Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2012 Vol. 2 (2) April-June, pp.164 -172 /Yadav et al.
Research Article

INFLUENCE OF EXTRANEOUS FGF-2 AND ITS ANTAGONIST ANTIFGF-2, ON THE PROGRESS OF TAIL REGENERATION IN *HEMIDACTYLUS FLAVIVIRIDIS*

M. Yadav[1], P. Anusree[1], I. Desai[2] and [*]B. Suresh[1]
[1]*Division of Developmental Physiology, Department of Zoology, The Maharaja Sayajirao University of Baroda, India*
[2]*Department of Bioscience, NVPAS, Anand 388 120, India*
**Author for Correspondence*

ABSTRACT
We studied the role of FGF-2 in reptilian regeneration. FGF-2 was found to enhance the process of wound healing and the formation of wound epithelium, followed by acceleration in blastema formation. In addition, it also increased the rate of growth of regenerate during early stages of tail regeneration. However, animals exogenously treated with antiFGF-2 delayed the healing of the wound. There was also a marked inhibition of the growth of regenerate in the lizards treated with antiFGF-2. Nevertheless, there was no perceivable influence of FGF-2 or antiFGF-2 on regeneration after the animals started redifferentiation of the blastemal cells to form the lost appendage. Thus, it could be construed that FGF-2 is one of the quintessential growth factors for the successful initiation of many downstream pathways that then work independently to complete the appendage regeneration in lizards.

Key Words: *Epimorphic Regeneration, Fgf-2, Wound Healing, Blastema, Hemidactylus Flaviviridis*

INTRODUCTION

The process of epimorphic regeneration has been widely studied in amphibians. During amphibian limb regeneration, after the formation of wound epithelium (WE) the dedifferentiated cells undergo a series of hasty cell divisions to accumulate a mass of pleuripotent blastemal cells, which eventually restore the lost limb (Song *et al*., 2010). The stages during tail regeneration in lizards are comparable to those seen in urodele amphibians viz. wound epithelium stage, blastemic stage and differentiation stage (Iten and Bryant, 1976; Bellairs and Bryant,1985; McLean and Vickaryous, 2011). In order to understand the similarity, if any, that exists in the process of regeneration between amphibian and reptilian regeneration, it was thought worth trying the role of FGF2 that is imperative for amphibian regeneration (Gardiner and Bryant, 1996; Mullen *et al*., 1996) in initiating and possibly maintaining the process of epimorphic regeneration in reptiles as well (Alibardi, 2009). Fibroblast growth factors (FGFs) are a family of about 23 small peptide growth factors that are potent regulators of a variety of cellular processes including proliferation, differentiation, migration, morphogenesis, tissue maintenance, wound healing and repair (Cuevas *et al*., 1988, Burgess and Maciag 1989; Rifkin and Moscatelli, 1989; Clarke *et al*., 1993). FGF2 (Basic fibroblast growth factor), a member of this family has pleiotropic effects in different cell and organ systems. It is a potent angiogenic molecule in vivo and in vitro stimulates smooth muscle cell growth, wound healing, and tissue repair (Basilico and Moscatelli, 1992; Schwartz and Liaw, 1993;, Yokoyama, 2008). FGFs are known to play significant role in epimorphic regeneration as well and of all the FGFs, FGF2 is the most influential factor and very important for epimorphic regeneration (Yamashita *et al*., 2000).

The process of wound healing predominantly, involves many events like apoptosis of damaged and deformed cells, proteolytic digestion of extracellular matrix and proliferation of cells to heal the wound. FGF2 has been localized to the WE and nerves of the regenerating amphibian limb (Mullen *et al*., 1996) and more recently it is shown to be localized in regenerating tissues during tail regeneration of lizard *L. guichenoti* (Alibardi and Lovicu, 2010) Further support for their importance is underscored by studies showing that exogenous FGF2, applied either in vivo or in vitro, induces blastema cell proliferation in the absence of the WE (Chen and Cameron, 1983; Albert *et al*., 1987). Moreover, it has been shown that in urodele amphibians one of the first proteins to be formed after

amputation is FGF2, which is a major regulator of the events happening during wound healing process. It is known to switch on and off different set of genes required during the early events of tail regeneration viz. Dlx-3, Msx, Hox genes, Shh, etc. (Gardiner *et al*., 1995, Gardiner and Bryant, 1996). Another critical event associated with the healing of the wound after amputation in amphibians, is marked induction of proteolytic activity. Several protein degrading enzymes are known to be involved in this process that permits cells to escape from the extracellular matrix (ECM) and migrate into the blastema. *In vitro* studies have shown that FGF-2 is one of the important regulatory factors for extracellular matrix turnover via modulation of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMP) secretion from subepithelial myofibroblasts (Yasui *et al*., 2004).

