INFLUENCE OF COX-2 INDUCED PGE_2 ON THE INITIATION AND PROGRESSION OF TAIL REGENERATION IN NORTHERN HOUSE GECKO, *HEMIDACTYLUS FLAVIVIRIDIS*

INTRODUCTION

Regeneration is defined as the ability to reproduce organs or structures after they have been lost through trauma or other causes (Belliars and Bryant, 1985). The invertebrates (planaria and hydra) show amazing power of regeneration by regenerating whole body from fragments of the body through cellular reorganization better known as morphallaxis (Goss, 1969; Baguna, 1998; Sanchez-Alvarado, 2000). Among the vertebrates, Urodele (caudate) amphibians and lizards express epimorphic regeneration. Epimorphic regeneration involves the 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 which 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 phenomena. A scan through the literature however, reveals that the tail regeneration in lizard is not studied as extensively as that of amphibian regeneration. Nevertheless, the process of regeneration is comparable between the lizards and amphibians (Ityen and Bryant, 1976). Regeneration in 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.

Inflammatory phase which is a hallmark of wound healing stage, leads to release of cytokines and growth factors leading to the permeability of blood vessels and chemotaxis of inflammatory cells. The cells at the edge of an epidermal wound migrate, proliferate, and differentiate to cover the exposed wound surface, and fibroblasts and capillaries produce a new granulation tissue (Clark, 1993; Martin, 1997). 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). Prostaglandin E_2 (PGE_2), which constitutes the major PGs in human and rat skin (Jouvenaz *et al.*, 1970; Jonsson and Änggård, 1972), affects keratinocyte cell proliferation (Lowe and Stoughton, 1977; Pentland and Needleman, 1986), differentiation (Evans *et al.*, 1993), and also promote angiogenesis *in vivo* together with PGE_1 (Ziche *et al.*, 1982; Form and Auerbach, 1983). 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 catalyzed 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). 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 is also reported of late 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 doest not appear to have significant involvement in tissue inflammation (Prisk and Hauard, 2003).

PGE_2 exerts its effects on cellular behaviour via E prostanoid receptors (EP1, EP2, EP3, and EP4). Prostaglandin receptors belong to the general category of G- coupled protein receptors (a 7 trans-membrane domain receptors allow for transduction of extracellular signals by coupling to G protein, which can subsequently activate multiple intracellular signaling pathways). Furthermore, receptors for PGE_2 mediate the effect of PGE_2 on keratinocyte growth (Konger *et al.*, 1998). Indeed, EP4 receptor mRNA showed upregulation in a fetal rabbit skin wound (Li *et al.*, 2000). These 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 like 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 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 closure and its effects are likely mediated by the EP1 and EP4 receptor subtypes. The stimulation of closure by prostanoid metabolites occur immediately after wounding and may stimulate spreading and migration of the cells. Recent research in skeletal muscle healing and regeneration also demonstrated that *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 number of cellular functions and their regulatory effects in many systems are mediated by cyclic AMP (cAMP). Thus, indicating 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 lizard (Appukuttan *et al.*, 1993).

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

Usage of selective and non-selective COX inhibitors was taken because, of the selectivity of their therapeutic action and also due to the presence of several isoforms of the enzyme. Non-specific COX inhibitor prevents the 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 of selectivity whereas etoricoxib being a second generation of NSAIDs has 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 complete lost part does not occur

naturally in humans. Moreover it is suggested that regenerating lizard tails are potentially useful models for studying molecular basis of regeneration with a view to develop possible treatments for human diseases (Daniels *et al.*, 2003).

MATERIALS AND METHODS

Experimental animals

Adult Northern House Geckos, *Hemidactylus flaviviridis*, of both the 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 experiment. The animals were fed within house reared cockroach nymphs twice a week and purified water was given daily, *ad libitum.*

Drug Administration and Experimental Procedure:

A total of thirty six animals were used for this experiment. They were randomly allocated into four groups of nine animals each based on 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 10gm 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 nonspecific 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 Spectoroscopy (FTIR) (see material and methods for details) (Figure 1.1).

Experiment I

Animals in each group were treated as follows:

Group I: This group of animals served as 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 50mg/kg body wt.

