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PGE-2 is neither a downstream agent of thyroxine nor is it involved in the molecular program of initiation of caudal regeneration: Timed temporal evaluation in *Hamidactylus flaviviridis*

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ABSTRACT

In the light of known involvement of PGE-2 in mammalian hepatic and muscle regeneration, the possible participation of PGE-2 in wound healing and initiation of caudal regeneration in the lizard, Hemidactylus flaviviridis has been evaluated by assaying the endogenous level in the immediate post-autotomy periods and timed temporal administration. Further, the importance of induced PGE-2 deficiency by the use of COX-2 inhibitor was also evaluated. Overall, the observations suggest no involvement of PGE-2 in the initiation of regeneration, as seen by the similar number of days taken to attain various arbitrary stages of regeneration in both control and experimental lizards, which is confirmed by the down regulation of endogenous PGE-2 level in the immediate post-autotomy periods. Further, the evaluation of PGE-2 as a possible downstream agent of thyroxine, in inducing ependymal growth, by way of administration in hypothyroid lizards also disproved the concept. The role of PGE-2 inferred in lizard tail regeneration is as a cytoprotectant, contributing to the formation of a robust regeneration blastema and augmented tail elongation with higher blastemal cell density, by reduced apoptosis. A schema of possible mechanism of regeneration initiation, in the light of available information, is proposed.

Keywords: - PGE-2, Regeneration, Lizards, Thyroid

INTRODUCTION

Can humans one day regrow extremities lost in accident or regenerate organs destroyed by illness? The realization of this fond hope lies in unraveling the molecular intricacies underlying appendage regeneration in our immediate vertebrate ancestor. Regeneration of tissues and lost

parts is of great adaptive value to animals and of great relevance to human reparative medicine. Animals with regenerative capacity serve as ideal models for understanding the mechanics of regeneration. Epimorphic regeneration of lizard tail is an ideal system to this end and, its evolutionary relationship to mammals provides further impetus. Endoperoxidase synthase, commonly called ass cyclooxygenase is the key enzyme required for the conversion of arachidonic acid to prostaglandins. The two known COX isoforms are, COX-1 produced constitutively and known to regulate most of the house keeping functions and COX-2, highly inducible at sites of inflammation [1]. The pro-inflammatory agents such as IL-1, TNFα and LPS as well as growth factors like TGF-β, EGF and FGF, have all been shown to induce COX-2 expression in synovial tissue from rheumatoid arthritis patients while, anti-inflammatory cytokines have been shown to decrease COX-2 levels [2]. Hepatocytes from regenerating liver have been shown to express COX-2 [3]. In addition, PGE-2 has been reported to be involved in various stages of myogenesis [4]. Studies have also reported role of COX-2 in corneal wound healing [5] and angiogenesis of neoplastic cells [6]. It is evident from literature that, COX-2 is very essential in controlled inflammation, muscle differentiation, wound healing, angiogenesis etc. Hence, we were tempted to evaluate role of PGE-2 on a temporal scale in the immediate post-autotomy periods during caudal regeneration in *Hemidactylus flaviviridis*.

The outgrowth of ependyma from the cut end of spinal cord towards the wound epithelium is considered essential for setting up a conductive environment for vascular and axonal growth, dedifferentiation and proliferation of tail stump tissues at the cut end. All these ultimately lead to the formation of a regeneration blastema, a crucial interphase between regressive and progressive phases. Previous studies from our laboratory have shown the permissive role of thyroxine in inducing ependymal out growth as, hypothyroidism results in delayed ependymal out growth and regenerative tail elongation [7, 8]. However, the exact role of thyroxine in inducing ependymal cell proliferation and out growth is not clear, but it is likely that thyroxine might employ some downstream signaling molecule to bring about its response. In this context, we have evaluated involvement of PGE-2 in relation to temperature and thyroid hormone during tail regeneration.

MATERIALS AND METHODS

Experimental animals

Adult *Hemidactylus flaviviridis* (10±2 g) of both the sexes with snout - vent length of 70-80 mm were used for the experiments. The cages housing the animals measured 18”X 15”X 10” with one side of transparent glass and ventilated on three sides. Six lizards were housed in each cage and were balanced for sex and size. The animals were maintained under a normal light-dark photoperiodic schedule in the summer (34-36°C) and monsoon (20-22°C) months and were fed on nymphs and provided with water *ad libitum.* Autotomy was performed by pinching off the tail three segments from the vent. The experimental protocol was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) India and approved by the animal ethical committee of Department of Zoology, The M.S. University of Baroda, Vadodara (Approval No.827/ac/04/CPCSEA).

Preparation of drugs and dosage

PGE-2:- Commercially available Dianoprostone tablet was dissolved in 0.6% Nacl and then diluted to obtain a final concentration of 2.5µg/100µl.

Methimazole: - Methimazole powder (Sigma Aldrich, Ltd, USA) was dissolved in 0.6% Nacl and then diluted to obtain a final concentration of 50µg/100µl.

Cox-2 Inhibitor: - Eterocoxib tablet (Sun Pharmaceuticals Ltd**.,** Baroda) was dissolved in 0.6% Nacl and then diluted to obtain a final concentration of 2.5µg/100µl.

Combined TNFα-Cox-2 inhibitor: - Commercially available Nimesulide tablet (Atmiya Pharmaceuticals, Baroda) was dissolved in 0.6% Nacl and diluted to obtain a final concentration of 50µg/100µl.

Experimental evaluations

Measurement of caudal PGE-2 level

Endogenous PGE-2 level was measured in unautotomised and autotomised tails as per the instruction of the manufacturer (R&D systems, USA) at 24, 36, 48 and 72 hr post autotomy.