The healing of the wound in amphibians is followed by the formation of blastema. Its growth is characterized by rapid cell proliferation. There are quite a lot of factors involved in the controlled proliferation of blastemal cells in amphibians, and FGF-2 is one factor which is thought to play role in the cell division cycles of blastemal cells. Its receptor, FGFR1, is expressed in blastema cells, suggesting that it could be acting on blastemal tissues to promote mitotic activity (Poulin *et al*., 1993). Ferretti *et al*., (2001) have shown that FGF-2, in addition to being up-regulated in the regenerating spinal cord in newts, is also expressed in a subset of blastemal cells and chondroblasts, in the basal epidermal layer and also in differentiating muscle. These results indicate that FGF-2 plays an important role in tail regeneration in newts and is likely to be involved both in proliferation and differentiation of tail tissues.

Further, angiogenesis at the site of wound is essential for the healing of wound. FGF-2 is considered a powerful stimulator of angiogenesis *in vivo* and it is also a pleotropic regulator of proliferation, migration, differentiation and survival of many cell types *in vitro*, including endothelial cells, smooth muscle cells and pericytes (D'Amore and Smith, 1993; Klein *et al*., 1993; Fernig and Gallagher, 1994; Friesel and Maciag, 1995; Slavin 1995; Biklfalvi *et al*., 1997; Iruela-Arispe and Dvorak, 1997; Webster and Donoghue, 1997).

Thus in the light of diverse functions of FGF2 during critical events of epimorphic regeneration like wound healing, cell proliferation, differentiation in amphibians, the present study was aimed at understanding the role of FGF2 in tail regeneration in *Hemidactylus flaviviridis*, an amniote model for studies on tissue regeneration. Since reptiles are higher in hierarchy and closer to mammals and the fact that some of them can regenerate lost body parts, makes them more suitable model for the study of regeneration and to disclose the secret of regenerating lost appendages.

MATERIALS AND METHODS

Adult Northern House Geckos, *Hemidactylus flaviviridis*, of both the sexes, with intact tail, weighing 10±2 gram were collected from the natural habitat. All animals were screened for parasitic infestation and the healthy ones were acclimated for a week before experiments were started. They were housed in well ventilated wooden cages of 45x30x60 cm with glass slider on one side for light and visibility, in the departmental animal house (827/ac/04/CPCSEA). The lizards were subjected to 12:12 hour light-dark cycles. Room temperature was maintained at 30±2ºC, as this temperature is necessary to produce optimum tail growth in lizards. The animals were fed with in-house reared cockroach nymphs twice a week and purified water was given daily, *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC). All procedures of amputation and treatments were done under hypothermic anaesthesia (Reilly, 2001) and were in complete compliance with the ethical guidelines of CPCSEA, India. At the end of the experiments, animals were rehabilitated for a period of one month followed by release to their natural habitat.

Experiment I

A total of twenty-four animals were used and they were divided into four groups of six animals each. Animals in each group were treated as follows:

Group I: This group of lizards served as the control group and were administered saline (0.6%) *in loco* (injection given at the first intact tail segment from the vent)

Group II: Animals received FGF-2 (Sigma,USA) *in loco* (25 µg/kg body weight).

Group III: The animals were injected with antiFGF-2 (Sigma, USA) *in loco* (25 mg/kg body weight).

Group IV: These animals served as a negative control and were given *in loco* injection of Rabbit anti-Rat IgG (Genei Products, Merck) (25 µg/kg body weight).

All the drugs were prepared in 0.6% saline every day immediately before use and were administered every alternate day at a dosage of 0.025ml/animal. After four doses of drug treatment autotomy was performed in all groups by pinching off the tail by exerting mild thumb pressure keeping three segments intact from the vent. The growth in the length of tail was measured at fixed intervals and time taken to reach the different stages during epimorphic regeneration was recorded.