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 0.05ml 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 till the termination of experiment. The growth of the regenerate was measured at fixed intervals using a calibrated digital Caliper (Mitutoyo, Japan) and time taken to reach different stages of epimorphic regeneration was recorded.

Experiment II

Autotomy was induced, as described earlier, on 150 lizards *H. flaviviridis*, and the regenerating animals were selected at three defined stages of regeneration *viz*., (i) just after amputation (ii) 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

Injection 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. These groups were treated as follows:

Group I: This group of animals served as control to the experimental groups and were injected with Tris Buffer (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 an injection of celecoxib at a dose of 50mg/kg body weight.

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

The treatment continued till the termination of experiment (i.e. when the animals in the control group reached differentiation stage). The number of days taken by the lizards to attain different stages was recorded and the length of the regenerate was documented at fixed intervals.

Series B

In loco injection of PGE_2 antagonists *viz*: colosprin, celecoxib and etoricoxib were administered at WE stage of epimorphic regeneration.

Thirty-six lizards which attained WE stage on the same day were selected and were divided into four groups of nine animals each.

Group I: This group of animals served as control to the experimental groups and received IL injection of vehicle (Tris Buffer pH 8.8).

Group II: Animals received colosprin (50 mg/kg body weight).

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

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

The treatment started at WE stage and was continued till the termination of experiment. The number of days taken by the lizards to attain different stages and the length of the regenerate was recorded at fixed intervals.

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 till the control animals reach differentiation stage.

The time taken to reach the various stages of tail regeneration and the rate of growth of regenerate was recorded at fixed intervals.

STATISTICAL ANALYSIS

Data was 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. A '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. falviviridis,* at all the stages, was found to hamper the process of regeneration as compared to

that of control animals. However, of all the inhibitors studied, etoricoxib, the 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 differentiation stage (Figure 1.2).

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 stage as compared to that of the groups subjected to colosprin and celecoxib (Figure 1.3). Etoricoxib treated animals took the maximum number of days to attain the differentiation stage as compared to other groups (Table 1.1a).

Rate of growth and percentage of growth inhibition were calculated for the growth (2 – 12mm) and differentiation (12 – 24mm) 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 growth rate during 2-12mm stage and 54% reduction during 12-24 mm stage of regenerate (Table 1.1b and Figure 1.4). 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 amputation leads to delay in the process of wound healing, blastema formation, growth and differentiation. It took on an average 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 1.2a). Control animals however, took only four days for the attainment of the same stage. Lowest rate of growth of regenerate (0.3 mm/day) was recorded in etoricoxib treated animals (Table 1.2b and Figure 1.5). Moreover, the percentage decrease in the growth of regenerate was maximum in this set of experiment.

Series B & C In these sets of 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 non-specific COX-2 inhibitors. Results were very much similar with the above experiment of series A where the drugs were given prior to and after amputation. Significant decrease was observed in the rate of growth of regenerate in animals that received etoricoxib during WE stage of regeneration (Table 1.3a, b and Figure 1.6).

Moreover, the 73% reduction in the rate observed during growth phase of animals that received etoricoxib at the blastema stage was the highest decrease among all the treated groups (Table 1.4a, b and Figure 1.7). 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 hemostasis 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 (Figure 1.4, 1.5, 1.6). It is apparent from the results of the present study that the second generation drug etoricoxib imparts more adverse effect on the progression of the process of regeneration, compared to other COX inhibitors studied (Table 1.1a), possibly by effective blocking of the downstream component of Prostaglandin pathway. The likely reason for the developmental anomaly could be due to the blockage of 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 few other known or unknown autocrine/ paracrine factors.

Injury activates multiple inflammatory cascades including induction of COX-2 (Branski *et al.*, 2005). A lipid-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 of as studied in 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 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 observed in the treated groups, in achieving the respective stages of regeneration. Shen (2006) has indicated that relatively low concentration of PGE_2 increased cell proliferation in both *in vivo* and *in vitro* studies. In addition, other finding concluded that during vertebrate appendage regeneration high activity of COX and PGE_2 were observed (Appukutan *et al.*, 1993).

Further, the data presented here provides insight into the role of PGE_2 in regulating the rate of growth of regenerate. In the current study significant difference has been observed among animals treated with different drugs used for inhibiting the COX-2 production. This is particularly important since colosprin, celecoxib and etoricoxib have difference in the percentage of selective inhibition of COX-2. Present study resulted into a marked delay in the rate of growth of regenerate with the treatment of colosprin, celecoxib and etoricoxib. However, as explained that 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).

