. Observe the scleroblasts (asterisk). Note in lepidotrichial matrix, two light regions, less electron-dense (l) and a central dark region (nucleation centre), more electron-dense (m). The arrow indicates the collagen fibrils arranged in various directions. Bar = 1.2 µm. For the group treated with indomethacin in the dose of 10 mg.L^{-1}, there are no differences with the control group. b) Electron micrograph of a longitudinal section of a regenerating lepidotrichia after 8 days of regeneration of a fish of group treated with indomethacin in the dose of 20 mg.L^{-1}. Observe the epidermis (E), the regenerating lepidotrichial hemisegment (L) with the collagen fibrils arranged in various directions (arrow) and the scleroblast (asterisk). Note the smaller width of the lepidotrichium, when compared to the control group, and the absence of nucleation centre. Compare with Figure 3a. Bar = 0.6 µm. For the group treated with indomethacin in the dose of 30 mg.L^{-1}, there are no differences with the group treated with indomethacin in the dose of 20 mg.L^{-1}.

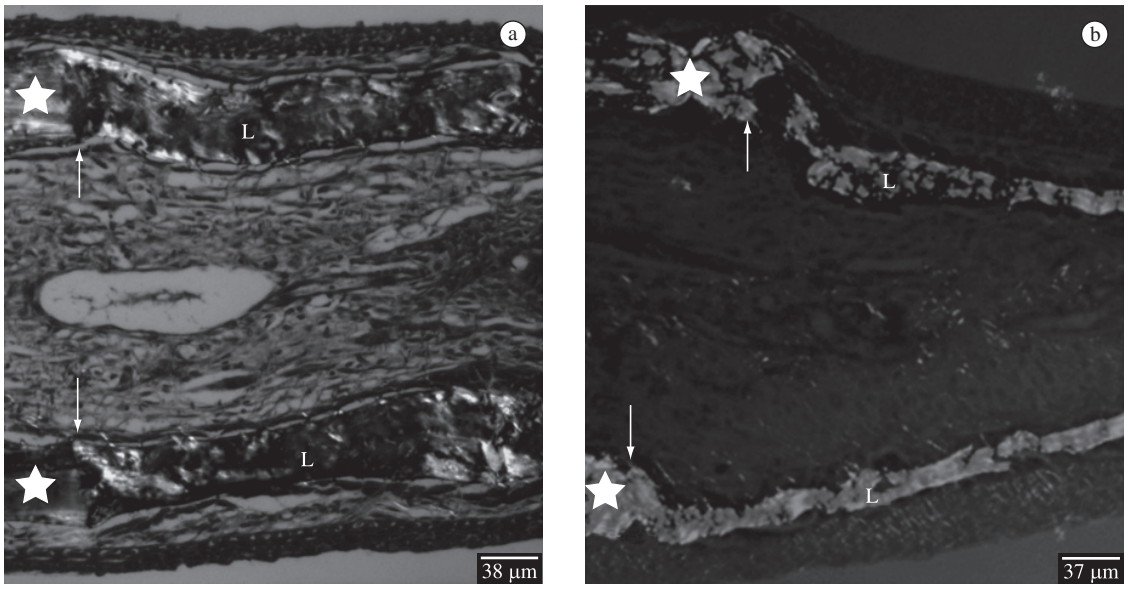

Figure 4. a) Longitudinal section of the tail fin of a fish of control group after 10 days of regeneration, stained with picrosirius-hematoxylin and observed under polarised light. Observe the old lepidotrichium (stars) and the regenerating lepidotrichium (L). Note the brightness of the collagen molecules present in the lepidotrichial matrix against a dark background. The arrows indicate the site of amputation. Bar = 38 µm. For the group treated with indomethacin in the dose of 10 mg.L^{-1}, there are no differences with the control group. b) Longitudinal section of the tail fin of a fish of group treated with indomethacin in the dose of 20 mg.L^{-1} after 10 days of regeneration, stained with picrosirius-hematoxylin and observed under polarised light. Observe the old lepidotrichium (stars) and the regenerating lepidotrichium (L). Note the brightness of the collagen molecules present in the lepidotrichial matrix against a dark background. The arrows indicate the site of amputation. Note the delay in the regenerating lepidotrichium, represented by the smaller width of the lepidotrichium when compared to the one from the control group. Bar = 37 µm. For the group treated with indomethacin in the dose of 30 mg.L^{-1}, there are no differences with the group treated with indomethacin in the dose of 20 mg.L^{-1}.

