Original Article

Influence of COX-2-Induced PGE_2 on the Initiation and Progression of Tail Regeneration in Northern House Gecko, *Hemidactylus flaviviridis*

(epimorphic / wound healing / blastema / differentiation / eicosanoids / cell proliferation)

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Abstract. We investigated the role of prostaglandin E_2 in reptilian regeneration. Prostaglandin E_2 is known to play a vital role during wound healing and cell proliferation. A significant delay in the rate of growth of regenerate after autotomy was observed when the production of prostaglandin E_2 was blocked by usage of specific cyclooxygenase inhibitors as compared to control animals and this delay continued to all the defined stages of regeneration. Therefore, prostaglandin E_2 could be one of the essential requirements for a successful process of regeneration.

Introduction

Regeneration is defined as the ability to reproduce organs or structures after they have been lost through trauma or other causes (Bellairs and Bryant, 1985). Invertebrates (planaria and hydra) show an amazing power of regeneration by regenerating the whole body from fragments of the body through cellular reorganization better known as morphallaxis (Goss, 1969; Baguna, 1998; Sanchez-Alvarado, 2000). Among vertebrates, Urodele (caudate) amphibians and lizards express epimorphic regeneration. Epimorphic regeneration involves generation of new stem cells, either by proliferation of the existing stem cells or by dedifferentiation of adult cells, which differentiate to form the lost appendage that is more or less similar in size and structure compared to the original lost structure (Brockes and Kumar, 2002; Bryant et al., 2002). The ability of many lizards to cast off (autotomize) their tail is a widely known phenomenon. A scan through the literature, however, reveals that tail regeneration in the lizard has not been studied as extensively as that of amphibians. Nevertheless, the process of regeneration is comparable between the lizards and amphibians (Ityen and Bryant, 1976). Regeneration in the lizard is lined by many definable phases: (1) Wound epithelium: during which wound closure, inflammation, dedifferentiation and blastemal cell accumulation occurs. (2) Blastema formation: proliferation of blastemal cells and elongation as well as growth of blastema. (3) Growth and differentiation phase: which is a morphogenetic phase leading to histogenesis. In order to execute all the above events, inputs of various factors are required.

The inflammatory phase, which is a hallmark of wound healing stage, leads to release of cytokines and growth factors ensuring permeability of blood vessels and chemotaxis of inflammatory cells. Each process may be regulated by many bioactive substances, including growth factors, extracellular matrix components, and eicosanoids. Eicosanoids such as prostaglandins (PGs), prostacyclins, and thromboxane have been implicated in wound healing in various tissues such as cornea (Joyce and Meklir, 1994), skin (Talwar et al., 1996), gastrointestinal tract (Zushi et al., 1996), and kidney (Cybulsky et al., 1992). Talwar and co-workers (1996) have found that synthetic PGE_2 facilitates fibrosis during healing of wounded rat skin.

Prostaglandin E_2 is a lipid-based soluble mediator synthesized from arachidonic acid (AA), a component of the cellular membrane released by phospholipase-A2 activity. Arachidonic acid is then modified enzymatically by cyclooxygenases (COX) and converted into an intermediate molecule, prostaglandin H_2 (PGH_2). The COX product PGH_2 may then be converted into various other prostaglandins. In most cells, the conversion of AA to prostanoids is catalysed by the COX enzyme isoform COX-1 found in normal cells and tissues, although several cell types use the isoform COX-2 for AA conversion when stimulated with cytokines or growth factors and inflammatory mediators (Shen et al., 2006).

Received : September 16, 2008. Accepted: November 25, 2008.

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Abbreviations: AA – arachidonic acid, AEC – apical epithelial cap, BL – blastema, COX – cyclooxygenases, IL – *in loco*, NSAIDs – nonsteroidal anti-inflammatory drugs, PGH_2 – prostaglandin H_2, WE – wound epithelium.

However, several studies on wound healing have suggested that COX-2 was the constitutive and dominant isoform in these cells (Hamasaki et al., 1993; Kwon et al., 1994; Asano et al., 1996). Recently, one more splice variant of COX-1 named COX-3 has also been reported, which appears to be involved in processes such as fever, and is inhibited by acetaminophen (Botting, 2000; Chandrasekharan et al., 2002). Unlike COX-1 and COX-2, COX-3 does not appear to have significant involvement in tissue inflammation (Prisk and Hauard, 2003).

