CHAPTER 1

Influence of extraneous FGF-2 and its antagonist antiFGF-2, on the progress of tail regeneration in *Hemidactylus flaviviridis*

INTRODUCTION

The events of the process of epimorphic regeneration are comparable with those occurring during development. However, an intriguing difference between the two is the events leading to the formation of blastema during epimorphic regeneration. The early events of epimorphic regeneration in amphibians are thought to be regulated by scores of factors, of which FGF-2 is of key importance, and it is one of the first molecules to be expressed during limb regeneration in amphibians (Bryant and Gardiner, 1992). Further, in lizards, immediately after amputation, the first activity to take place is the healing of the wound. The release of growth factors at the injured site is an important step in the initiation of the healing process (Li *et al.,* 2002). The role of FGF-2 in the healing of the wounds has been studied in various animal models. Moreover, the factors associated with wound include TGF-β1, FGF-2, EGF, TGF-α, and heparin binding EGF and IGF-1, which stimulate intensive migration of scratch-wounded astrocytes with consequent closure of wound in rats (Faber-Elman *et al.,* 1996). Several *in vitro* as well as studies on mammalian system have also shown that FGF-2 plays a very crucial role during healing of the wound (Marks *et al.,* 1991; Stenberg *et al.,* 1991; Chen *et al.,* 1992; Kurita *et al*, 1992; Pierce *et al.,* 1992, Slavin *et al.,* 1992; Tsuboi *et al.,* 1992, Albertson *et al.,* 1993; Legrand *et al.,* 1993; Phillips *et al.,* 1993; Gibran *et al.,* 1995) Moreover, FGF-2 also plays role in skin wound healing in mice (Ortega *et al.,* 1998) whereas healing is delayed in mice lacking FGF-2 Similarly, van der Bas *et al.,* (2004) showed that FGF-2 is present transiently during wound repair *in vivo* in porcine model. Further, exogenous FGF-2 is known to accelerate wound healing in different animal models as well (Tsubai *et al.,* 1990; Nissen *et al.,* 1996).

The process of wound healing predominently, involves many events like apoptosis of damaged and deformed cells, proteolytic digestion of extracellular matrix and proliferation of cells to heal the wound Topical application of FGF-2 to the animals has been shown to result in a modest acceleration of healing in incisional wounds (McGee *et al.,* 1988). Contrary to this, in normal rats, antiFGF-2 antibodies retard granulation tissue formation (Broadley *et al.,* 1988; Broadley *et al.,* 1989; Klingbeil *et al.,* 1991). Further, topical

application of FGF-2 directly to the wound site in rats, augments the endogenous supply of FGF-2 causing the recruitment and division of cells required for granulation tissue and subsequent contraction of the wound (Kuhn *et al.,* 2001). Several investigators have opined that FGF-2 expression is up regulated after brain injury in rats (Zhang *et al.,* 2000; Yoshimura *et al.,* 2001) and is known to induce wound healing (Miller *et al.,* 2000). It is also speculated that in rats, brain injury not only up regulates synthesis of FGF-2 intracellularly, but also promotes cell secretion and dissociation of cells from extracellular matrix. Furthermore, FGF-2 has been detected at the wound site early in healing and its rapid appearance after injury suggests that pre-existing tissue FGF-2 may be important in healing rather than that synthesized *de novo* by inflammatory macrophages (Yoshimura *et al.,* 2001)