4. Discussion

Some inferior vertebrates, such as urodelic amphibians and the teleost fish, have a high ability to regenerate great variety of tissues and organs, while mammals have a limited capacity. This difference is understood as part of a process where the animals, during evolution, started gradually losing their ability to regenerate lost or wounded parts. This change in regenerative capacity during the course of evolution is strongly linked to the conservation of many genes and the expression and function of these among the vertebrate species (Nakatani et al., 2007).

Teleost tail fins are organs that present a relatively simple and symmetric structure with limited cell types. Furthermore, it is an organ of easy access for manipulation and, possible wounds in its structure do not compete to the survival of the animal. Therefore, the tail fin represents a simple model of the system in regeneration that can be useful to illustrate the biological principles involved in the regeneration, and, also, for the study of the effect of substances that can, perhaps, interfere in this regenerative process.

Fin regeneration can be summed up into four stages (Poss et al., 2000b): firstly, epidermic cells migrate to cover the wound and form a multistratificated layer; secondly, the mesenchymal tissue between both hemisegments located right beneath the epidermal layer suffers a disorganisation and an undifferentiation and, mesenchymal cells migrate distally in the direction of the amputation (Poleo et al., 2001); thirdly, these cells accumulate to form the blastema, a tissue mass through which new structures of the fin are derived; and finally, the regeneration is complete on the growing phase, composed of an intensively integrated proliferation, differentiation and restoration (Poss et al., 2002).

It is known that the regeneration of teleost fin is extremely sensitive to physical and chemical external factors, such as temperature variation (Johnson and Weston, 1995; Nechiporuk and Keating, 2002), the action of some environmental contaminants, such as TCDD (Fingerman, 1980; Zodrow and Tanguay, 2003) and the action of some drugs that inhibit collagen synthesis and regeneration (Bechara et al., 2000, 2003).

Indomethacin is a nonsteroid and nonspecific anti-inflammatory drug that causes three kinds of effects: analgesic, antipyretic and anti-inflammatory. It is utilised in the treatment of diseases such as osteoarthritis, rheumatic arthritis (Sadowski and Steinmeyer, 2001), gout, cancer and in the prevention of cardiovascular diseases and gynaecologic diseases (Pai et al., 2001) and also used to alleviate the pain after damage or surgery in the tendons and ligaments (Riley et al., 2001).