Many findings indicate that PGE_2 production is essential for cutaneous wound healing. There are reports that COX-2 is present in the margin of healing ulcers and that COX-2 products such as PGE_2 might contribute to the resolution of inflammation in the gastrointestinal tract (McCarthy, 1995; Bamba et al., 1998) and elsewhere (Appleton et al., 1995). However, the role of many inflammatory components including COX-2-induced PGE_2 in the prostaglandin pathway is not well understood in lizard tail regeneration.

It is known that COX products are essential for rapid wound repair. The prostanoid PGE_2 provides a significant stimulation for wound. The stimulation of closure by prostanoid metabolites occurs immediately after wounding and may stimulate spreading and migration of the cells. Recent research in skeletal muscle healing and regeneration also demonstrated that the *in vivo* effect of COX-2 inhibitors resulted in the delay in muscle regeneration (Shen et al., 2005). It is also being reported that PGs are local regulators of a number of cellular functions and their regulatory effects in many systems are mediated by cyclic AMP (cAMP). This indicates that PGs produced during cell aggregation are involved in cell differentiation by acting via local modulators of cAMP during blastema and differentiation stages of caudal regeneration in the lizard (Appukuttan et al., 1993).

The present study was undertaken to ascertain the role of prostanoids, particularly PGE_2, in the regulation of epimorphic tail regeneration in lizards. The effect of different nonsteroidal anti-inflammatory drugs (NSAIDs), including non-specific inhibitor of cyclooxygenase such as colosprin and specific cyclooxygenase-2 inhibitors celecoxib and etoricoxib, was studied during the successive stages of regeneration.

Usage of selective and non-selective COX inhibitors was applied because of the selectivity of their therapeutic action and also due to the presence of several isoforms of the enzyme. A non-specific COX inhibitor prevents generation of prostaglandins by direct action on the COX enzyme (Flower, 2003). According to Warner et al. (1999), celecoxib inhibits COX-2 with a 5–50-fold selectivity, whereas etoricoxib being a second generation of NSAIDs displays 80-fold selective inhibition of COX-2.

The current attempt to understand the basic principles and pathways behind caudal regeneration may improve our understanding of different types of tissue regeneration in human and also provide insight into why regeneration of a completely lost part does not occur naturally in humans. Moreover, it is suggested that regenerating lizard tails are potentially useful models for studying the molecular basis of regeneration with a view to develop possible treatments for human diseases (Daniels et al., 2003).

Material and Methods

Experimental animals

Adult Northern House Geckos, *Hemidactylus flaviviridis*, of both sexes with normal intact tail were collected from the natural habitat. All animals were screened for parasitic infestation and the healthy ones were acclimated for a week before the commencement of the experiment. The animals were fed with in-house reared cockroach nymphs twice a week and purified water was given daily, *ad libitum*. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The experiments were performed in accordance with the guidelines of the Animal Care.

Drug administration and experimental procedure

A total of 36 animals were used for this experiment. They were randomly allocated into four groups of nine animals each based on the body weight stratification method using in-house made validated statistical software. At the commencement of treatment, the mean body weight of animals in each group was 10 g and the variation among the animals was within 20 % of the mean body weight.

All animals were given *in loco* (IL) injections of the specific and non-specific COX inhibitors. The doses were selected based on the reference data for the drugs (etoricoxib data sheet 2005) and also following a dose range study. The presence of the drug in the tissue (regenerate) was confirmed by Fourier Transform Infrared Spectroscopy (FTIR) (Fig. 1).

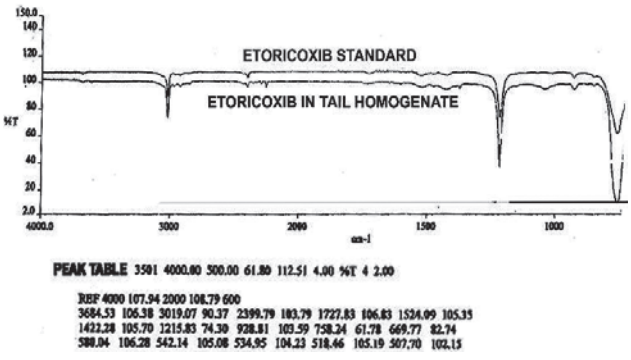

Fig. 1. Drift spectra of etoricoxib in lizard tail homogenate showing characteristic C═ N, S═O, C-Cl stretching vibration

Experiment I

Animals in each group were treated as follows:

Group I: This group of animals served as a control to the experimental groups and was injected with vehicle (Tris buffer of pH 8.8).