Blockage of PGE_2 expression resulted in hampering the milestone of regenerative process in lizard. The delay in the formation of wound epithelium can be indicative of changes in cell expression for migration and proliferation (Shen, 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. Cyclooxygenase (COX) is the rate–limiting enzyme in the production of prostaglandin and also a key target for many anti-inflammatory drugs. There are two known isoform of COX: COX-1 and COX-2, which have quite distinct expression pattern and biological activities. COX-1 is a constitutively expressed protein found in most tissue, whereas COX-2 expression can be induced by variety of mitogens including cytokines and hormones (Kujubu *et al.*, 1991; O'Banion *et al.*, 1992). Inflammatory stimuli has been found to have little effect on COX-1 expression but rapid rise in COX-2 mRNA, suggesting an important role of COX-2 in the process of inflammation (O'Banion *et al.*, 1992). Previous studies have demonstrated that exogenous administration of FGF-2 in lizard resulted into a faster rate of migration of cells and differential modulation of migration by extrinsic cues (Yadav, 2005). 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 is 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-epithelialization in the early phase of wound healing and also inhibited angiogenesis.

The negative influence caused by specific and non-specific COX-2 inhibitors can be explained by previous studies utilizing extensive pharmacological experimentation. They 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). 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 cells. Aspirin and indomethacin also reduced the proportion of cells in G_2/M. In 1980, DeMello *et al.*, reported that anti-inflammatory drugs arrested the growth of rat hepatoma and human fibroblast cultures in the G_1 phase. The effect was reversed by washing out of 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 cell cycle during cell proliferation for the formation of blastema and successive stages of regeneration. Similar finding was also accounted by Appukuttan *et al.*, (1993) in lizard regenerating tail.

According to them PGE_2 levels were high during cell aggregation period. High level of PGE_2 may later stimulate cAMP production resulting in cytodifferentiation of blastemal cells.

Inhibition of fibroblast migration was found to correspond to 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 fibers (Sandulache, 2006).This is consistent with previous studies linking cAMP release to PKA activation and cytoskeletal rearrangement (Lamb *et al.*, 1988; Iwamoto *et al.*, 1993; Kondo and Yonezawa, 1995; Smith *et al.*, 1995; Edin *et al.*, 2001; Dormond *et al.*, 2002; Glenn and Jacboson, 2002). It is likely that this process can be responsible for the impairment of fibroblast motility for migration towards the wound bed and thus augmenting the adverse effect during caudal regeneration.

The impairment in the rate of growth of regenerate with the blockage of PGE_2 can further regulate the functional activites of cells via binding to cell surface receptors (e.g. EP1, EP2, EP3 and EP4) (Negishi *et al.*, 1995; Narumiya *et al.*, 1999; Abramovtiz *et al.*, 2000). Thus it could be possible that the treated animals showed less pool of cells in cell cycle both in apical epithelial cap (AEC) which is the main source of cells to differentiate and the underlining mesenchyme. This delay in attaining the specific stages of regeneration further resulted in string desire to work on the reasons behind the hampered epimorphic regeneration.

Table 1.1a: 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)

Table 1.1b: 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)		% increase/decrease compared to control	
	From 2-12 mm	From 12-24 mm	From 2-12 mm	From 12-24mm
IL Control	1.20± 0.018[@]	2 ± 0.024	-	-
IL Colosprin	0.46± 0.003**↓	1.11± 0.005**↓	62↓	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

* Values are corrected to the nearest whole number

Values are expressed as mode and range in parenthesis

Table 1.2a: 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	No. of Days		
	WE	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)

Table 1.2b: 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)		% increase/decrease compared to control	
	From 2-12 mm	From 12-24 mm	From 2-12 mm	From 12-24mm
IL Control	0.85 ± 0.011[@]	2.42 ± 0.022	-	-
IL Colosprin	0.36± 0.010**↓	0.86 ± 0.006**↓	58↓	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≤0.05, ** p≤0.01 ; n=5

* Values are corrected to the nearest whole number

Values are expressed as mode and range in parenthesis

Table 1.3a: 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	No. of Days		
	WE	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)