Experiment II

Autotomy was performed on sixty lizards *viz*. *Hemidactylus flaviviridis*, and the regenerating animals were selected at two stages *viz*. (i) completion of wound healing and appearance of wound epithelium (WE) stage, and (ii) lizards at early blastema (BL) stage. Only those animals, which attained the above stages on the same day, were selected and grouped. Immediately after amputation, the process of wound healing initiates, followed by the formation of wound epithelium. The wound epithelium appears as a smooth shining surface and is accompanied by the process of dedifferentiation. Blastema stage is characterized by conical aggregation of blastemal cells, (approx. 1-2 mm in length from the stump) which have been formed as a result of dedifferentiation process. The blastema further grows in size and later on differentiates to replace the missing structures.

Series A: Injection of FGF-2 and antiFGF-2 at WE stage

Twenty-four lizards which attained WE stage on the same day were selected and divided into four groups of six animals each. These groups were treated as follows: -

Group I: Injected with saline *in loco*

Group II: Administered FGF-2 *in loco* (25µg/kg body wt).

Group III: *In loco* administration of antiFGF-2 (25 mg/kg body wt).

Group IV: *In loco* administration of Anti-rat IgG (25 mg/kg body wt).

The treatment started at WE stage and was continued every alternate day till 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 B: Injection of FGF-2 and antiFGF-2 at early blastema (BL) stage

Twenty-four lizards, which attained the blastema stage on the same day, were selected for the experiment. They were divided into four groups of six animals each and treated as described in Series A starting from blastema stage. The time taken to reach the various stages of tail regeneration and the rate of growth of tail was measured every alternate day after the first injection.

Statistical analysis

The data were subjected to Bartlett test for homogeneity and the significance level of the treatment groups with control group was evaluated through Student's 't' test with 95% confidence limit. The values are expressed as either Mean ± SE or as Mode with range in parenthesis. All statistical analyses were done using a statistical program SPSS, 11.5 (SPSS Inc. Chicago, IL, USA).

RESULTS AND DISCUSSION

The exogenous administration of FGF-2 prior to autotomy reduced the time taken by the animals to heal the wound (Table 1). The lizards treated with FGF-2, showed wound healing three days ahead compared to control lizards. However, treatment with antiFGF-2 delayed the healing of the wound compared to animals of control group. The blastema formation was also accelerated in FGF-2 treated animals, which took only six days to reach the early blastema stage, whereas in antiFGF-2 treated animals, the formation of blastema was significantly delayed. Similarly the attainment of differentiation (DF) stage was also hastened in FGF-2 treated animals, while the results were exactly opposite for the animals treated with antiFGF-2, where the attainment of DF stage was significantly delayed as compared to control animals (Figure 1). The progression of the regenerate (from 2-12 mm) was found to be accelerated in the animals treated with FGF-2 during the first fifteen days post-autotomy. However, treatment with antiFGF-2 significantly decreased ($p \leq 0.01$) the rate of growth of the regenerate in the first fifteen days compared to control lizards.

Further, the rate of growth of regenerate from 12-24 mm was significantly higher in animals treated with FGF-2 ($p \leq 0.01$). But, antiFGF-2 treatment did not significantly affect growth rate during this late differentiation period. There was approximately 91% increase in the growth rate of regenerate from 2-12 mm in the FGF-treatment group while treatment with antiFGF-2 showed 43% inhibition of growth of regenerate. However, the rate of growth of regenerate from 12-24 mm showed 28% increase in FGF-2 treated lizards whereas there was 9% inhibition in antiFGF-2 treated lizards. Animals treated with Anti-IgG serving as negative control attained various stages of regeneration in a comparable time as control. Their rate of growth of regenerate also did not show any significant increase or decrease as compared to control animals (Table 1, Figure 1).

Table 1: Onset and progression of regeneration in *H. flaviviridis*, subjected to *in loco* (IL) injection of FGF-2 and Anti- FGF-2 before amputation

Treatment	No. of Days		
	WH	BL (2mm)	DF (12mm)
IL Control	6 (5-6)[#]	10 (9-11)	16 (16-17)
IL FGF-2	3 (3-4)	6 (5-6)	14-13-14)
IL Anti FGF-2	11 (10-11)	14 (14-15)	20 (19-21)
IL Anti IgG	5 (5-6)	10 (10-11)	16 (16-17)

Treatment	No. of Days		
	WH	BL (2mm)	DF (12mm)
IL Control	6 (5-6)[#]	10 (9-11)	16 (16-17)
IL FGF-2	3 (3-4)	6 (5-6)	14-13-14)
IL Anti FGF-2	11 (10-11)	14 (14-15)	20 (19-21)
IL Anti IgG	5 (5-6)	10 (10-11)	16 (16-17)