Group II: The animals of this group received colosprin (50 mg/kg body weight).

Group III: The animals of this group received celecoxib at a dose of 50 mg/kg body weight.

Group VI: The animals of this group were injected with etoricoxib (25 mg/kg body weight).

All the drugs were prepared fresh in Tris buffer of pH 8.8 immediately before use and were administered every day at a maximum quantity of 0.05 ml per animal. After a week of drug treatment, autotomy was induced in all groups of animals by exerting mild thumb pressure on the normal intact tail three segments away from the vent. The treatment was continued until the termination of the experiment. The growth of the regenerate was measured at fixed intervals using a calibrated digital Caliper (Mitutoyo, Kawasaki, Japan) and time taken to reach different stages of epimorphic regeneration was recorded.

Experiment II

Autotomy was induced, as described earlier, in 150 lizards *H. flaviviridis*, and the regenerating animals were selected at three defined stages of regeneration *viz*., (i) just after amputation, (ii) at completion of wound healing and appearance of wound epithelium (WE) stage, and (iii) in lizards at early blastema (BL) stage. Only those animals that attained the above stages on the same day were selected and grouped.

Series A

Injections of PGE_2 antagonists *viz*: colosprin, celecoxib and etoricoxib were given just after amputation. Thirty-six lizards were selected and divided into four groups of nine animals each. All the groups were given *in loco* (IL) injections and the animals were divided evenly in the same four groups as in Experiment I.

Series B

In loco injections of PGE_2 antagonists *viz*: colosprin, celecoxib and etoricoxib were administered at WE stage of epimorphic regeneration. The numbers of animals and groups were the same as in Series A.

Series C

Thirty-six lizards that attained the blastema stage on the same day were selected for the experiment. They were divided into four groups of nine animals each and treated as described earlier until the control animals reached differentiation stage. The time taken to reach the various stages of tail regeneration and the rate of growth of regenerate were recorded at fixed intervals.

Statistical Analysis

Data were subjected to Bartlett's test to meet homogeneity of variance before conducting Analysis of Variance (ANOVA) and Duncan's multiple range test. The values were expressed as mean ± SE. The P value of 0.05 or less was considered statistically significant.

Results

Exogenous administration of specific and non-specific inhibitors of COX-2 in the lizard, *H. flaviviridis,* at all stages was found to hamper the process of regeneration as compared to that of control animals. However, of all the inhibitors studied, etoricoxib, second-generation COX-2-specific inhibitor, was found to be the most potent inhibitor of regeneration. A dose-dependent retardation in the progression of caudal regeneration was evident in the present study. Moreover, etoricoxib at a dose of 25 mg/kg body weight and beyond was found to arrest the entire process of regeneration. The inflammatory tissue (tail stump) of the animals that received such higher doses of etoricoxib remained at the wounded stage with no further progression even after the controls reached the differentiation stage (Fig. 2).

Experiment I: In experiment I, where the drugs were administered prior to amputation, a significant delay in attaining various stages of regeneration was observed as compared to vehicle-treated controls. The delay was more pronounced in animals injected with etoricoxib, which took more time to reach wound epithelium, blastema, growth and differentiation stages as compared to that of the groups subjected to colosprin and celecoxib (Fig. 3). Etoricoxib-treated animals took the maximum number of days to attain the differentiation stage as compared to other groups (Table 1).

The rate of growth and percentage of growth inhibition were calculated for the growth (2–12 mm) and differentiation (12–24 mm) stages of regenerating tail. A significant decrease was observed in the length of the regenerating tails of all treated animals, and the highest percentage decrease in the rate of growth was seen in etoricoxib-injected animals with 71% reduction in

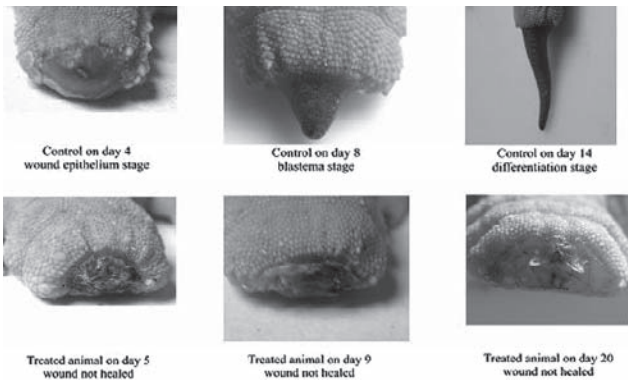

Fig. 2. Total suppression of epimorphosis in lizards treated with a high dose of selective COX-2 inhibitor