Table 1.3b: 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)		%increase/decrease compared to control	
	From 2-12 mm	From 12-24 mm	From 2-12 mm	From 12-24mm
IL Control	0.96 ± 0.019[@]	2.034 ± 0.020	-	-
IL Colosprin	0.37 ± 0.005**↓	0.82 ± 0.004**↓	61↓	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, * $p \leq 0.05$, ** $p \leq 0.01$; n = 5

Values are corrected to the nearest whole number

Values are expressed as mode and range in parenthesis

Table 1.4a: 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	No. of Days		
	WE	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)

Table 1.4b: 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)		% increase/decrease compared to control	
	From 2-12 mm	From 12-24 mm	From 2-12 mm	From 12-24mm
IL Control	1.07 ± 0.002[@]	1.89 ± 0.001	-	-
IL Colosprin	0.46± 0.015**↓	0.89± 0.001**↓	57↓	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≤0.05, ** p≤0.01

* Values are corrected to the nearest whole number

\# Values are expressed as mode and range in parenthesis

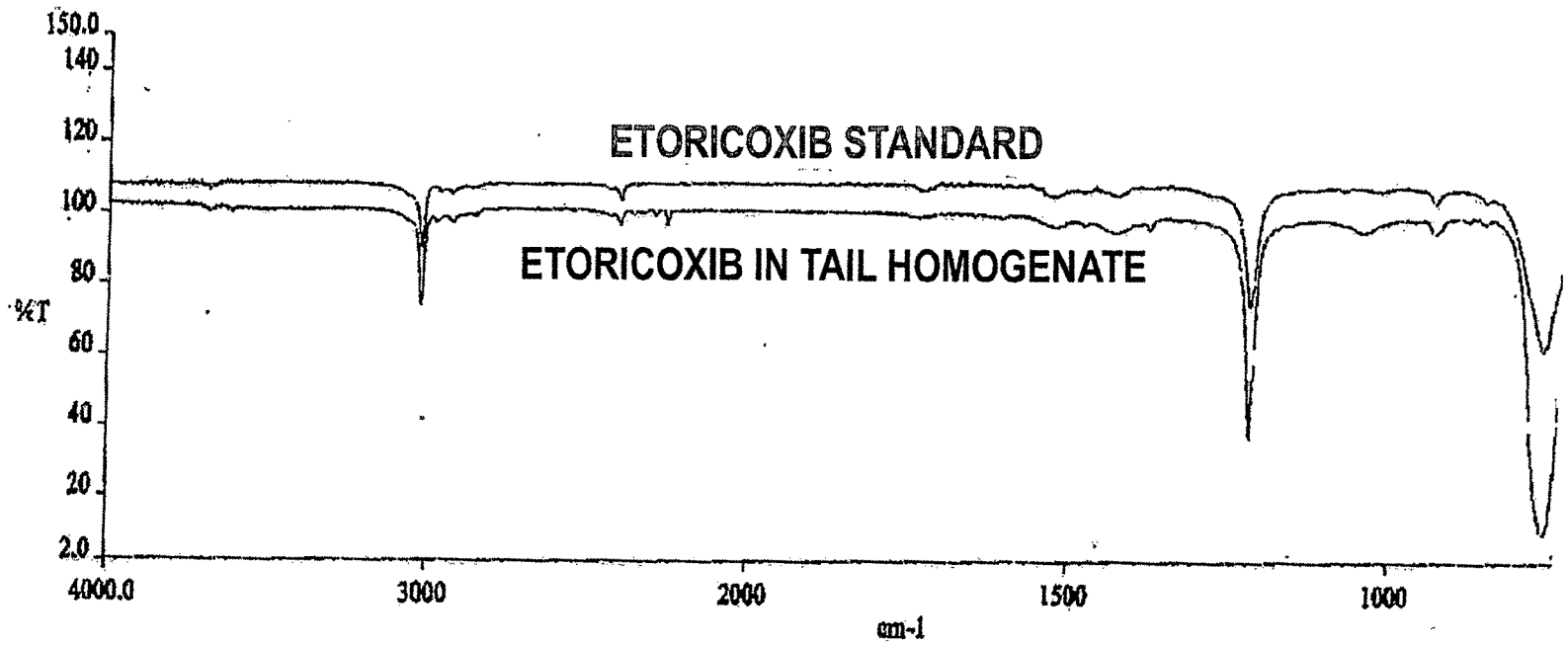

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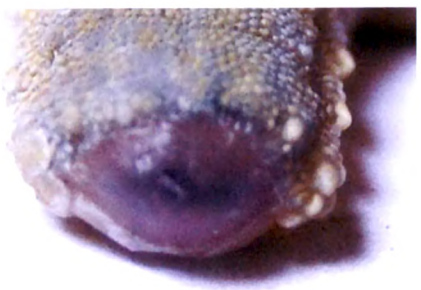
Figure 1.1: Drift spectra of Etoricoxib in lizard tail homogenate showing characteristic C= N, S=O, C-Cl stretching vibration



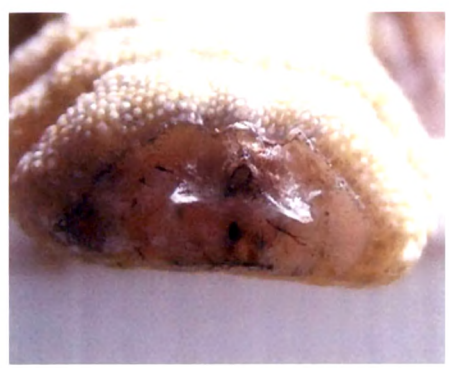
Control on day 4
wound epithelium stage

Control on day 8
blastema stage

Control on day 14
differentiation stage

Treated animal on day 5
wound not healed

Treated animal on day 9
wound not healed



Treated animal on day 20
wound not healed

Figure 1.2 Total suppression of epimorphosis in lizards treated with high dose of selective COX-2 inhibitor