Single administration of PGE-2

Exogenous PGE-2 was administered *in loco* at 12, 24, 36 or 48 hr post autotomy, animals were observed for attainment of various arbitrary stages (wound healing, pre blastama, blastema and initiation of growth), and tail length was measured by using graduated scale until 30th day post autotomy. Results were compared with control animals that were administered with 0.6% Nacl.

Multiple administrations of PGE-2

Exogenous PGE-2 was administered *in loco* or intraperitoneally *(i.p.*) at 12 & 24, 24 & 36 or 36 & 48 hr post autotomy and animals were observed for the attainment of various arbitrary stages (wound healing, pre blastama, blastema and initiation of growth) and tail length was measured by using graduated scale till 30th day post autotomy. Results were compared with control animals that were administered with 0.6% Nacl.

Multiple administrations of Cox-2 or combined Cox-2-TNFα inhibitor

Cox-2 and combined Cox-2-TNFα inhibitors were administered *in loco* at 12 & 24, 24 & 36 or 36 & 48 hr post autotomy and animals were observed for the attainment of various arbitrary stages (wound healing, pre blastama, blastema and initiation of growth) and tail length was measured by using graduated scale till 5th day after initiation of growth. Results were compared with control animals that were administered with 0.6% Nacl.

Continuous administration of PGE-2 or Cox-2 inhibitor

Two sets of lizards were administered PGE-2 or COX-2 inhibitor respectively every 12 hr post autotomy till 15 days and observations were made.

Hypothyroidism and administration of PGE-2

Methimazole was administered 7 days prior to and at intervals of 48 hr post autotomy until 15th day. A set of animals was administered with PGE-2 every 12 hr post autotomy along with methimazole treatment and were observed for attainment of various arbitrary stages (wound healing, pre blastama, blastema and initiation of growth) and tail length was measured by using graduated scale till 30th day post autotomy. Results were compared with control animals that were administered with 0.6% Nacl. These experiments were conducted in the summer months (32-34°C). Same set of experimental schedule was performed in the winter months at 22-24°C and were observed for attainment of various arbitrary stages (wound healing, pre blastama,

blastema and initiation of growth) and tail length was measured by using graduated scale till 15th day post autotomy.

Temperature variation and PGE-2 administration

Animals were divided into 3 groups of 8 animals each. Group I was maintained under normal temperature condition (32-34°C) while groups II and III were maintained under cold temperature (20-22°C). Group III was administered with PGE-2 *in loco* at an interval of 12 hr post autotomy until 34th day post autotomy and attainment of various arbitrary stages (wound healing, pre blastama, blastema and initiation of growth) were studied

Statistical analysis

Data are expressed as Mean±S.E.M for n=8 animals and were analyzed by Student's *t* test using Graph Pad Prism-3 software.

RESULTS

Endogenous PGE-2 level

Measurement of endogenous PGE-2 level in the post autotomy periods recorded lower levels at 24, 36 and 48 hr post autotomy compared to unautotomised samples and the level returned to near normal level by 72 hr (Figure. 1).

Single or multiple administration of PGE-2

As shown in Figure. 2, there was no difference in the number of days taken to attain various arbitrary stages of regeneration either after single or multiple administrations of PGE-2 compared to control animals. However, significant augmented growth was observed in animals treated with PGE-2 at 36 & 48 hr post autotomy.

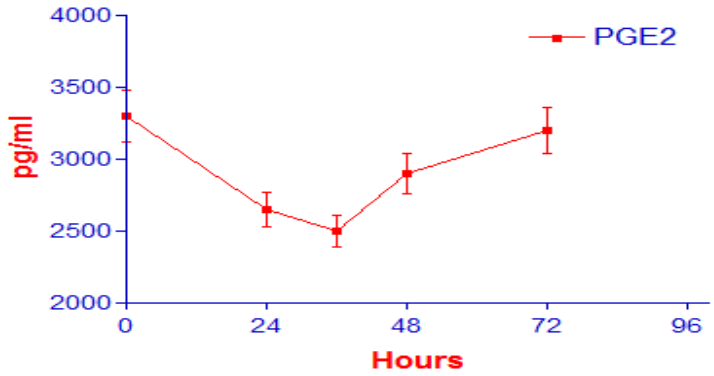

Figure.1 Changes in endogenous PGE-2 levels in the immediate post autotomy periods.

Multiple administrations of Cox-2 and Cox-2-TNFα inhibitor

Except for a slight delay in wound healing, no significant differences in the number of days taken to attain various stages of regeneration were observed. The length of tail regenerated showed widening difference from 16th day of initiation of growth, with the regrowth of experimental lizards depicting progressive retardation (Figure. 3).

Continuous administration of PGE-2 or Cox-2 inhibitor

Regeneration was inhibited by either of the two treatments with lizards showing only a delayed wound healing with no growth (Figure. 4)