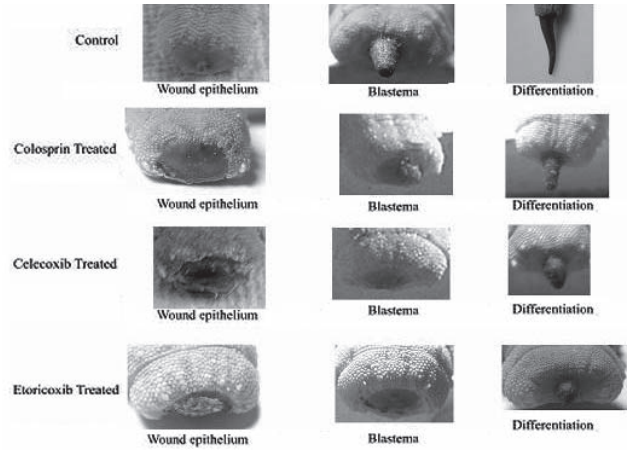

Fig. 3. Dorsal view of the regenerating tail of the Northern House Gecko; images taken at different stages

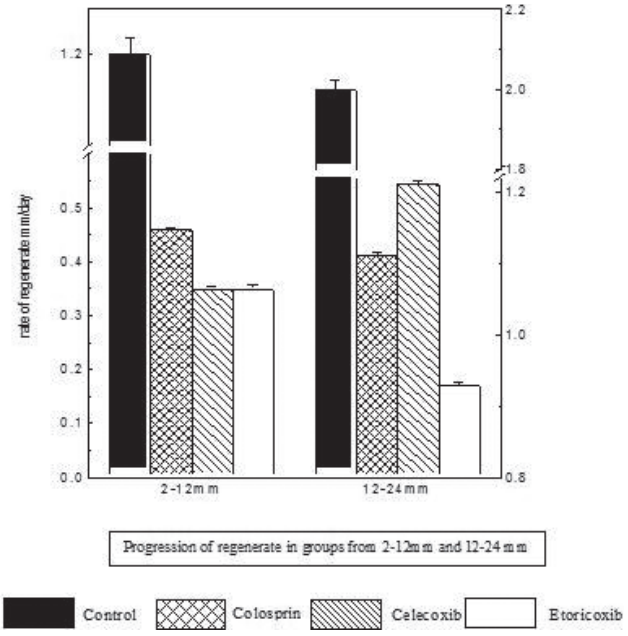

Fig. 4. Progression of tail regeneration in wall lizard, *Hemidactylus flaviviridis*, subjected to *in loco* injection of specific and non-specific COX inhibitors before amputation

growth rate during 2–12 mm stage and 54% reduction during 12–24 mm stage of regenerate (Table 2, Fig. 4). The heightened negative effect of second-generation drug etoricoxib on regeneration could be attributed to its higher specificity for COX-2 inhibition.

*Experiment II***:** In order to unravel the effect of COX-2-induced autocoid PGE_2 at different stages of caudal regeneration, animals were injected with the drugs at specific stages of regeneration.

Series A: Exogenous administration of colosprin, celecoxib and etoricoxib just after the amputation led to a delay in the process of wound healing, blastema formation, growth and differentiation. On average, it took eight days for colosprin-treated animals to attain the wound-healing stage, whereas celecoxib- and etoricoxib-injected animals took ten days to reach the wound-healing stage (Table 3). Control animals, however, took only four days for the attainment of the same stage. The lowest rate of growth of regenerate (0.3 mm/day) was recorded in etoricoxib-treated animals (Table 4, Fig. 5). Moreover, the percentage decrease in the growth of regenerate was maximum in this set of the experiment.

Series B & C: In these sets of the experiment, the role of PGE_2 was ascertained at wound epithelium and blastema stages, respectively. The animals on achieving these stages were subjected to specific and

Table 1. Number of days taken to reach various regenerative stages in wall lizard, Hemidactylus flaviviridis, subjected to in loco (IL) injection of colosprin, celecoxib and etoricoxib before amputation

Treatment	No. of days WE	BL (2 mm)	DF (12 mm)
IL control	5 (6–5)[#]	7 (8–9)	12 (13–12)
IL colosprin	10 (9–10)	14 (14–15)	25(25–26)
IL celecoxib	9 (9–10)	17 (16–17)	28 (27–28)
IL etoricoxib	10 (10–11)	18 (18–19)	30 (29–30)

[#] Values are expressed as mode and range in parenthesis

Table 2. Length of tail regenerated in wall lizard, Hemidactylus flaviviridis, after in loco (IL) treatment with various inhibitors (colosprin, celecoxib and etoricoxib) before amputation. The average tail length is in mm.