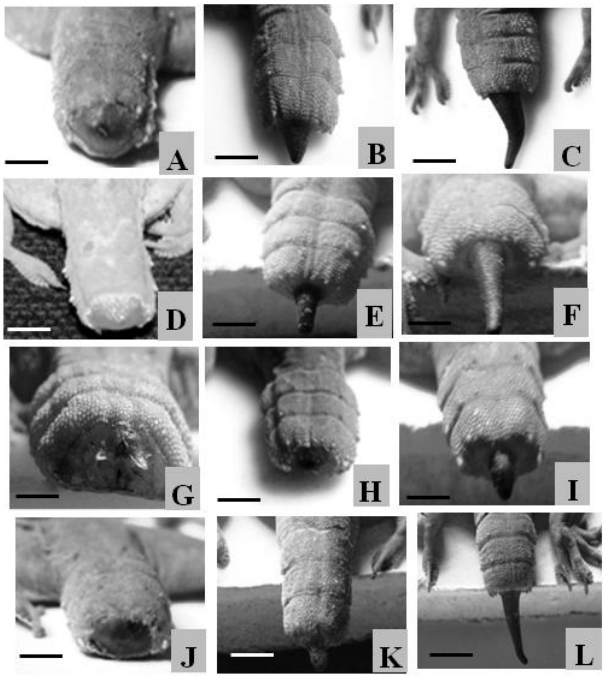

Figure 1: Dorsal view of the regenerating tail of *Hemidactylus* at different stages. A, B, C – Control animal on 6th (wound epithelium), 10th (blastema) and 16th (differentiation) day post amputation (DPA) respectively; D, E, F – FGF-2 treated animal on 3rd , 6th and 14th DPA respectively

showing accelerated regeneration as compared to control; G, H, I – Anti-FGF-2 treated animal on 6th, 10th and 16th DPA respectively showing delayed regeneration as compared to control; J, K, L – Negative Control animal on 6th, 10th and 16th DPA respectively (Scale bar =1mm)

In the experiment whereby treatment started at the WE stage, FGF-2 treated lizards showed hastening of the regenerative process. These lizards reached blastema stage faster, taking only eight days as compared to control lizards, which took ten days for the same (Table 2).

Table 2: Onset and progression of regeneration in *H. flaviviridis*, subjected to *in loco* (IL) injection of FGF-2 and Anti- FGF-2 at WE stage

Treatment	No. of Days		
	WH	BL (2mm)	DF (12mm)
IL Control	**6 (6-7)#**	**10 (9-10)**	**17 (16-17)**
IL FGF-2	**6 (6-7)**	**8 (7-8)**	**14 (13-14)**
IL Anti FGF-2	**6 (6-7)**	**14 (13-14)**	**20 (19-20)**
IL Anti IgG	**6 (6-7)**	**10 (9-10)**	**16 (16-17)**

Treatment	Rate of Growth of Regenerate (mm/day)		% increase/decrease compared to control	
	From 2-12mm	From 12-24mm	From 2-12mm	From 12-24mm
IL Control	1.568±0.051@	0.925±0.020	-	-
IL FGF-2	2.132±0.145*↑	1.076±0.033*↑	36↑$	16↑
IL Anti FGF-2	1.004±0.028**↓	0.847±0.020*↓	36↓	8↓
IL Anti IgG	1.550±0.036	0.928±0.021	1↓	0

However, the results were entirely reverse with lizards treated with antiFGF-2, which took three more days to attain blastema stage as compared to control lizards. The DF stage was attained earlier by the animals treated with FGF-2, while the same was delayed in antiFGF-2 treated animals. The rate of growth of regenerate from 2-12 mm was significantly higher ($p \leq 0.05$) in FGF-2 treated animals. But, treatment with antiFGF-2 showed a significant inhibition ($p \leq 0.01$) of growth of regenerate. There was approximately 36% increase in the rate of growth of regenerate from 2-12 mm in FGF-2 treated animals. Alternatively, treatment with antiFGF-2 showed 36% decrease in growth rate. Similar results were obtained for the growth of regenerate from 12-24 mm in both the treatments as compared to control animals, with 16% increase in the regenerate in FGF-2 treated animals and 8% decrease in the antiFGF-2 treated animals respectively. Similar to the first experiment, the animals treated as the negative control did not show significant changes in attaining the regeneration stages as compared to control.

Treatment at BL stage with both the drugs showed little influence on the progress of tail regeneration in *Hemidactylus flaviviridis* (Table 3).