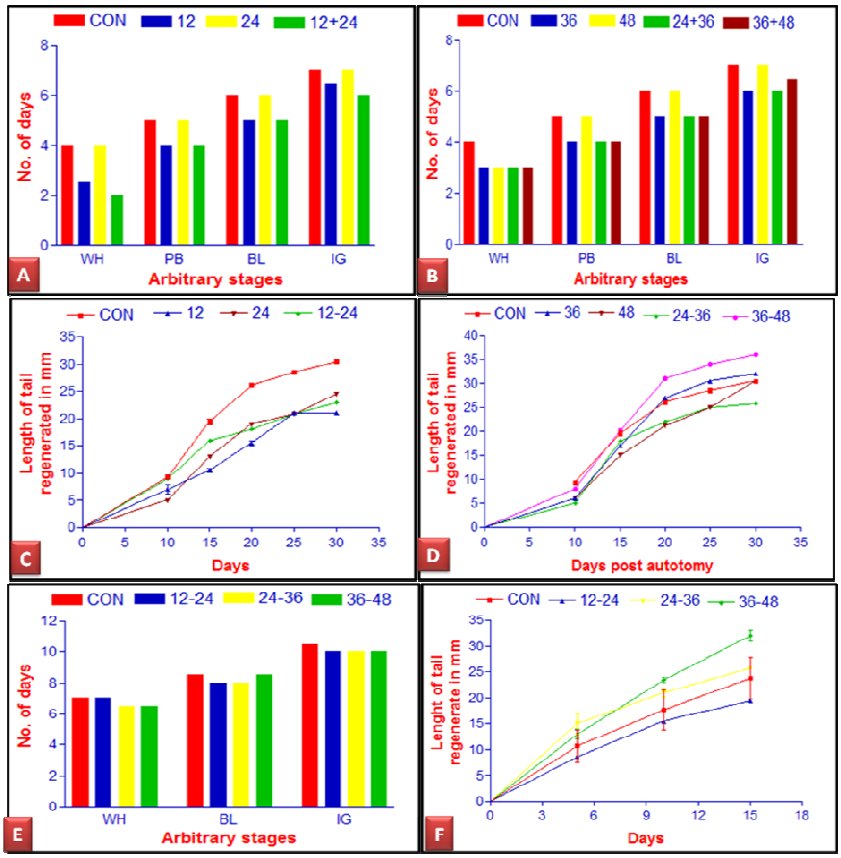

Figure.2A & B. number of days taken to attain various arbitrary stages of regeneration on PGE-2 administration *in loco* at 12, 24 or 12 & 24 hr and 36, 48 or 24 & 36 or 36 & 48 hr post autotomy. C & D. length of tail regenerated in the above groups of lizards at the end of 30 days post initiation of growth. E & F. number of days taken to attain various arbitrary stages in lizards administered PGE-2 intraperitoneally at 12 & 24 or 24 & 36 or 36 & 48 hr post autotomy and the length of tail regenerated at the end of 15 days post initiation of growth in the above groups of lizards.

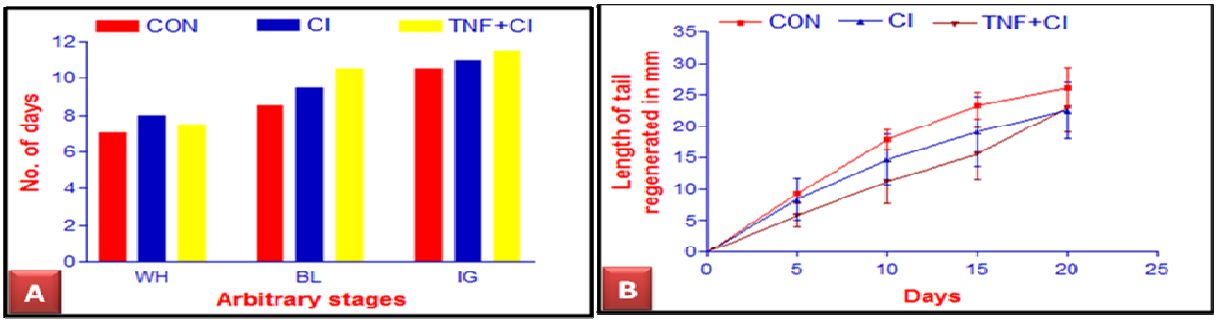

Figure.3A & B. number of days taken to attain various arbitrary stages in lizards treated with COX-2 inhibitor or COX-2 and TNF*α* inhibitor and the length of tail regenerated at the end of 20 days post initiation of growth in the above groups of lizards.

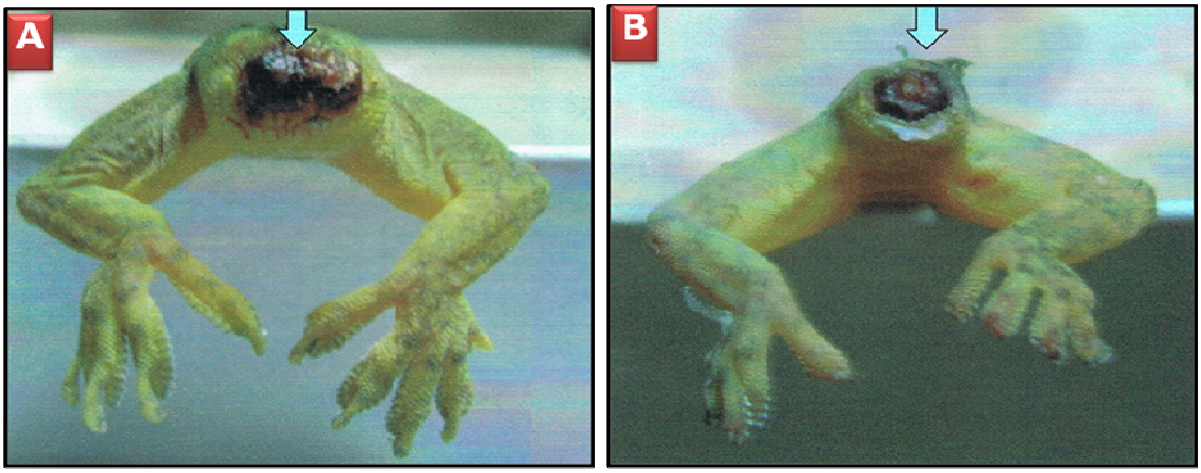

Figure.4 Photomicrographs of regenerating tail of lizards administered with (A) COX-2 inhibitor or (B) PGE-2 continuously for 15 days post autotomy at 12 hr interval.