Treatment	Rate of growth of regenerate (mm/day) 2–12 mm	12–24 mm	% decrease (↓) compared to control 2–12 mm	12–24 mm
IL control	1.20 ± 0.018[@]	2.00 ± 0.024	-	-
IL colosprin	0.46 ± 0.003**↓	1.11 ± 0.005**↓	62↓[b]	45↓
IL celecoxib	0.35 ± 0.003**↓	1.21 ± 0.004**↓	71↓	40↓
IL etoricoxib	0.35 ± 0.006**↓	0.93 ± 0.004**↓	71↓	54↓

[@] Values are expressed as mean ± SE, * P ≤ 0.05, ** P ≤ 0.01; N = 5

[b] Values are corrected to the nearest whole number

Table 3. Number of days taken to reach various regenerative stages in wall lizard, Hemidactylus flaviviridis, subjected to in loco (IL) injection of colosprin, celecoxib and etoricoxib after amputation

Treatment	WE	No. of days BL (2 mm)	DF (12 mm)
IL control	4 (4–5)[#]	8 (9–8)	14 (15–16)
IL colosprin	8 (9–8)	14 (13–14)	27 (26–27)
IL celecoxib	10 (10–11)	15 (14–15)	27 (26–27)
IL etoricoxib	10 (10–11)	15 (14–15)	30 (29–30)

[#] Values are expressed as mode and range in parenthesis

Table 4. Length of tail regenerated in wall lizard, Hemidactylus flaviviridis, after in loco (IL) treatment with various inhibitors (colosprin, celecoxib and etoricoxib) after amputation. The average tail length is in mm.

Treatment	Rate of growth of regenerate (mm/day) 2–12 mm	12–24 mm	% decrease (↓) compared to control 2–12 mm	12–24 mm
IL control	0.85 ± 0.011[@]	2.42 ± 0.022	-	-
IL colosprin	0.36 ± 0.010**↓	0.86 ± 0.006**↓	58↓[b]	64↓
IL celecoxib	0.32 ± 0.006**↓	0.75 ± 0.005**↓	62↓	69↓
IL etoricoxib	0.30 ± 0.002**↓	0.85 ± 0.004**↓	65↓	65↓

[@] Values are expressed as mean ± SE, * $P \leq 0.05$, ** $P \leq 0.01$; N = 5
[b] Values are corrected to the nearest whole number

non-specific COX-2 inhibitors. The results were very much similar with the above experiment of series A, where the drugs were given prior to and after amputation. A significant decrease was observed in the rate of growth of regenerate in animals that received etoricoxib during WE stage of regeneration (Table 5, Table 6, Fig. 6).

Moreover, the 73% reduction in the rate observed during the growth phase of animals that received etoricoxib at the blastema stage was the highest decrease among all the treated groups (Table 7, Table 8, Fig. 7).

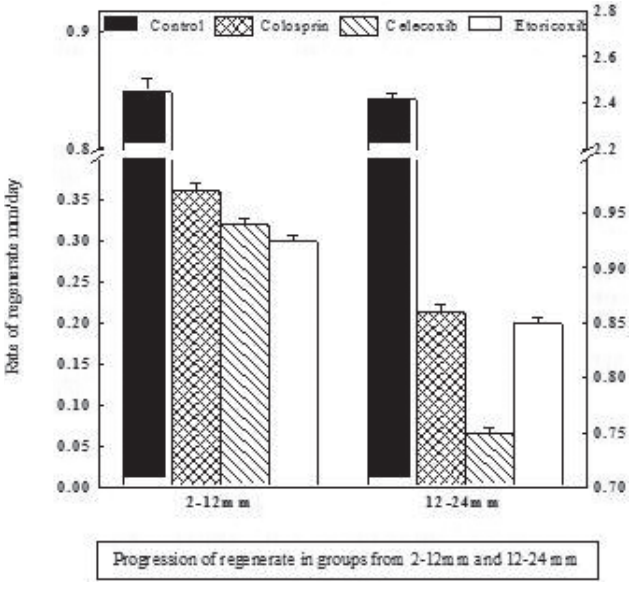

Fig. 5. Progression of tail regeneration in wall lizard, *Hemidactylus flaviviridis,* subjected to *in loco* injection of specific and non-specific COX inhibitors after amputation

This could be because during blastema stage the cells undergo aggregation, migration and cell proliferation, where PGE_2 might be playing a cardinal role.