Studies carried out in various animal models reveal that the healing of retinas and corneas is also accelerated by FGF-2 (Fiddes *et al.,* 1991; Mazue *et al.,* 1991, Rich *et al.,* 1992; Rieck *et al.,* 1993, Rieck *et al.,* 1993; Hoppenreijs *et al.,* 1994; Schuschereba *et al.,* 1994). Moreover, apoptosis regulation following FGF-2 administration to an incisional wound, may lead effectively to granulation tissue formation and promote a scar-less repair process in rats (Akasaka *et al.,* 2004). Further, angiogenesis at the site of wound is essential for the healing of wound and FGF-2 has been known as a potent angiogenic molecule *in vivo*, and *in vitro*, it stimulates smooth muscle cell growth, wound healing, and tissue repair (Basilico *et al.,* 1992, Schwartz *et al.,* 1993). According to some other studies, the ruptured blood vessels in rats, release FGF-2 at the site of injury that play an important role in the autoregulation of angiogenesis after injury (Villaschi and Nicosia, 1993) Similarly, autotomy of the tail in lizards might also be triggering the release of endogenous FGF-2, which might play role in healing of the wound and further events of tail regeneration. Also, FGF-2 promotes vascular repair and angiogenesis and can induce *in vitro* tissue factor (TF), a potent agent, initiating thrombogenesis, which probably plays a role in angiogenesis. In addition, Angiotensin-II and Nor-epinephrin (NE) cooperate in promoting vascular smooth muscle cell (VSMC) growth and FGF-2 upregulation is known to be involved in this effect (Parenti *et al.,* 2001). Furthermore, basic fibroblast growth factor (FGF-2) is known to be important in the regeneration of granulation tissue. Although FGF-2 has been shown to be a potent angiogenic agent in mammals, it does not produce a change in capillary density or granulation tissue thickness, though the capillaries appear to be larger in the FGF-2 treated animals (Gospodarowicz *et al.,* 1986, Folkman and Klagsbrun, 1987; Gospodarowicz *et al.,* 1989; Hayward *et al.,* 1992) Besides, 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; Fernig *et al.,* 1994; Friesel *et al.,*

1995, Slavin, 1995; Bikfalvi *et al.*, 1997, Galzie *et al.*, 1997; Klein *et al.*, 1997; Iruela-Arispe and Dvorak, 1997; Webster *et al.*, 1997, Burke *et al.*, 1998).

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. For example, a matrix metalloprotease has been shown to be expressed in the mesenchyme as early as 3 to 4 hours after amputation in newts (Yang and Bryant, 1994) These proteolytic activities permit cells to escape from the extracellular matrix (ECM) and migrate into the blastema. Moreover, *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 (SEMFs) (Yasui *et al.*, 2004). Likewise, changes in MMP-2, 7 and TIMP-2 expressions are an important process of wound repair, which are closely related to the acceleration of wound healing by the application of FGF-2 (Cheng *et al.*, 2003). The healing of the wound in amphibians is followed by the formation of a mass of pleuripotent cells called blastema.

The growth of blastema is characterized by rapid cell proliferation. Although during epimorphic regeneration in urodeles, blastemal cells are able to engage in multiple reversible episodes of cell cycle reentry, it has long been recognized that this is not associated with susceptibility but with marked resistance to tumor formation (Prehn, 1971; Tsonis and Eguchi, 1983) For example, after application of chemical carcinogens to the blastema, the mesenchymal cells retain their ability to undergo differentiation and morphogenesis, and in some cases supernumerary regenerates are formed. Further, all systems of epimorphic regeneration show the ability to sustain multiple cycles of regeneration with little change in time course, a feature possibly inconsistent with the finite proliferative potential of most animal cells (Zilakos *et al.*, 1992). Indeed, newt limb blastemal cells can be maintained in culture for more than 200 generations without signs of crisis or senescence (Ferretti and Brockes, 1988). 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 Besides, FGF-2 is also known to be mitogenic for many other cells as well For example, FGF-2 is most effective in promoting proliferation of human bone marrow stromal cells *in vitro* (Martin *et al.*, 1997). Moreover, FGF-2 and Fibroblast growth factor receptors (FGFR) 1 and 2 have been shown to be involved in prostatic (Giri *et al*, 1999) and pancreatic cancer in humans (Kormann *et al.*, 1998). In cell culture, FGF-2 induces a dose-dependent mitogenic response on bovine and human corneal endothelial cells (Hoppenreijs *et al.*, 1994). Further, FGF-2 binds with cell membrane monosialoganglioside (GM1) and this binding is required for the mitogenic

activity of the growth factor and hence GM1 acts as a functional FGF-2 co-receptor in different cell types *in vitro* (Rusnati *et al.,* 2002) Furthermore, the proliferation and differentiation of bovine osteoblasts are stimulated by FGF-2 (Globus *et al.,* 1988) while it is an exogenous regulator of smooth muscle cell migration and proliferation in humans (Blaes and Allera, 1997)