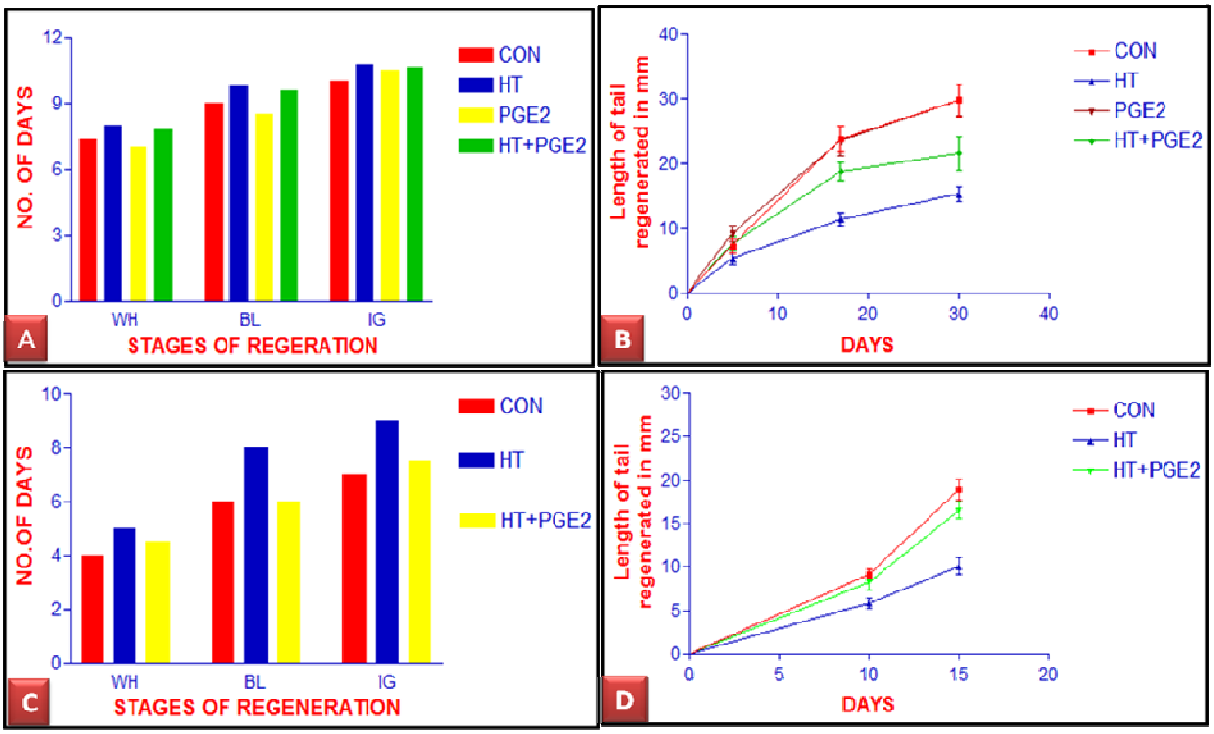

Figure.5A & B. number of days taken to attain various arbitrary stages in control (CON), hypothyroid (HT), PGE-2 administered (PGE2) and PGE-2 administered hypothyroid lizards (HT+PGE2) and, the length of tail regenerated after initiation of growth in above-mentioned groups of lizards in summer months. C & D. number of days taken to attain various arbitrary stages in control (CON), hypothyroid (HT) and PGE-2 administered hypothyroid lizards (HT+PGE2) and, the length of tail regenerated after initiation of growth in above-mentioned groups of lizards in monsoon months.

Hypothyroidism (HT) and PGE-2 administration

Methimazole administered animals showed a delayed attainment of various arbitrary stages compared to control animals while, PGE-2 administration to methimazole treated animals improved hypothyroidism induced delay in the initiation of growth during both summer and monsoon months. Observations on growth rate revealed a compromised growth in HT lizards compared to controls and PGE-2 treated HT animals during both summer and monsoon months (Figure.5).

Temperature variation and PGE-2 administration

Whereas in the summer temperature, the autotomized tail healed in seven days, formed a well-formed blastema in nine days and initiation of growth commenced by eleven days, lizards in the winter temperature healed their wound by nineteen days and formed an incipient blastema with no growth by twenty-eight days. PGE-2 treated lizards in the winter temperature, though healed their wound by nineteen days, a scarred incipient blastema with no further growth was formed by thirty-four days (Figure. 6).

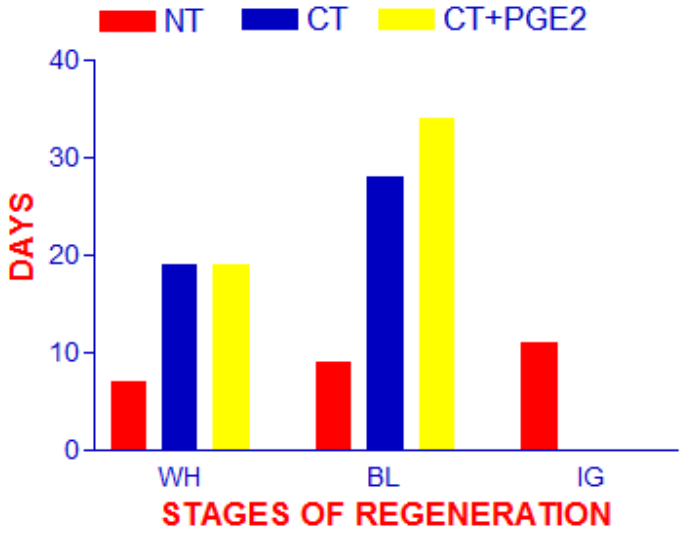

Figure.6 number of days taken to attain various arbitrary stages in control lizards maintained under normal temperature (NT), under cold temperature (CT) and PGE-2 administered lizards maintained under cold temperature (CT+PGE2).