Discussion

Induced autotomy in lizard tail results in a cascade of events beginning with haemostasis and inflammation and concluding with growth and differentiation. Whereas inflammation eventually subsides as wound healing progresses, it has lasting effects on the final wound healing outcome. Inflammatory mediators, released by macrophages and neutrophils, serve as chemotactic cues for invading fibroblasts and later regulate cell proliferation and cell migration in the wound bed (Sandulache, 2006). Exogenous administration of COX-2 inhibitors to *H. flaviviridis,* prior to amputation or later at wound epithelium and blastemal stages, led to the retardation in the progression of the regenerate (Figs. 4, 5, 6). It is apparent from the results of the present study that the second-generation drug etoricoxib imparts a more adverse effect on the progression of the process of regeneration, compared to other COX inhibitors studied (Table 1), possibly by effective blocking of the downstream component of the prostaglandin pathway. The likely reason for the developmental anomaly could be the blockage of the COX-2 enzyme, which catalyses the reaction for the formation of PGE_2. Therefore, from the present study it is evident that COX-2-induced PGE_2 is essential for the formation and maintenance of apical epithelial cap (AEC). Hence PGE_2 can be considered as a key modulator of epimorphic regeneration in tandem with a few other known or unknown autocrine/paracrine factors.

Injury activates multiple inflammatory cascades including induction of COX-2 (Branski et al., 2005). Lip-

Table 5. Number of days taken to reach various regenerative stages in wall lizard, Hemidactylus flaviviridis, subjected to in loco (IL) injection of colosprin, celecoxib and etoricoxib at WE stage

Treatment	WE	No. of days BL (2 mm)	DF (12 mm)
IL control	5 (5–6)[#]	9 (9–10)	15 (15–16)
IL colosprin	5 (5–6)	14 (13–14)	26 (25–26)
IL celecoxib	5 (5–6)	15 (14–15)	27 (26–27)
IL etoricoxib	5 (5–6)	15 (14–15)	27 (26–27)

[#] Values are expressed as mode and range in parenthesis

Table 6. Length of tail regenerated in wall lizard, Hemidactylus flaviviridis, after in loco (IL) treatment with various inhibitors (colosprin, celecoxib and etoricoxib) at WE stage. The average tail length is in mm.

Treatment	Rate of growth of regenerate (mm/day) 2–12 mm	m 12–24 mm	% decrease (↓) compared to control 2–12 mm	12–24 mm
IL control	0.96 ± 0.019[@]	2.034 ± 0.020	-	-
IL colosprin	0.37 ± 0.005**↓	0.82 ± 0.004**↓	61↓[b]	60↓
IL celecoxib	0.35 ± 0.003**↓	0.86 ± 0.004*↓	64↓	58↓
IL etoricoxib	0.32 ± 0.004**↓	0.84 ± 0.005*↓	67↓	59↓

[@] Values are expressed as mean ± SE, * P ≤ 0.05, ** P ≤ 0.01; N = 5
[b] Values are corrected to the nearest whole number

id-based mediator PGE_2, a product of COX-2 activation, has a more ubiquitous role in wound healing and may be expressed in both early and later stages, as studied in the rabbit model (Branski et al., 2005). Prostaglandin E_2 is thought to be the most important COX-2 product during dermal wound healing (Wilgus et al., 2004). Prostaglandin E_2 has been implicated in inhibiting profibrotic responses, including collagen production, contraction of extracellular matrix, and fibroblast proliferation in human (Kohyama et al., 2001). Interestingly, the application of exogenous PGE_2 has been shown to stimulate