The FGF-2 treated animals showed signs of differentiation two days prior than control animals, while it was delayed in antiFGF-2 treated animals. Lizards treated with FGF-2 when they reached blastema stage, showed a significant increase ($p \leq 0.05$) in the growth rate of the regenerate from 2-12 mm, as compared to saline treated animals in the initial stages of tail regeneration, whereas treatment with antiFGF-2 was found to decrease the rate of growth significantly ($p \leq 0.05$). However, there was not any significant influence on the rate of growth of regenerate from 12-24 mm, in either treatment. The lizards treated with FGF-2 showed approximately 17% increase in the rate of growth from 2-12 mm, but those treated with antiFGF-2 showed approximately 13% decrease in the growth rate. Furthermore, there was 1% increase in the rate of growth of regenerate from 12-24 mm in FGF-2 treated animals, while those treated with antiFGF-2, also showed no change in the growth rate. Here also, negative control group did not show any significant changes from the control group.

Table 3: Onset and progression of regeneration in *H. flaviviridis*, subjected to *in loco* (IL) injection of FGF-2 and Anti- FGF-2 at Blastema stage

Treatment	No. of Days		
	WH	BL (2mm)	DF (12mm)
IL Control	7 (6-7)[#]	10 (9-10)	17 (16-17)
IL FGF-2	7 (6-7)	10 (9-10)	15 (14-15)
IL Anti FGF-2	7 (6-7)	10 (9-10)	19 (18-19)
IL Anti IgG	7 (6-7)	10 (9-10)	17 (16-17)

Treatment	Rate of Growth of Regenerate (mm/day)		% increase/decrease compared to control	
	From 2-12mm	From 12-24mm	From 2-12mm	From 12-24mm
IL Control	1.439±0.058[@]	0.925±0.020	-	-
IL FGF-2	1.682±0.082	0.938±0.013**↑	17↑[$]	1↑
IL Anti FGF-2	1.258±0.045*↓	0.927±0.029	13↓	0
IL Anti IgG	1.426±0.056	0.921±0.012	1↓	0

[#]*Values are expressed as mode and range in parenthesis*
[@]*Values are expressed as Mean±SE; *p≤0.05; **p≤0.01;* [$]*Values are corrected to the nearest whole number*

Thus, in the present study, the extraneous administration of FGF-2 significantly influenced the process of tail regeneration in gekkonid lizard, *Hemidactylus flaviviridis.* The administration of FGF-2 prior to amputation was found to accelerate the healing of wound and formation of blastema. These observations lead to two very obvious influences of FGF-2 on regenerating tail - i) The healing of the wound and formation of WE, and ii) dedifferentiation of adult stump cells, if any, and formation of blastema. Since, FGF-2 administration showed early healing of the wound and it is a potent mitogen, it might be involved in the epithelial cell proliferation and migration, taking place during healing of the wound, as has been reported by several investigators (Dignas *et al.*, 1994; Bikfalvi *et al*., 1997; Burgess, 1998; Werner, 1998; Jones *et al*., 1999). Besides, the process of wound healing is known to be controlled by critical events like reepithelization, angiogenesis and matrix deposition (Miller and Gay, 1992), and FGF-2 might be involved in these processes. However, the treatment of animals with antiFGF-2 delayed the healing of the wound. This observation further strengthens the current notion that FGF-2 might be a key player in the healing process during tail regeneration in lizards.

Wound epithelium provides the necessary signals for the underneath tissues to dedifferentiate, proliferate and to form the blastema (Lo *et al*., 1993; Kumar *et al*., 2000). The present study revealed that administration of extraneous FGF-2, before amputation and at WE stage, to the animals hastened the formation of blastema. This faster process might be due to vital signals from the apical epithelial cap (AEC), which is a mass of pleuripotent cells formed by the repeated divisions of cells of WE. These signals include retinoic acid (Niazi and Saxena, 1978), hedgehog protein (Riddle *et al*., 1993) and FGF-2 (Boilly *et al*., 1991). While, retinoic acid and hedgehog protein respecify the proximo-distal axis during limb regeneration in amphibians, FGF-2 plays many other significant roles. The injury to blood vessels and nerves, which occurs as a result of amputation, is thought to be a trigger for the release of FGF-2 (Zhang *et al*., 2000;Yoshimura *et al*., 2001). Once this preformed FGF-2 is released, it further activates the synthesis and release of more FGF-2. Hence it is thought to work in an autocrine manner. Thus, extraneous FGF-2 might be adding on to the effects of the endogenous FGF-2 and hence, could bring about acceleration in the process of regeneration in the early stages. Furthermore, treatment with antiFGF-2 delayed the formation of blastema in animals. This might be caused partly by inhibition of endogenous FGF-2, which in turn, might have interfered with the FGF-2 signaling. Further, the formation of blastema requires recruitment of cells from the stump and this