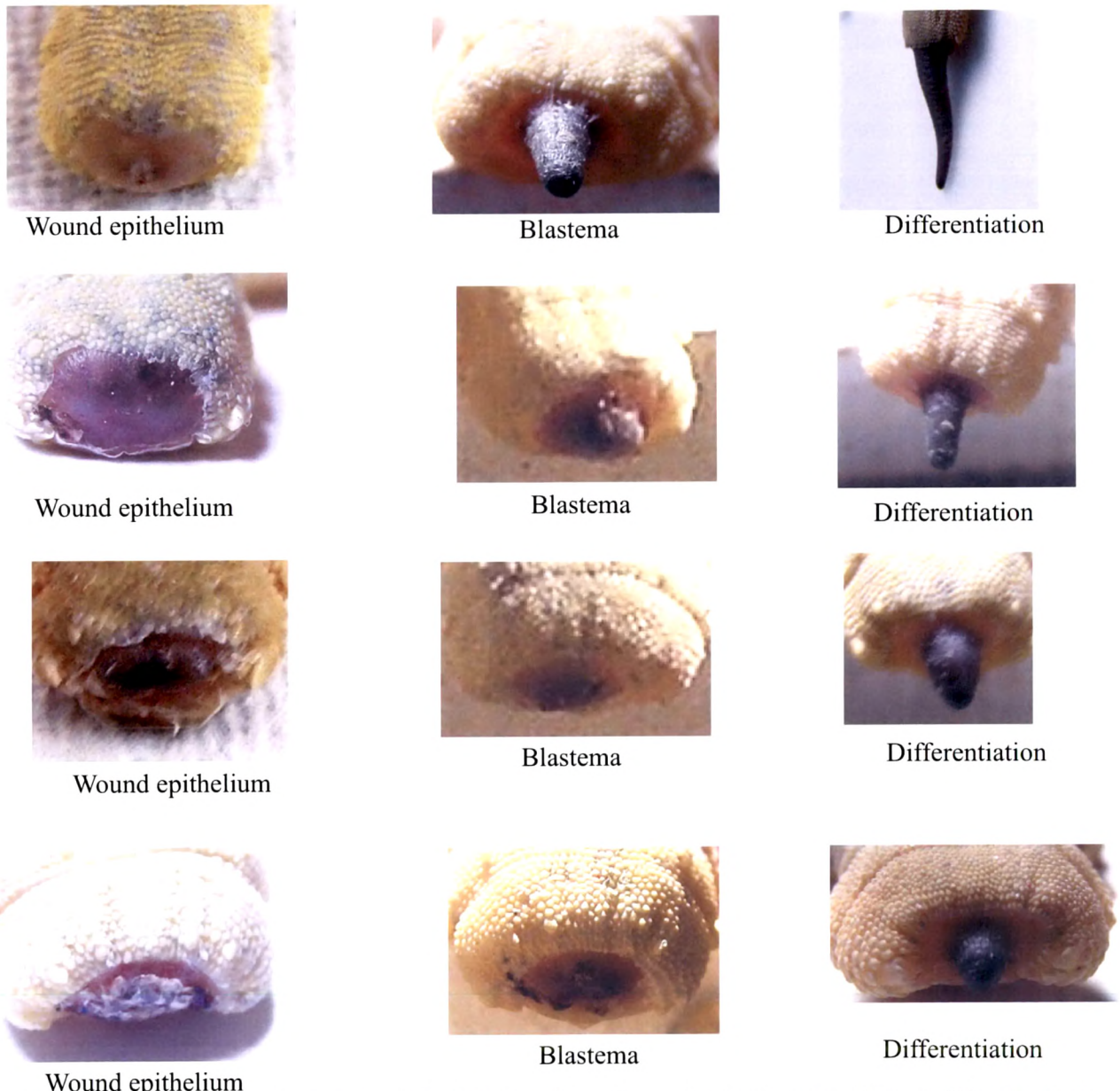


Figure 1.3 Dorsal view of the regenerating tail of the Northern House Gecko; images taken at different stages

Figure 1.4: Progression of tail regeneration in wall lizard, *Hemidactylus flaviviridis*, subjected to *in loco* injection of