Adding to the roles of FGF-2 in proliferation and differentiation, basic fibroblast growth factor is found to stimulate proliferation as well as differentiation of mesodermal tissues, such as fibroblasts and endothelial cells, as well as neuroectodermal cells in humans (Montesano, 1986, Baird and Walicke, 1989, Bennett and Schultz, 1993; Bennett and Schultz 1993; Bhora *et al.,* 1995, Gibran *et al.,* 1995). Likewise, *in vitro* studies using FGF-2 have demonstrated stimulation of fibroblast, vascular endothelial cell, and keratinocyte division, while *in vivo* studies have demonstrated granulation tissue formation and epidermal regeneration in mammals (Uhl *et al.,* 1993; Nicosia *et al.,* 1994; Danilenko *et al.,* 1995; Roesel and Nanney, 1995; Fu *et al.,* 1998). Further, in mammalian system, neural precursors isolated from adult rat brain are induced to proliferate and to differentiate by FGF-2 (Richards *et. al.,* 1992); likewise, the proliferation and differentiation of normal human melanocytes are dependent on FGF-2 (Halaban *et. al.,* 1992). *In vitro,* FGF-2 is mitogenic for immature oligodendrocytes of rats and promotes their survival while blockes oligodendrocytes maturation/differentiation (Saneto and DeVellis, 1985; McKinnon *et al.,* 1990; Goddard *et. al.,* 1998) Contrary to this, FGF-2 inhibits proliferation of rat chondrosarcoma cells and arrests cell cycle at G1 phase (Aikawa *et. al.,* 2001). Thus, FGF-2 promotes cell proliferation in some cell types, while inhibits in others. Likewise, Maher, (1999) extrapolated that in cell cultures, FGF-2 stimulates cell proliferation but not differentiation. However, during amphibian tail regeneration, FGF-2 has been shown to increase the proliferation of cells and accelerate the regeneration process (Ferretti *et al.,* 2001).

Moreover, the early stages of tail regeneration in lizards are characterized by rapid cell proliferation and are suggested to be partly under the influence of FGF-2. 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, Weyman *et al.,* (1998) have shown that FGF-2 inhibits skeletal muscle differentiation *in vitro,* whereas another study showed that FGF-2 induces transdifferentiation of retinal pigment epithelium (Sakaguchi *et al.,* 1997). Further, *in vitro* studies have also shown that FGF-2 regulates cell differentiation and

formation of actin-based cellular processes during morphogenesis in podocytes as well (Davidson *et al.*, 2001).

From all these studies and their observations, an obvious involvement of fibroblast growth factor-2 in wound healing, cell proliferation and differentiation in different animal models is apparent. Moreover, FGF-2 is also known to influence the key events during the process of epimorphic regeneration in amphibians. Hence, the present study was conducted to ascertain a similar role of FGF-2 during tail regeneration in house lizard, *Hemidactylus flaviviridis*

MATERIALS AND METHODS

Adult wall lizards, *Hemidactylus flaviviridis,* of both the sexes, with intact tail and weighing an average 10±2 gram, were selected. They were acclimated for a week before experiments were started. The animals were fed with cockroaches as and when required (2 times a week) and water was given daily, *ad libitum.*

Experiment I:

Adult wall lizards, *Hemidactylus flaviviridis,* weighing an average 10 ± 2g were selected and acclimated for a week. A total of thirty-six animals were used and they were divided into six groups of six animals each. Animals in each group were treated as follows:

Group I. These groups of animals served as control to the experimental groups and were injected intraperitoneally with 0.6% saline only.
Group II: The animals received an intraperitoneal (IP) injection of Fibroblast Growth Factor-2 at a dosage of 50µg/kg body wt.
Group III: The animals of this group received an IP injection of antiFGF-2 at a dose of 50mg/kg body wt
Group IV: Lizards were administered saline *in loco.*
Group V. Animals received FGF-2 *in loco* (25 µg/kg body weight).
Group VI The animals were injected with antiFGF-2 *in loco* (25 mg/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 0.05 ml per animal The animals were fed with cockroaches 2-3 times a week and water was given daily. After four days 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 treatment was continued

for six days post-autotomy. 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 eighty 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

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

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

Group I. The animals were injected with saline (0.6%) intraperitoneally.
Group II: Lizards were administered FGF-2 intraperitoneally (50µg/kg body wt)
Group III Administered anti FGF-2 intraperitoneally (50mg/kg body wt)
Group IV: Injected with saline *in loco*
Group V: Administered FGF-2 *in loco* (25µg/kg body wt)
Group VI: *In loco* administration of antiFGF-2 (25 mg/kg body wt).