needs extensive remodeling of the extracellular matrix (ECM) of the amputated stump. In the present study, accelerated blastema formation with FGF-2 treatment might have been possible due to faster reshuffling of the ECM, which made the cells free from the matrix and provided a platform for further events leading to the proliferation of blastemal cells. FGF-2 is also known to increase the activity of MMPs (Palmon *et al.*, 2000; Nishida *et al.*, 2011). However, the animals treated with antiFGF-2 showed a delay in the formation of blastema. This delay might be due to insufficient FGF-2 that is needed for further events of tail regeneration.

Once the blastema has been formed, the cells get engaged in repeated cycles of cell division, which result in the increase in length of the regenerate. The animals injected with FGF-2, before autotomy and at WE stage, were found to show an enhanced growth of the regenerate, while treatment with antiFGF-2 curtailed the rate of growth of regenerate from 2-12 mm. However, treatment with FGF-2 at BL stage, showed an increase in the rate of growth of regenerate from 2-12 mm, but had no influence in the later stages of growth. The proliferative role of FGF-2 might be due to its direct effect on the synthesis of DNA, which is needed by rapidly dividing cells of the regenerate. Unlike the FGF-2 treated animals, the animals treated with antiFGF-2 showed hampered growth of the regenerate from 2-12 mm, when injected before amputation, at WE stage and at BL stage. This hindrance might be due to inadequate signals for the proliferation of blastemal cells. However, once the regenerate attained a certain length it showed signs of differentiation and growth. The process of differentiation was found to be initiated earlier in the animals treated with FGF-2, before amputation and at WE stage. But treatment with FGF-2 at BL stage had little influence on the onset of differentiation. However, the rate of growth of regenerate form 12-24 mm was found to be enhanced in the animals treated with FGF-2 before amputation and at WE stage, while treatment at BL stage did not show any significant influence. These results reflected that though FGF-2 showed a noteworthy influence in the early events of tail regeneration in *Hemidactylus*, it did not have much influence on the regeneration of tail after the onset of differentiation process. Furthermore, animals treated with antiFGF-2 before amputation, at WE stage and at BL stage delayed the initiation of the process of differentiation. However, the rate of growth of regenerate from 12-24 mm was not influenced significantly. All these results reflected that FGF-2, by and large, is not involved in the process of differentiation of the regenerate as has been supported by the observations of Kruzhkova and Burgess (2000) who showed that FGF-2 inhibited the process of skeletal muscle differentiation in chick. It has also been shown that HGPG glypican-1 acts as a positive regulator of muscle differentiation by sequestering FGF-2 in lipid rafts and preventing its binding and dependent signalling (Gutierrez and Brandan, 2010). In all the experiments, the process of regeneration in animals treated as negative control was not significantly different from the control group, thereby validating that the results obtained with Anti-FGF-2 treatment were due to specifically antagonized FGF-2 and not due to any antibody toxicity.

Therefore, it can be hypothesized that FGF-2 significantly influenced the process of tail regeneration in gekkonid lizard, *Hemidactylus flaviviridis*. More importantly, the early events appeared critically under the influence of FGF-2. Furthermore, FGF-2 was found to accelerate the process of wound healing and the formation of wound epithelium and blastema. In addition, it also increased the rate of growth of regenerate during early stages of tail regeneration. Conversely, antiFGF-2 delayed the healing of the wound and the formation of WE was also delayed. There was also a marked inhibition of the growth of regenerate in the lizards treated with antiFGF-2. However, there was not much influence of FGF-2 or antiFGF-2 after the animals started redifferentiation of the blastemal cells to form the lost appendage.

ACKNOWLEDGEMENTS

The work was supported by grant no. SP/S0/AS/18/2007 awarded to SB by the Department of Science and Technology (DST), New Delhi, India. One of the Authors (MY), is also grateful to the help provided by the UGC in the form of Junior Research Fellowship

REFERENCES

Albert P, Boilly B, Courty J and Barritault D (1987). Stimulation in cell culture of mesenchymal cells of newt limb blastemas by EDGF I or II (basic or acidic FGF). *Cell Differentiation* **21** 63-68.