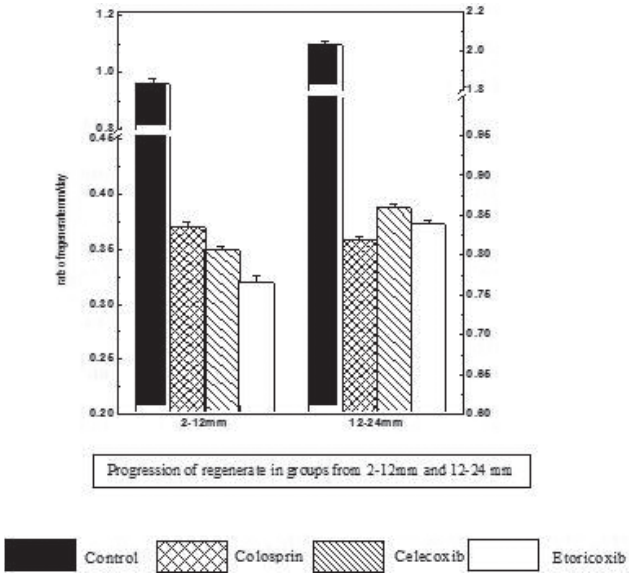

Fig. 6. Progression of tail regeneration in wall lizard, *Hemidactylus flaviviridis,* subjected to *in loco* injection of specific and non-specific COX inhibitors at wound epithelium (WE) stage

epithelial cell migration, suggesting its involvement in the wound-healing response (Savla et al., 2001).

Apart from its action on wound healing, PGE_2 also plays a pivotal role in the recruitment and proliferation of blastemal cells, as evidenced by the significant delay in achieving the respective stages of regeneration observed in the treated groups. Shen (2006) indicated that a relatively low concentration of PGE_2 increased cell proliferation in both *in vivo* and *in vitro* studies. In addition, other findings concluded that during vertebrate appendage regeneration, high activities of COX and PGE_2 were observed (Appukutan et al., 1993).

Further, the data presented here provide insight into the role of PGE_2 in regulating the rate of growth of the regenerate. In the current study, a significant difference was observed among animals treated with different drugs used for inhibiting the COX-2 production. This is particularly important since colosprin, celecoxib and etoricoxib display differences in the percentage of selective inhibition of COX-2. The present study resulted in a marked delay in the rate of growth of the regenerate after the treatment with colosprin, celecoxib and etoricoxib. However, as explained, etoricoxib is 80% more specific in COX-2 inhibition with respect to colosprin, which has 10–100-fold lowered sensitivity for COX-2 as compared to COX-1 (Simmons et al., 2004).

The blockage of PGE_2 expression resulted in hampering the milestone of regenerative process in the lizard. The delay in the formation of wound epithelium can be indicative of changes in cell expression for migration and proliferation (Shen et al., 2006). However, the marked deceleration in the progress of regeneration observed in etoricoxib-treated animals could be in response to specific cues such as growth factors or inflammatory mediators like PGE_2. Previous studies have demonstrat-

Table 7. Number of days taken to reach various regenerative stages in wall lizard, Hemidactylus flaviviridis, subjected to in loco (IL) injection of colosprin, celecoxib and etoricoxib at BL stage.

Treatment	WE	No. of days BL (2 mm)	DF (12 mm)
IL control	7 (6–7)[#]	10 (9–10)	15 (16–15)
IL colosprin	7 (6–7)	10 (9–10)	26 (25–26)
IL celecoxib	7 (6–7)	10 (9–10)	26 (26–27)
IL etoricoxib	7 (6–7)	10 (9–10)	27 (26–27)

[#] Values are expressed as mode and range in parenthesis

Table 8. Length of tail regenerated in wall lizard, Hemidactylus flaviviridis, after in loco (IL) treatment with various inhibitors (colosprin, celecoxib and etoricoxib) at BL stage. The average tail length is in mm.

Treatment	Rate of growth of regenerate (mm/day) 2–12 mm	12–24 mm	% decrease (↓) compared to control 2–12 mm	12–24 mm
IL control	1.07 ± 0.002[@]	1.89 ± 0.001	-	-
IL colosprin	0.46 ± 0.015**↓	0.89 ± 0.001**↓	57↓[b]	53↓
IL celecoxib	0.31 ± 0.001**↓	1.00 ± 0.001**↓	71↓	47↓
IL etoricoxib	0.29 ± 0.002**↓	0.93 ± 0.003**↓	73↓	51↓

[@] Values are expressed as mean ± SE, * $P \leq 0.05$, ** $P \leq 0.01$
[b] Values are corrected to the nearest whole number

ed that exogenous administration of FGF-2 into lizards resulted in a faster rate of migration of cells and differential modulation of migration by extrinsic cues (Yadav et al., 2008). Using this as a baseline, the effects of PGE_2 on plastic blastemal cells was determined. However, the percentage inhibition of growth in regenerating tail in our experiment was significantly much higher in etoricoxib-treated animals as compared to celecoxib- and colosprin-treated ones. These findings are important for establishing a COX-2-prostaglandin signalling pathway in regeneration. These results are consistent with data reported by Futagami et al. (2002) showing that administration of the COX-2 inhibitor delayed re-epithelization in the early phase of wound healing and also inhibited angiogenesis.