The treatment started at WE stage and was continued for ten days. 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

Thirty-six lizards, which attained the blastema stage on the same day, were selected for the experiment. They were divided into six groups of six animals each and treated for ten days from attainment of blastema as in series A.

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 was subjected to Student's't' test, with a 95% confidence limit. The values are expressed as Mean ± SE. A 'p' value of 0.05 or less was considered statistically significant.

RESULTS

Experiment I: The exogenous administration of FGF-2 prior to autotomy reduced the time taken by the animals to heal the wound (Table 1.1). 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 by four days compared to animals of control group, which took seven days for the healing of the wound and formation of wound epithelium The blastema formation was also accelerated in FGF-2 treated animals, which took only seven 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. The progression of the regenerate (from 2-12 mm) was found to be accelerated in the animals treated with FGF-2 (both IP and *in loco* treatments) during the first fifteen days post-autotomy (Figure 1.1, 1.3). 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 ($p \leq 0.05$) in animals treated with FGF-2 intraperitoneally and it was even more significant ($p \leq 0.01$) in the *in loco* treatment (Figure 1.2). But, treatment with antiFGF-2 reduced the rate of growth significantly ($p \leq 0$ 01) in IP treated lizards, while was ineffective in the *in loco* treated lizards. There was approximately 90% and 91% increase in the growth rate of regenerate from 2-12 mm, in IP and *in loco* FGF-2 treated animals respectively. However, treatment with antiFGF-2 showed 36% and 43% inhibition of growth of regenerate in IP and *in loco* treated animals However, the rate of growth of regenerate from 12-24 mm showed 24% and 28% increase in IP and *in loco* FGF-2 treated lizards whereas there was 7% and 9% inhibition in IP and *in loco* antiFGF-2 treated lizards respectively

Experiment II

(i) Injection at Wound Epithelium (WE) stage:

In this group, 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 1.2). 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 all FGF-2 treated animals (Figure 1.1, 1.3). But, treatment with antiFGF-2 showed a significant inhibition ($p \leq 0.01$) of growth of regenerate. The acceleration in the rate of growth of tail from 12-24 mm, was more significant ($p \leq 0.01$) in IP FGF-2 treated animals than *in loco* ($p \leq 0.05$) treated animals (Figure 1.2). However, treatment with antiFGF-2 showed a significant inhibition of growth of regenerate in IP ($p \leq 0.01$) and *in loco* ($p \leq 0.05$) treatments There was approximately 29% and 36% increase in the rate of growth of regenerate from 2-12 mm in IP and *in loco* FGF-2 treated animals respectively Alternatively, treatment with antiFGF-2 showed 30% and 36% decrease in IP and *in loco* treatments respectively. Similar results were obtained for the growth of regenerate from 12-24 mm in both the treatments as compared to control animals, with 12% and 16% increase in the regenerate in FGF-2 treated animals and 8% decrease in the antiFGF-2 treated animals respectively.

(ii) Injection at Blastemic (BL) stage:

Treatment at BL stage with both the drugs showed little influence on the progress of tail regeneration in *Hemidactylus flaviviridis* (Table 1 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 ($p \leq 0.05$) (Figure 1.1). However, there was not any significant influence on the rate of growth of regenerate from 12-24 mm, in either treatment (Figure 1.2). The lizards treated with FGF-2 showed approximately 15% increase in the rate of growth from 2-12 mm, but those treated with antiFGF-2 showed approximately 11% decrease in the growth rate. Furthermore, there was 1.4% increase in the rate of growth of regenerate from 12-24 mm in FGF-2 treated animals, while those treated with antiFGF-2, both IP and *in loco,* also showed an increase in the growth rate, *viz.* 1.4% and 0.24% respectively.