specific and non-specific COX inhibitors before amputation

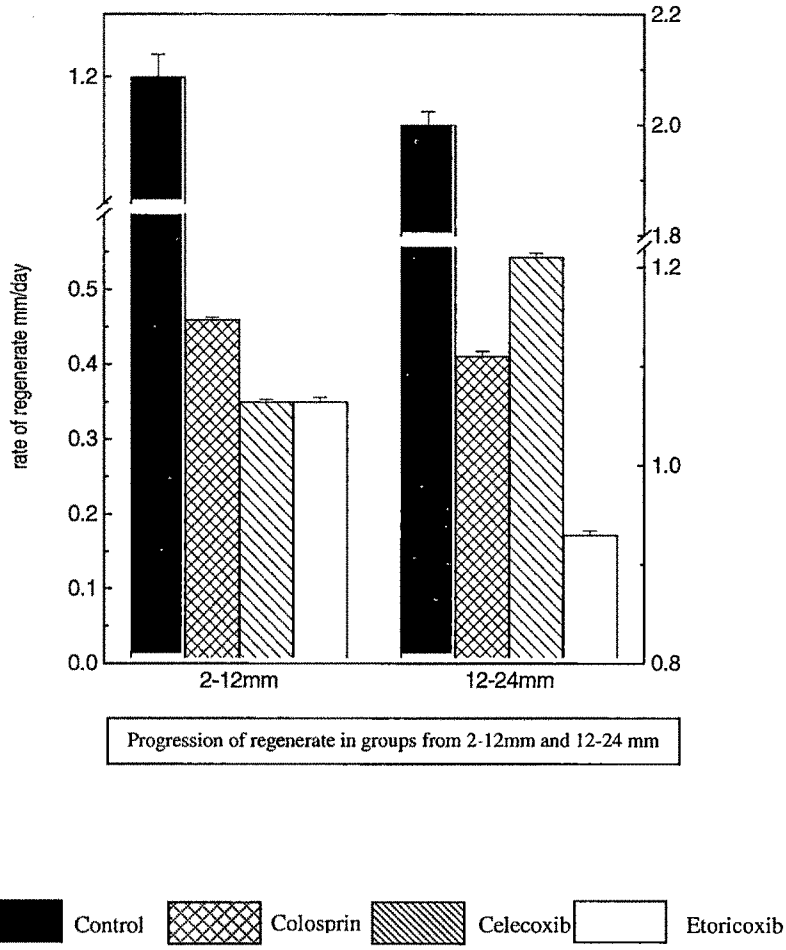


Figure 1.5 Progression of tail regeneration in wall lizard, *Hemidactylus flaviviridis*, subjected to *in loco* injection of specific and non-specific COX inhibitors after amputation