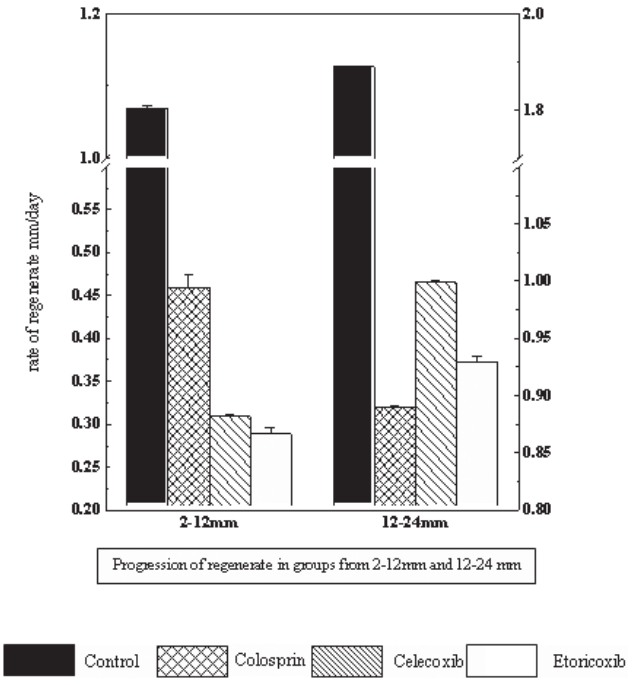

Fig. 7. Progression of tail regeneration in wall lizard, *Hemidactylus flaviviridis*, subjected to *in loco* injection of specific and non-specific COX inhibitors at blastema (BL) stage

The negative influence caused by specific and non-specific COX-2 inhibitors can be explained by previous studies utilizing extensive pharmacological experimentation. Shiff and co-workers have demonstrated that other NSAIDs including aspirin, indomethacin, naproxen and piroxicam all reduced proliferation and altered the morphology of HT-29 cells (Shiff et al., 1996). The decrease in cell proliferation could be explained by alteration of cell cycle distribution by these drugs to increase the proportion of cells in G_0/G_1 with a reduction of cells in S phase. Aspirin and indomethacin also reduced the proportion of cells in G_2/M. In 1980, de Mello et al. reported that anti-inflammatory drugs arrested the growth of rat hepatoma and human fibroblast cultures in G_1 phase. The effect was reversed by washing out the drug followed by resumption of cell growth. Therefore, it could be possible that with the exogenous administration of COX inhibitors there are amendments in the cell cycle during cell proliferation for the formation of blastema and successive stages of regeneration. Thus, it could be possible that the treated animals showed a lower pool of cells in cell cycle both in the apical epithelial cap (AEC), which is the main source of cells to differentiate, and the underlining mesenchyme. A similar finding was also encountered by Appukuttan et al. (1993) in lizard regenerating tail. According to them, PGE_2 levels were high during the cell aggregation period. A high

level of PGE_2 may later stimulate cAMP production resulting in cytodifferentiation of blastemal cells.

Inhibition of fibroblast migration was found to correspond with the obvious morphological alteration in the actin cytoskeleton. PGE_2, likely through a cAMP-mediated pathway, destabilizes the actin cytoskeleton and depolymerizes existing actin stress fibres (Sandulache, 2006). This is consistent with previous studies linking cAMP release to protein kinase A (PKA) activation and cytoskeletal rearrangement (Edin et al., 2001; Dormond et al., 2002; Glenn and Jacobson, 2002). It is likely that this process can be responsible for the impairment of fibroblast motility needed for migration towards the wound bed, and thus for augmenting the adverse effect during caudal regeneration.

Acknowledgment

The authors are highly appreciative to the UGC-DSA Phase-III programme of the Department of Zoology, Faculty of Science, M.S. University of Baroda, for providing all the financial help for procuring chemicals and animals. One of the authors (S.P.) is also grateful to the help provided by the M.S. University of Baroda in the form of Junior Research Fellowship.

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