DISCUSSION

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, both IP and IL, 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. The process of

wound healing requires the proliferation of epithelial cells to cover the exposed wound surface 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). Moreover, Oda *et al.,* (2004) have shown that FGF-2 significantly accelerates granular tissue formation and reepithelization during wound healing. Besides, the process of wound healing is known to be controlled by critical events like reepithelization, angiogenesis and matrix deposition (Cohn *et. al.,* 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

Thus, after amputation of the tail, the wound is quickly covered by the specialized epithelium called wound epithelium (WE). It is strongly believed that this epithelium provides the necessary signals for the underneath tissues to dedifferentiate, proliferate and to form the blastema. 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). Wherein, retinoic acid and hedgehog protein respecifiy 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. This can be accomplished only if the cells become free from the matrix. There are many proteolytic enzymes that play role in making cells free from cell-cell adhesion and reorganizing the matrix, during epimorphic regeneration.

The early events of tail regeneration in lizards require extensive remodeling of the extracellular matrix (ECM) of the amputated stump. Many ECM degrading enzymes like collagenases, elastases, matrix metalloproteinases (MMPs) are known to be involved in this

process. MMPs are special matrix degrading enzymes, which play specific roles during matrix reorganization. Thus, these MMPs appear to be the principle enzymes playing role in matrix reorganization during epimorphic regeneration, as has been noticed in amphibian limb regeneration. For example, Yang *et al.,* (1999) have reported the expression of MMP9 during axolotl limb regeneration. Similarly, Xmmp-9 has been shown to be expressed in the ectoderm and mesoderm at the tip of the amputated limb, very early during limb regeneration in *Xenopus*, where it is argued to play a role in matrix reorganization (Carinato *et al.,* 2000). As observed in the present study, the administration of FGF-2 accelerated the formation of blastema. This 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. The faster reorganization of ECM, might have happened under the influence of FGF-2, as FGF-2 is known to increase the activity of MMPs (Palmon *et al.,* 2000) 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 in gekkonid lizards.

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 Similarly, the rate of growth was also found to be higher in the regenerate of the FGF-2 treated lizards, while treatment with antiFGF-2 curtailed the rate of growth of regenerate from 2-12 mm These observations found support from the experiments of many investigators who reported that FGF-2 is mitogenic and increases the proliferation of different kinds of cells in culture (Neufeld *et al.,* 1988; Sasada *et al.,* 1988; Quarto *et al.,* 1991, Nguyen *et al.,* 1994; Bikfalvi *et al.,* 1997). 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. Hence, a separate experiment was conducted to study the influence of FGF-2 on the levels of nucleic acids and protein in the regenerate (Chapter II). The FGF-2 might have exerted its mitogenic influence by binding to its specific receptor tyrosine kinase (RTK) that triggered the MAPK (Mitogen Activated Protein Kinase) signaling cascade (Antoniotti *et al.,* 2003). In turn, MAPK might have phosphorylated and activated cytosolic phospholipase A2 (cPLA2), which then released arachidonic acid (AA) from plasma membrane phospholipids as reported by Sa *et al.,* (1995). Further, AA metabolites might have been involved in the control of cell motility (mostly via cyclooxygenase pathway - COX pathway) and proliferation (via lipoxygenase cascade - LOX pathway) (Antoniotti *et al.,* 2003). Thus, FGF-2 might have stimulated proliferation of cells through COX-PGE2 pathway during tail regeneration in

lizards. Moreover, several studies have also shown that both AA and eicosanoids of the COX and LOX pathway are critical intermediaries in the stimulation of endothelial cell proliferation promoted by FGF-2 (Fafeur *et al.,* 1991; Dethlefsen *et al.,* 1994; Whatley *et al.,* 1994, Nie *et al*, 2000, Antoniotti *et al.,* 2003). Further, FGF-2 is known to induce COX-2 expression through p38 pathway (Tessner *et al.,* 2003), which is thought to be involved in arachidonic acid metabolism. Similarly, other cell culture studies have shown that cell proliferation is induced by FGF-2 mainly through MAPK-cPLA2 pathway but phospholipase C (PLC) and phospholipase D (PLD) pathways might also be involved as suggested by Antoniotti *et al.* (2003)

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 the animals treated 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 didn't show any significant influence. These results reflected that though FGF-2 showed a noteworthy influence in the early events of tail regeneration in *Hemidactylus flaviviridis,* 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 *et al.,* (2000) who showed that FGF-2 inhibited the process of skeletal muscle differentiation in chick.