DISCUSSION

Prostaglandins are known to be associated with a number of functions like an inflammatory cytokine, involvement in oxidative stress and apoptosis, as a cytoprotectant, control of cell cycle etc. [9, 10, 11, 12]. In the model of mammalian liver regeneration, administration of exogenous PGE-2 has been shown to hasten the process of liver regeneration [13]. In the absence of any report on the role of PGE-2 in initiation of epimorphic regeneration, the present study was taken up to ascertain the possible involvement of PGE-2 in tail regeneration in the Gekkonid lizard, *Hemidactylus flaviviridis.*

To this end, endogenous caudal PGE-2 level was measured at different periods post-autotomy and compared with the basal level in the unautotomised state. Autotomy results in significant decline in the level of PGE-2. In other words, the level of PGE-2 is significantly lower in the post-autotomised tail stump until 72 hr. Taking cue from this noticeable change in endogenous PGE2 levels, exogenous PGE2 was administered at different time intervals to ascertain its temporal role under conditions of PGE-2 excess. The results clearly indicate that PGE-2 has no influence on wound healing to initiation of growth. From the result of *in loco* administration of PGE-2 on a chronological time scale, it is evident that 36 -48 hour is the most optimal phase of PGE-2 mediation as marked by a slightly hastened wound healing and augmented regenerative growth at the end of 30 days. Though the exogenous administration of PGE-2 has not shown any favorable influence in relation to wound closure and blastema formation at all time periods of study, an optimal effect of PGE-2 seems to be between 36 - 48 hour as marked by a better initiation of growth and relatively greater total tail replacement at the end of 30 days. It is also interesting to note that, PGE-2 administration either post wound healing or post blastema formation has no significant effect on the course of regeneration (Data not shown). As a further complementation to this study, the regenerative response was studied under the influence of specific Cox-2 inhibitor (eterocoxib) or TNFα & Cox-2 blocker. Administration of Cox-2 inhibitor or TNFα & Cox-2 blocker did not affect the growth kinetics of the regenerating tail. Based on these results, it can be assumed that, PGE-2 is not involved in the early preparative events leading to initiation of tail regeneration in *Hamidactylus flaviviridis.* The regenerative capacity of skeletal muscle was found delayed when COX-2 inhibitor was applied in the initial stages of injury suggesting the importance of COX-2 induced prostaglandins in the initial phases of tissue repair [14, 15]. Hatazawa et al. [16] have reported a delay in the healing of gastric ulcers on administration of Cox-2 blockers. They have highlighted the up regulation of vascular endothelial growth factor (VEGF), a key inducer of angiogenesis, under the direct influence of Cox-2 induced prostaglandins. It is worthwhile to note that, VEGF expression has been seen as a key to regeneration in the regenerating spinal cord of *Ambystoma mexicanum* [17]. The basis for the observed discrepancy in the results obtained from the present study with those discussed above, is due to the distinct nature of epimorhic regeneration, which is devoid of inflammatory response to the magnitude seen in mammalian models of tissue repair and regeneration. Apparently, PGE-2 participation in the regressive phase of epimorphic regeneration is unimportant while, it seems to have some role in the post blastemic progressive phase of regeneration. In keeping with the importance in the progressive phase, exogenously induced PGE-2 excess between 36-48 hrs shows a significantly augmented growth rate. The recovery in endogenous PGE-2 level noted during these periods after precipitous fall until 24 hr tends to substantiate this influence. Growth of blastema is dependent on the active proliferation of the accumulated mesenchymal cells. The quality of blastema is a function of the quantum of viable cells. This requires a check in apoptosis and maintenance of the cells under viable condition. PGE2 has been shown to inhibit apoptosis [18]. In addition, in one of our previous reports we have demonstrated down regulation of caspase 3 in the initial periods post caudal autotomy [19]. Thus, timed PGE-2 administration from 36 hr presumably enhances the quality of regenerative blastema by increasing the number of viable cells and decreasing the degree of apoptosis. This essentially points towards a favorable role of Cox-2 induced PGE-2 in the progressive phase of regeneration.

The apparent reason behind the suggestive down-regulation of PGE-2 in the initial hours post autotomy entails consideration of the basic difference in the pattern of wound healing between epimorphic regeneration and generalized tissue repair following wounding or inflammation in mammals. The wound healing that occurs during epimorphic regeneration is alike fetal wound healing in mammals, which is devoid of fibrosis and scar formation, distinctly different from adult wound repair, which takes place with an inflammatory response, re-epithelialization and the formation of a permanent scar. Several reports implicated PGE-2 as a major player in the Cox-2 pathway of inflammatory wound healing [20, 21, 22, 23]. Even trace quantity of exogenous PGE-2 is shown to result in scarring of wound in adult dermal injury while, a similar injury in the fetal stage was refractory to exogenous administration of PGE-2 due to absence of inflammatory events in fetal wound healing [24]. With the modalities of epimorphic regeneration being very similar to fetal wound repair, it can be construed that, there is an essential down regulation of PGE-2, principally to prevent scarring. Apparently, participation of PGE-2 and other inflammatory cytokines in the initial phase of regeneration could lead to scarring, which is a deterrent to regeneration (Ramachandran, 2010; communicated). Further, continuous administration of PGE-2 twice a day for 15 days showed scar formation with no signs of blastema formation while, continuous administration of Cox-2 inhibitor once every day for 20 days also showed incomplete wound closure with an exposed cut. Hence, continued excess of PGE-2 or even total absence is detrimental for effective wound closure and initiation of regeneration.

Involvement of spinal cord in providing impetus for initiation of lizard tail regeneration is clearly established. The immediate response to caudal autotomy has been shown as an outgrowth of the ependymal lining of the spinal cord in the form of stalked vesicle, as the cut surface is being covered by wound epithelium [25]. The outgrowth of ependyma towards the wound epithelium is expected to set up an environment conducive for vascular and axonal growth, dedifferentiation and proliferation of tail stump tissues at the cut end, migration of nomadic pluripotent or stem cells from elsewhere and, prevention of the formation of collagenous dermal substance below the wound epithelium. All these ultimately lead to the formation of a regeneration blastema, a crucial interphase between regressive and progressive phases. Studies from this laboratory on *Hemidactylus flaviviridis* and *Mabuya carinata* have shown the permissive role of thyroxine in inducing ependymal out growth as, hypothyroidism resulted in delayed ependymal out growth and regenerative tail elongation [7, 8, 26]. How exactly thyroxine modulates ependymal cell proliferation and out growth is not clearly established. It is likely that, thyroxine might employ some downstream signaling molecule to bring about its response. It is in this context, the present study on the role of PGE-2 as a possible downstream agent of thyroxine has been investigated.