Alibardi L (2009). *Morphological and cellular aspects of tail and limb regeneration in lizard* (Springer, New York).
Alibardi L and Lovicu FJ (2010). Immunolocalization of FGF1 and FGF2 in the regenerating tail of the lizard *Lampropholis guichenoti*: Implications for FGFs as trophic factors in lizard tail regeneration. *Acta Histochemica* **112**(5) 459-473.
Basilico C and Moscatelli D (1992). The FGF family of growth factors and oncogenes. *Advances in Cancer Research* **59** 115-165.
Bellairs ADA and Bryant SV (1985). Autotomy and regeneration in reptiles. In: *Biology of Reptilia,* edited by Gans C and Billet F (John Wiley and sons, New York) 301-410.
Bikfalvi A, Klein S, Pintucci G and Rifkin DB (1997). Biological roles of FGF-2. *Endocrine Reviews* **18**(1) 26-45.
Boilly B, Cavanaugh KP, Thomas D, Hondermarck H, Bryant SV and Bradshaw RA (1991). Acidic fibroblast growth factor is present in regenerating limb blastemas of axolotls and binds specifically to blastema tissues. *Developmental Biology* **145**(2) 302-310.
Burgess AW (1998). Growth control mechanisms in normal and transformed intestinal cells. *Philosophical Transactions of the Royal Society B: Biological Sciences* **353**(1370) 903-909.
Burgess WH and Maciag T (1989). The heparin-binding (fibroblast) growth factor family of proteins. *Annual Review of Biochemistry* **58** 575-606.
Chen KE and Cameron JA (1983). Increase in mitotic activity of regenerating axolotl limbs by growth factor-impregnated implants. *Journal of Experimental Zoology* **226**(2) 325-329.
Clarke MS, Khakee S and McNeil PL (1993). Loss of cytoplasmic basic fibroblast growth factor from physiologically wounded myofibres of normal and dystrophic muscle. *Journal of Cell Science* **106**(1) 121-33.
Cuevas P, Carceller F, Ortega S and Nieto I (1988). Basic fibroblast growth factor promotes cartilage repair in vivo. *Biochemical and Biophysical Research Communications* **156**(2) 611-618.
D'Amore PA and Smith SR (1993). Growth factor effects on cells of the vascular wall: A survey. *Growth Factors* **8**(1) 61-75.
Dignas AU, Tsunekawa S and Podolsky DK (1994). Fibroblast growth factors modulate intestinal epithelial cell growth and migration. *Gastroenterology* **106**(5) 1254-1262.
Fernig DG and Gallagher JT (1994). Fibroblast Growth Factors and their receptors: an information network controlling tissue growth, morphogenesis and repair. *Progress in Growth Factor Research* **5**(4) 353-377.
Ferretti P, Zhang F, Santer-Ruiz L and Clarke JDW (2001). FGF signaling and blastema growth during amphibian tail regeneration. *International Journal of Developmental Biology* **45**(S1) S127-S128.
Friesel RE and Maciag T (1995). Molecular mechanism of angiogenesis: Fibroblast growth factor signal transduction. *The FASEB Journal* **9**(10) 919-925.
Gardiner DM, Blumberg B, Komine Y and Bryant SV (1995). Regulation of HoxA expression in developing and regenerating axolotl limbs. *Development* **121**(6) 1731-1741.
Gardiner DM and Bryant SV (1996). Molecular mechanisms in the control of limb regeneration: the role of homeobox genes. *International Journal of Developmental Biology* **40**(4) 797-805.
Gutierrez J and Brandan E (2010). A novel mechanism for sequestering fibroblast growth factor-2 by glypican in lipid rafts, allowing skeletal muscle differentiation. *Molecular biology of the cell* **30**(7) 1634-1649.
I**ruela-Arispe ML and Dvorak HF (1997).** Angiogenesis: a dynamic balance of stimulators and inhibitors. *Journal of Thrombosis and Haemostasis* **78**(1) 672-677.
Iten LF and Bryant SV (1976). Stages of tail regeneration in the adult newt *Notopthalmus viridescens*. *Journal of Experimental Zoology* **196**(3) 283-292.
Jones MK, Tomikawa M, Mohajer B and Tarnawski AS (1999). Gastrointestinal mucosal regeneration: role of growth factors. *Frontiers in Bioscience* **4** 303-309.
Klein S, Giancotti FG, Presta M, Albelda SA, Buck CA and Rifkin DB (1993). Basic fibroblast growth factor modulates integrin expression in microvascular endothelial cells. *Molecular Biology of the Cell* **4**(10) 973-982.