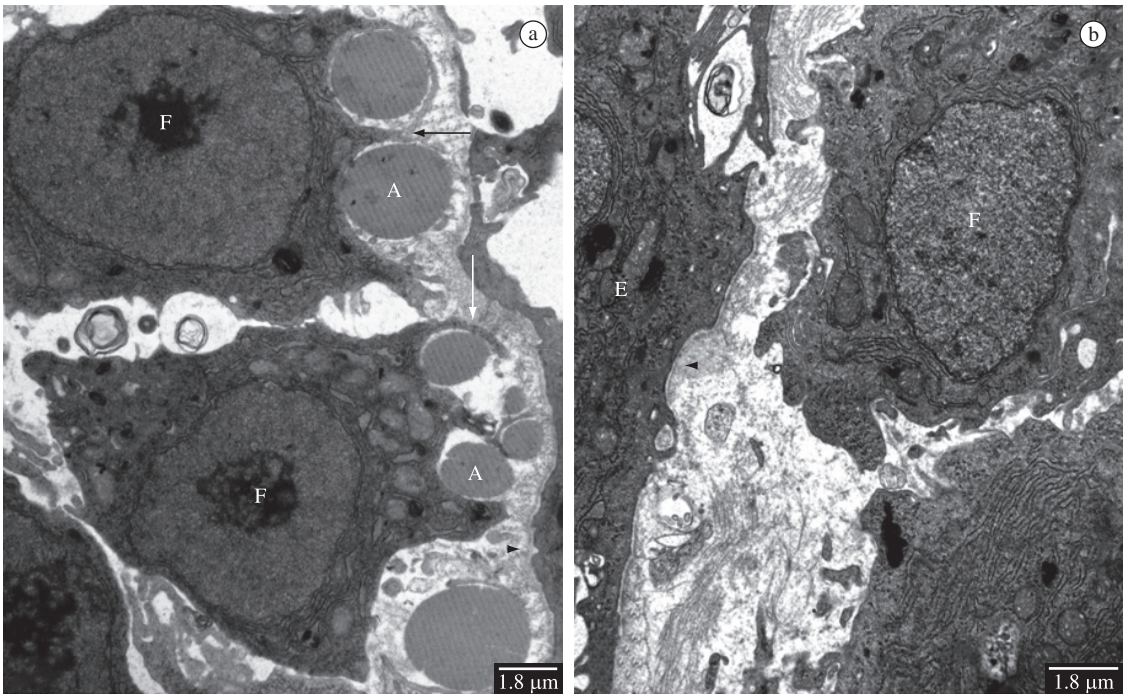

Figure 5. a) Electron micrograph of a transversal section of the tail fin of a fish of control group after 5 days of regeneration. Observe the regenerating actinotrichia (A) surrounded by cytoplasmic processes (arrows) of connective tissue cells, similar to fibroblasts (F). Observe the basal lamina of the epidermis (arrowhead). Bar = 1.8 µm. For the group treated with indomethacin in the dose of 10 mg.L^{-1}, there are no differences with the control group. b) Electron micrograph of a transversal section of the tail fin of a fish of group treated with indomethacin in the dose of 20 mg.L^{-1} after 5 days of regeneration. Observe the epidermis (E), the basal lamina of the epidermis (arrowhead), the connective tissue cells, similar to fibroblasts (F) and note the absence of regenerating actinotrichia under the basal lamina of the epidermis. Bar = 1.8 µm. For the group treated with indomethacin in the dose of 30 mg.L^{-1}, there are no differences with the group treated with indomethacin in the dose of 20 mg.L^{-1}.

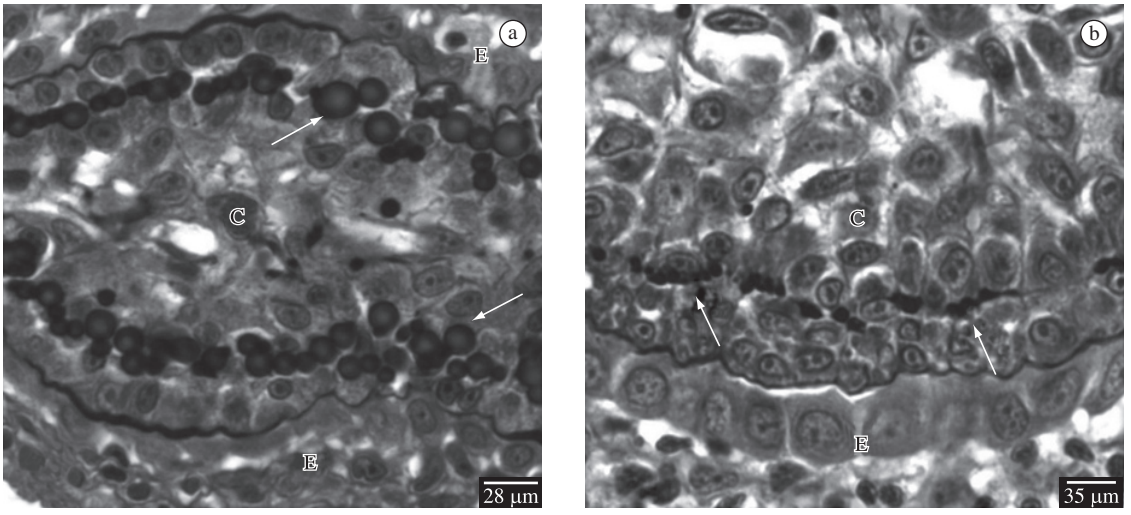

Figure 6. a) Transversal section of the regenerating tail fin of a fish of control group after 8 days of regeneration stained with picrosirius-hematoxylin. Observe the epidermis (E), the connective tissue (C) and the regenerating actinotrichia (arrows). Bar = 28 µm. For the group treated with indomethacin in the dose of 10 mg.L^{-1}, there are no differences with the control group. b) Transversal section of the regenerating tail fin of a fish of group treated with indomethacin in the dose of 20 mg.L^{-1} after 8 days of regeneration stained with picrosirius-hematoxylin. Observe the epidermis (E), the connective tissue (C). Note the delay in the regenerating actinotrichium (arrows), represented by the smaller diameter of the actinotrichia. Bar = 35 µm. For the group treated with indomethacin in the dose of 30 mg.L^{-1}, there are no differences with the group treated with indomethacin in the dose of 20 mg.L^{-1}.

Table 1. Morphometric results comparing the mean of the areas in μm^2 of regenerating fins of the control and indomethacin treated fishes in the doses of 10, 20 and 30 $mg.L^{-1}$, on the intervals of 4, 6 and 8 days after amputation of tail fin. Five fish in each treatment and 25 longitudinal fin sections for each fish were compared.

Days after amputation	Treatments			
	Control	10 $mg.L^{-1}$	20 $mg.L^{-1}$	30 $mg.L^{-1}$
4	79239 ± 17624	77430 ± 22084	76929 ± 45373	76529 ± 41609
6	211165 ± 69119	210958 ± 21276	206037 ± 75803	203530 ± 58000
8	245709 ± 104775	227347 ± 118796	223187 ± 25543	222475 ± 41100

Results expressed in Mean ± SEM using one-way analysis of variance (ANOVA) followed by Tukey's mean comparison test. The level of significance was set at 5% ($p < 0.05$). When compared the areas did not indicate any significant alteration ($p > 0.05$).