In recent times, PGE-2 has been implicated in both hepatic and muscle regeneration in general [3, 4, 27, 28]. In all these cases, PGE-2 has been considered as an early mediator of regeneration. During liver regeneration its role in increased synthesis of hepatic growth factor (HGF) and transition of primed hepatic cells to S phase of DNA cycle is reported [3]. However, during muscle regeneration, it is found important for recruitment of macrophages, promotion of angiogenesis to, and at the site of muscle injury, promotion of myoblast formation and proliferation and even mediation of recruitment and proliferation of satellite cells [29]. Since, similar events are also expected to occur during lizard tail regeneration, the present study has

attempted to evaluate the role of PGE-2 in euthyroidic and hypothyroidic lizards. Previous seasonal studies on lizard tail regeneration in euthyroid and hypothyroid lizards had shown retardation in initiation of regeneration during monsoon months but not during summer months. This was related to decreasing TH level and sensitivity to TH during the monsoon months [8]. It was inferred that, the prevailing higher temperature in the summer months (above 30□C) induces greater sensitivity to TH and hence even a basal TH level (hypothyroidism) is able to trigger formation of blastema without delay and initiate regeneration. The present study carried out in the summer months confirms the above observation and interpretation as, hypothyroidic lizards formed a blastema in same number of days as did the euthyroid lizards. In addition, exogenous PGE-2 administration did not have any effect on the time course of WH, BL formation and IG either in hypothyroidic or euthyroidic lizards. Apparently, PGE-2 has no additive effect in triggering initiation of regeneration under the prevailing TH levels characteristic of euthyroidic or hypothyroidic lizards. Had PGE-2 been involved in triggering lizard tail regeneration independently, or even as a downstream agent of thyroxine, there should have been an early formation of blastema and initiation of growth. Since, the present observations do not subscribe to these paradigms, it is safe to conclude that PGE-2 induction or its mediation is not involved in the scheme of molecular events required to trigger the initiation of lizard tail regeneration. The observed down regulation of PGE-2 level in the immediate post autotomy periods (12 to 72 hr) provides compelling evidence and gives credence to the concept of no PGE-2 involvement in the regressive phase of lizard tail regeneration. Clearly, the factors in operation in lizard tail regeneration are quite distinct from those involved in mammalian organ regeneration.

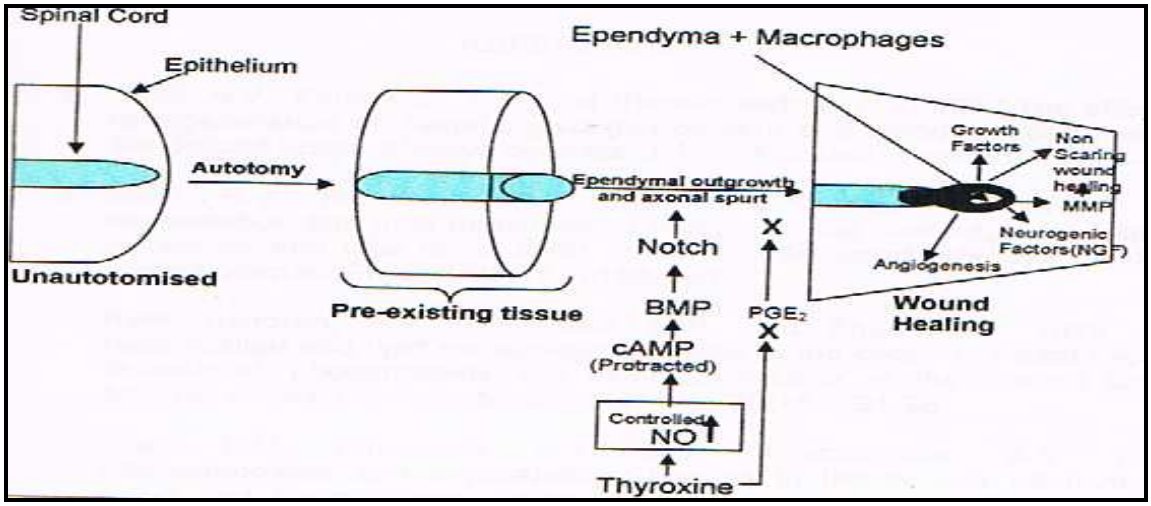

Figure 7: Schematic representation of hypothetical Post-autotomy events.

Recent molecular analysis of amphibian tail regeneration has brought out the activation of BMP and Notch signaling. It is also inferred that, Notch signaling is required for ependymal growth/spinal cord regeneration with BMP as an upstream activator and, that, a later BMP activation is required for progressive phase of regeneration involving tissue differentiation with Msx-1 as its downstream agent [30, 31]. Further, early NO induction in the injured area of spinal cord as well as of FGF-1 and FGF-2 for ependymal out growth along with MMP activation and