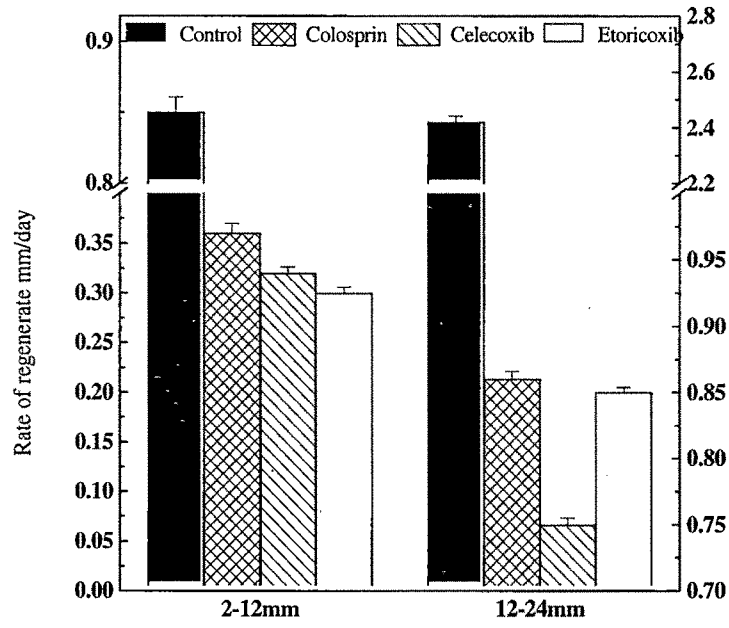


Figure 1.6 Progression of tail regeneration in wall lizard, *Hemidactylus flaviviridis*, subjected to *in loco* injection of specific and non-specific COX inhibitors at wound epithelium (WE) stage.

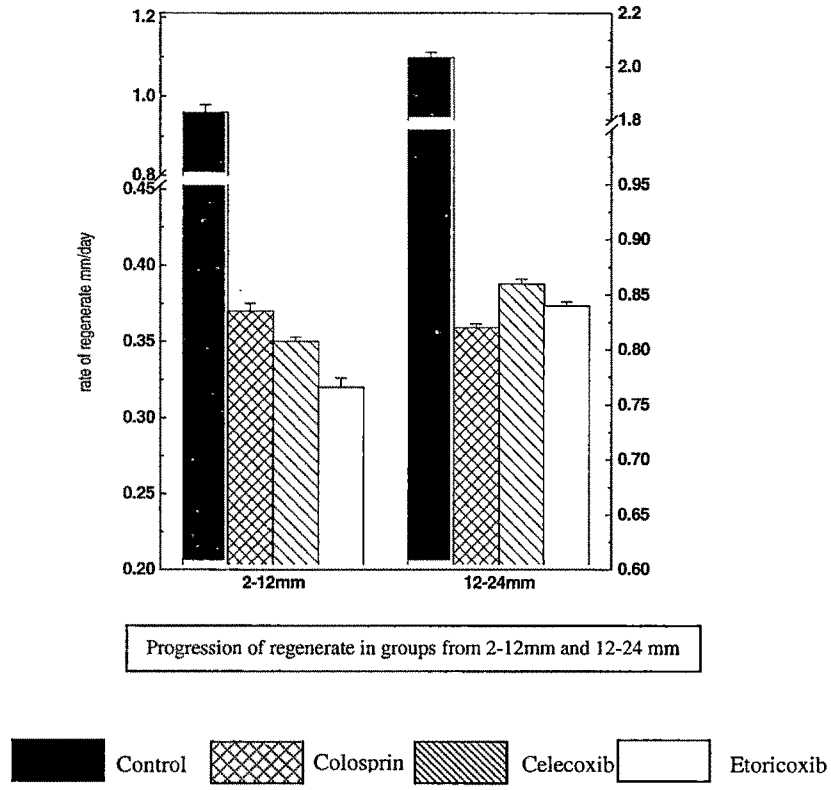


Figure 1.7 Progression of tail regeneration in wall lizard, *Hemidactylus flaviviridis*, subjected to *in loco* injection of specific and non-specific COX inhibitors at blastema (BL) stage.