Kruzhkova L and Burgess P (2000). The role of FGF2 in skeletal myogenesis of chick presomitic mesoderm. *Developmental Biology* **255** 1707-1710.
Kumar A,Vellosco CP, Imokawa Y and Brockes JP (2000). Plasticity of retrovirus-labelled myotubes in the newt limb regeneration blastema. *Developmental Biology* **218** (2) 125-136.
Lo DC, Allen F and Brockes JP (1993). Reversal of muscle differentiation during urodele limb regeneration. *Proceedings of the National Academy of Sciences* **90** (15) 7230-7234.
McLean KE and Vickaryous MK (2011). A novel amniote model of epimorphic regeneration: the leopard gecko, *Eublepharis macularius*. *BMC Development Biology* **11** 50-73.
Miller E and Gay S (1992). Collagen structure and function. In: *Wound Healing, Biochemical and Clinical Aspects*, edited by Cohen IK, Diegelman RF and Lindlab WJ (WB Saunders, London) 130-151.
Mullen LM, Bryant SV, Torok MA, Blunberg B and Gardiner DM (1996). Nerve dependency of regeneration: The role of distal-less and FGF signaling in amphibian limb regeneration. *Development* **122** (11) 3487-3497.
Niazi A and Saxena S (1978). Abnormal hindlimb regeneration in tadpoles of the toad *Bufo andersonii* exposed to excess vitamin A. *Folia Biologica* **26** 3-8.
Nishida T, Kubota S, Aoyama E, Janune D, Maeda A and Takigawa M (2011). Effect of CCN2 on FGF-2 induced proliferation and MMP9 and MMP13 productions by chondrocytes. *Endocrinology* **151** (11) 4232-4241.
Palmon A, Ross H, Edel J, Sax B, Savion N, Grosskop A and Pitaru S (2000). Inverse dose and time dependent effect of Bfgf on the gene expression of collagen type I and MMP-1 by periodontal ligament cells in culture. *Journal Periodontology* **71** (6) 974-980.
Poulin ML, Patrie KM, Botelho MJ, Tassava RA and Chiu IM (1993). Heterogeneity in the expression of fibroblast growth factor receptors during limb regeneration in newts (*Notophthalmus viridescens*). *Development* **119** (2) 353-361.
Reilly JS (2001). *Euthanasia of animals used for scientific purposes* (ANZCCART, Adelaide).
Riddle RD, Johnson RL, Laufer E and Tabin C (1993). Sonic hedgehog mediates the polarizing activity of the ZPA. *Cell* **75** (7) 1401-1416.
Rifkin DB and Moscatelli D (1989). Recent developments in the cell biology of basic fibroblast growth factor. *Journal Cell Biology* **109** (1) 1-6.
Schwartz SM and Liaw L (1993). Growth control and morphogenesis in the development and pathology of arteries. Journal of Cardiovascular *Pharmacology* **21** (S1) S31-S49.
Slavin J (1995). *Fibroblast growth factors: at the heart of angiogenesis. Cell* Biology International **19** (5) 431-444.
Song F, Li B and Stocum DL (2010). Amphibians as research models for regenerative medicine. *Organogenesis* **6** (3) 141-150.
Webster MK and Donoghue DJ (1997). Fibroblast growth factor receptor activation in skeletal disorders: too much of a good thing. *Trends in Genetics* **13** (5) 178-182.
Werner S (1998). Keratinocyte growth factor: A unique player in epithelial repair processes. *Cytokine & Growth Factor Reviews* **9** (2) 153-165.
Yamashita T, Yoshioka M and Itoh N (2000). Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain. *Biochemical and Biophysical Research Communications* **277** (2) 494-498.
Yasui H, Andoh S, Bamba S, Inatomi O, Ishida H and Fujiyama Y (2004). Role of fibroblast growth factor-2 in the expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human intestinal myofibroblasts. *Digestion* **69** (1) 34-44.
Yoshimura S, Takagi Y, Harada J, Teramoto T, Thomas SS, Waeber C, Bakowska JC, Breakefield XO and Moskowitz MA (2001). FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. *Proceedings of the National Academy of Sciences* **98** (10) 5874-5879.
Zhang F, Clarke JDW and Ferretti P (2000). FGF-2 up regulation and proliferation of neural progenitor in the regenerating amphibian spinal cord *in vivo*. *Developmental Biology* **225** (2) 381-391.