matrix degradation have all been reported during amphibian tail/spinal cord regeneration [32, 33]. Since the genetic programs controlling the process of appendage morphogenesis during development are highly conserved, it is safe to assume that Notch and BMP signaling are involved in lizard tail morphogenesis as well, and that, they may be reactivated during regeneration. Controlled NO production might be involved in the activation of BMP-Notch signaling pathway to initiate ependymal outgrowth by way of cAMP and cGMP mediated pathways. There are enough evidences available to support involvement of NO and cAMP in spinal cord and neuronal repair and regeneration in amphibians [32, 34, 35]. These events might contribute to angiogenesis, induction of MMPs and growth factors, prepare an environment conducive for dedifferentiation of locally resident cells, as well as, migration of nomadic cells, and activate cell cycle mechanisms for their proliferation. Interestingly, our own studies have reveled up regulation of NO and cAMP between 24 and 72 hr after caudal autotomy along with up regulation of cGMP at 24 hr post-autotomy [19]. Based on these observations, a tentative hypothetical scheme for initiation of ependymal outgrowth and triggering regeneration starting with thyroxine is presented (Fig.7). According to this hypothetical schema, it is presumed that, thyroxine, with its high affinity receptors during summer months, may induce controlled NO expression leading to up-regulation of cAMP principally and cGMP very transiently in the injured spinal cord, which result in BMP expression and, increased expression of BMP employs Notch as its downstream agent to induce ependymal outgrowth. The outpouring ependymal cells and the axonal sprouts together with the incoming macrophages would serve as a source of various cytokines, growth factors like NGF and other neurogenic factors [17], FGFs 1 & 2 [25], TGF-β (Ramachandran et al., 2010; communicated) etc, angiogenic factors and, MMP 2 & 9 [36], all of which together could contribute to non-scarring wound healing, growth of capillaries, matrix remodeling (replacing collagen, laminin etc with hyaluronan based matrix), dedifferentiation of cells and expression of FGF-10 in the so formed pluripotent mesenchymal cells which in turn can induce FGF-8 expression in the wound epithelium. This results in the establishment of a regeneration blastema wherein the FGF-10 – FGF-8 interactions (between mesenchymalcells and blastimic epithelium akin to AEC of tail bud) would maintain tail elongation. Apparently, PGE-2 is not involved in the molecular schema of events in the immediate post autotomy periods and, neither is it a probable downstream agent of thyroxine during lizard tail regeneration. However, immediate pre-wound healing period could be favorable for regenerative growth by probably decreasing apoptotic cell loss and acting as a cytoprotectant and also favoring cell proliferation as noted by the hastened tail elongation in a timed PGE-2 administration study (fig.8).

In the experiments on the role of PGE-2 in removing cold temperature mediated block in regeneration, has shown that, PGE-2 is unable to remove this block. Despite administration of exogenous PGE-2, there is no apparent sign of initial growth. The influence of external factors like temperature, humidity and electric current on appendage regeneration has been well documented [37] and the inhibitory effect of cold temperature on regenerative ability on *Hemidactylus flaviviridis* was reported [38, 39]. It can therefore be presumed that cold temperature induced inhibition of regeneration seems to be unrelated with PGE-2 signaling as exogenous PGE-2 supplementation is incapable of reversing the hypothermia induced inhibition in regeneration.

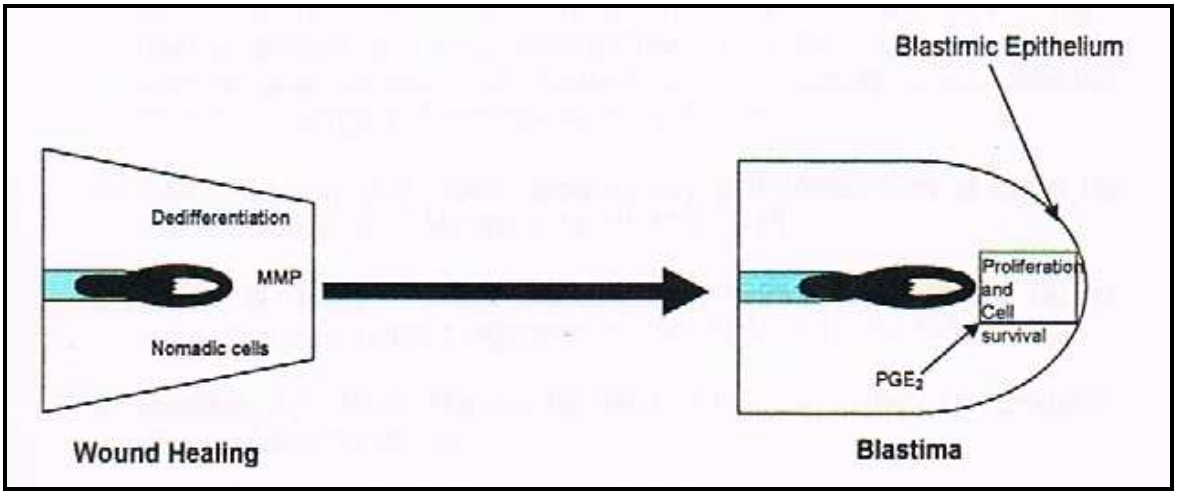

Figure 8: Possible role of PGE_2 in promoting increased blastimal cell density.

CONCLUSION

PGE-2 is not involved in the overall scheme of molecular mechanism contributing to initiation of lizard tail regeneration and is at best a favorable agent for post-wound healing regenerative growth. Neither is PGE-2 a possible downstream agent of thyroxine in inducing ependymal outgrowth.