According to some studies (Collier and Ghosh, 1991; Leroux and Saffar, 1993; Guez et al., 2001; Pai et al., 2001; Riley et al., 2001; Forslund et al., 2003; Cohen et al., 2006; Azoubel et al., 2007) indomethacin can interfere in the restoration of the tissue of many organisms, since it inhibits the action of the ciclooxygenase enzyme and consequently the conversion of arachidonic acid into prostaglandin, elements that perform important functions in cell protection, growth, angiogenesis and extracellular matrix production (Savla et al., 2001).

In the present study it was shown that the fish treated with a dose of 10 $mg.L^{-1}$ of indomethacin, did not suffer damage on the regenerative process of their tail fins, thus, this dose did not affect the formation of the epidermal cap, connective tissue, blastema, blastemal cells differentiation and formation of actinotrichia, as well as synthesis, deposition, organisation and mineralisation of the components of the lepidotrichial matrix. Similarly, the fish treated with doses of 20 and 30 $mg.L^{-1}$ showed a complete formation of epidermal cap, blastema formation and a regeneration of the connective tissue. However, these fish presented a delay in the process of dermal skeleton regeneration (lepidotrichia and actinotrichia) of the tail fins.

As mentioned above, Guez et al. (2001) analysed the action of indomethacin in a sample of bone remodelling in rats and showed that the drug affected various stages of this remodelling sequence, such as bone resorption and formation. Various studies have shown the influence of indomethacin in the synthesis of the extracellular matrix of the connective tissue (Arumugham and Bose, 1981; Riley et al., 2001).

During fin regeneration, a large number of genes, including signalling molecules and transcription factors, are expressed with the end of restoring loss and damaged parts (Poss et al., 2000a; Nechiporuk and Keating, 2002). Laforest et al. (1998) analysed the expression of genes involved in the pathway of signalling SHH (*Sonic Hedgehog)* and observed that *shh, ptc* and *bmp2* are expressed during the fin regeneration in a coordinated way, suggesting the function of these genes in the interaction epidermis-blastema, which leads to the synthesis and to the restoration of the ray. Quint et al. (2002) investigated the effects of cyclopamine under the expression of signalling genes *shh* and noticed that there was a reduction followed by inhibition in fin regeneration, as well as the formation of little or no actinotrichia and a distal accumulation of pigment cells. This data suggest that a delay or even an inhibition in the fin regenerative process is strongly linked to an alteration in the expression of these genes.

Although the doses here used delayed the regeneration of the dermal skeleton of the tail fin, they did not interfere with the migration of the epidermal cells and consequently with the formation of the epidermal cap. They did not inhibit the formation of the blastema nor even the synthesis and the disposition of the connective tissue matrix presented between two lepidotrichial hemisegments and between two rays. This is to say that probably doses of 20 and 30 $mg.L^{-1}$ did not interfere in the expression of the genes required to the formation of the epidermal cap and the blastema, but could have interfered, momentarily, during transcriptions or translations or even complexing with proteins inhibiting their expression as an enzyme or structural functions as a protein in the process of blastemal cells differentiation on the cells responsible for the synthesis and deposition of actinotrichia and lepidotrichia, or even in the necessary signalling to differentiated cells to trigger the synthesis of the components of the dermal skeleton.

The morphometric analysis done in the fish treated with the 3 different doses of indomethacin did not indicate any significant alteration in relation to the total area of the fin regeneration. This occurred because in the fish treated with doses of 20 e 30 $mg.L^{-1}$, the region that should have been filled with dermal skeleton in regeneration was filled by the connective tissue in regeneration, without altering the total area of the fin.

The histological studies above offer an indication that there are various genes involved in the expression of this regenerative process. The synthesis of the actinotrichia, as well as the dermal bone formation (lepidotrichia), was affected (smaller diameter of the actinotrichia and smaller width of the lepidotrichia at the doses of 20 and 30 $mg.L^{-1}$ of indomethacin) but not the intra-rays connective tissue, despite having both (lepidotrichia and intra-rays connective tissue) collagen as their main component, though synthesised by different cells. Showing that tail fin is a good model to study the morphogenic toxicity of drugs and for the study of regenerative processes, we acknowledge that much more has to be done in order to understand the

mechanism involved in the process at a molecular and a histological level.

Detailed studies about the mechanisms of nonsteroid anti-inflammatory drug action and the action of these drugs under the expression or inhibition of expression of some genes involved in the teleost tail fin regenerative process could explain more precisely the influence of indomethacin in the regenerative process.

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