REFERENCES

[1] M. E. Turini, R. N. Dubois, *Annu. Rev. Med*., **2002**, 35,57
[2] L.J. Crofford, R.L. Wilder, A.P. Ristimäki, H. Sano, E.F. Remmers, H.R. Epps, T. Hla, *J. Clin. Invest.*, **1994,**93,1095.
[3] M. Casado, N. A. Callejas, J. Rodrigo, X. Zhao, S.K. Dey, L. Boscá, P. Martín-sanz. *The FASEB Journal*., **2001,** 15, 2016.
[4] B.A. Bondesen, S.T. Mills, K.M. Kegley, G.K. Pavlath, *Am. J. Physiol. Cell. Physiol*., **2004,** 287, C475.
[5] C. Amico, M. Yakimov, M.V. Catania, R. Giuffrida, M. Pistone, V. Enea., *Tissue. Cell*., **2004**,36,1.
[6] S. Tsuji, M. Tsujii, J. Gastroenterol, 39, 1224.
[7] A.V. Ramachandran, M.S. Swamy, R.V. Shah, Amphibia Reptilia., **1984**, 5,135.
[8] A.V. Ramachandran, A. Kurup, *J Endocrine Reproduction*., **2006**, 10,134.
[9] A.A. Weber, T.P. Zucker, A. Hasse, D. Bönisch, M. Wittpoth, K. Schrör, *Basic. Res. Cardiol*., **1998**, 93, s054.
[10] Z. Xiao, P. Biancani, J.Behar, *Am. J. Physiol. Gastrointest. Liver. Physiol*., **2004**, 286, 82.
[11] G. L. Larsen, J. Loader, C. Fratelli, J. B. Kang, A. Dakhama, G. N. Colasurdo, *Am. J. Physiol. Lung. Cell. Mol. Physiol.*, **2007**, 293, L239.
[12] E. Candelario-Jalil, S. Taheri, Y. Yang, R. Sood, M. Grossetete, E.Y. Estrada, B.L. Fiebich, G. A. Rosenberg, *J. Pharmacol. Exp. Ther*., **2007**, 323, 488.
[13] M. Jodi, S. Schoen, W. W. Lautt, *Nitric Oxide*., **2005**, 13, 111.

[14] J.G. Tidball, *Med. Sci. Sports. Exerc.*,**1995,** 27, 1022.
[15] J. Bondeson, S.D. Wainwright, S. Lauder, N. Amos, C.E. Hughes, *Arthritis. Res. Ther*., **2006,** 8, R187.
[16] R. Hatazawa, A.Tanaka[1], M. Tanigami, K. Amagase, S. Kato, Y.Ashida, K. Takeuchi, *Am. J. Physiol. Gastrointest. Liver. Physiol.*, **2007**, Doi:10.1152/ajpgi.00131.2007.
[17] E.A. Chernoff, D.L. Stocum, H.L. Nye, J.A. Cameron, *Dev. Dyn*., **2003**,226,295.
[18] V. Leone, A. Palma, P. Ricchi, F. Acquaviva, M. Giannouli, A. M. D. Prisco, F. Iuliano, A. M. Acquaviva. *Am. J. Physiol. Gastrointest. Liver. Physiol.*, **2007**, 293, G673.
[19] P.A.Deshmukh,I.Y.Bhatt,R.R.Jape,P.N.Jivani,H.R.Kavale,V.V.Nambiar,S.S. Prakashkar, A.V. Ramachandran, *J. Endocrine, Reproduction*., **2008**, 12, 31.
[20] M.J. Morykwaz, S.L. Perry, L.C. Argenta. *Int J Tissue React*., **1993,** 15, 151.
[21] N. S. Adzick, H. P. Lorenz, *Ann. Surg*., **1994**, 220, 10.
[22] H.S. Li, P.A. Hebda, L.A. Kelly, G.D. Ehrlich, D.C. Whitcomb, J.E. Dohar, *Arch. Otolaryngol. Head. Neck. Surg*., **2000**, 126, 1337.
[23] T.A. Wilgus, Y. Vodovotz, E. Vittadini, E.A. Clubbs, T.M. Oberyszyn, *Wound Repair Regen*., **2003**, 11, 25.
[24] V.C. Sandulache, A. Parekh, H.S. Li-Korotky, J.E. Dohar, P.A. Hebda, *Wound Repair Regen*. **2006**, 14, 633.
[25] L. Alibardi, F. J. Lovicu, *Acta Histochem.*,**2010,** 459.
[26] A.V. Ramachandran, S. Abraham, *Acta. Embryol. Morphol. Exp*., **1990**, 11, 45.
[27] K. Matsumoto, H. Okazaki, T. Nakamura, *J. Biochem*., **1995**, 117, 458.
[28] D.A. Rudnick, D.H. Perlmutter, L.J. Muglia, *Early Edition of the Proceedings of the National Academy of Sciences*., **2001**, 2, 2179.
[29] S. B. P. Chargé, M. A. Rudnicki, *Physiol. Rev.*,**2004**, 84, 209.
[30] C.W. Beck, J.M. Slack, *Int. J. Dev. Biol*., **2002**, 46, 255.
[31] C. L. Stoick-Cooper, R. T. Moon, G. Weidinger, *Genes & Dev*., **2007,** 21, 1292.
[32] L. Cristino, V. Guglielmotti, A. Cotugno, C. Musio, S. Santillo, *Brain. Res.*,**2008**, 1225, 17.
[33] F. Zhang, J. D. W. Clarke, P. Ferretti, *Developmental Biology*., **2000**, 225, 381.
[34] D. Cai, J. Qiu, Z. Cao, M. Mcatee, B.S. Bregman, M. T. Filbin. *The. J. Neuroscience.*, **2001**, 21, 4731.
[35] T. Spencer, M. T. Filbin. *J. Anat.*, **2004**, 204, 49.
[36] V.V. Nambiar, I.Y. Bhatt, P.A. Deshmukh, R.R. Jape, P.N. Jivani, H.R. Kavale, S.S. Prakashkar and A.V. Ramachandran, *J. Endocrinol. Reprod.*, **2008**, 2, 67.
[37] R. B. Borgens, J. W.Vanable, L. F. Jaffe, *J. Exp. Zool*., **1979**, 207, 217.
[38] P. I. Ndukuba, A. V. Ramachandran, *Gen. Comp. Endocrinol.*,**1989**, 192.
[39] Kurup, A. A Ph.D thesis submitted to The M. S. University of Baroda, India. **1996**