Moreover, the systematic progression of tail regeneration requires not only accurate signaling, but also maintenance of each stage for a stipulated duration so that the tissue can prepare itself to move onto next stage of tail regeneration. The cellular processes occurring during the tail regeneration produce many by-products that can interfere with these signaling mechanisms One such by-product is the formation of reactive oxygen species (ROS) that are considered highly deleterious to the tissue. However, cells possess defense mechanisms against these ROS. Hence, a separate experiment was planned to study the effect of FGF-2 on the antioxidant defenses during various stages of tail regeneration (Chapter III) Furthermore, FGF-2 is not the only growth factor playing role in epimorphic

regeneration. There are several other growth factors that influence epimorphic regeneration and epidermal growth factor (EGF) is also one of them. Therefore, the role of EGF in tail regeneration in *Hemidactylus flaviviridis* was also studied separately (Chapter IV).

In conclusion, it can be hypothesized that basic fibroblast growth factor 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. The formation of blastema was also accelerated by the FGF-2 supplementation. 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.

TABLE 1 1. The onset and progression of regenerate in wall lizards, *Hemidactylus flaviviridis*, subjected to *in loco* (IL) and intraperitoneal (IP) injection of FGF-2 and antiFGF-2 before amputation.

Treatment	No. of Days		
	WH	BL (2 mm)	DF (12 mm)
IP Control	7(6-7)#	10 (9-10)	17 (16-17)
IP FGF-2	4 (3-4)	7 (6-7)	13 (13-14)
IPantiFGF-2	10 (9-10)	13 (12-13)	20 (19-20)
IL Control	6 (5-6)	10 (9-11)	16 (16-17)
IL FGF-2	3 (3-4)	6 (5-6)	14 (13-14)
IL antiFGF-2	11 (10-11)	14 (14-15)	20 (19-21)

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
IP Control	1.439 ± 0 058@	0.953 ± 0.017	-	-
IP FGF-2	2.732 ± 0 233**↑	1.182 ± 0.040*↑	90↑*	24↑
IPantiFGF-2	0.926 ± 0 029**↓	0.883 ± 0.014**↓	36↓	7↓
IL Control	1.568 ± 0.051	0.941± 0 024	-	-
IL FGF-2	2.998 ± 0.182**↑	1.204 ± 0.034**↑	91↑	28↑
IL antiFGF-2	0.892 ± 0 028**↓	0.859 ± 0.017	43↓	8↓

@ 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

TABLE 1 2· The onset and progression of regenerate in wall lizard, *Hemidactylus flaviviridis*, subjected to *in loco* (IL) and intraperitoneal (IP) injection of FGF-2 and antiFGF-2 at WE stage

Treatment	No. of Days		
	WH	BL (2 mm)	DF (12 mm)
IP Control	6 (6-7)#	10 (9-10)	16 (15-16)
IP FGF-2	6 (6-7)	8 (7-8)	14 (13-14)
IPantiFGF-2	6 (6-7)	13 (12-13)	19 (18-19)
IL Control	6 (6-7)	10 (9-10)	17 (16-17)
IL FGF-2	6 (6-7)	8 (7-8)	14 (13-14)
IL antiFGF-2	6 (6-7)	14 (13-14)	20 (19-20)

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
IP Control	1 521 ± 0 051@	0.925 ± 0.020	-	-
IP FGF-2	1.964 ± 0 138*↑	1.036 ± 0.020**↑	29↑*	12↑
IPantiFGF-2	1.066 ± 0 024**↓	0.847 ± 0.020**↓	30↓	8↓
IL Control	1 568 ± 0.051	0.925 ± 0.020	-	-
IL FGF-2	2 132 ± 0 145*↑	1 076 ± 0.033*↑	36↑	16↑
IL antiFGF-2	1.004 ± 0 028**↓	0.847 ± 0.020*↓	36↓	8↓

@ 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

TABLE 1 3 The onset and progression of regenerate in wall lizards, *Hemidactylus flaviviridis*, subjected to *in loco* (IL) and intraperitoneal (IP) injection of FGF-2 and antiFGF-2 at BL stage

Treatment	No of Days		
	WH	BL (2 mm)	DF (12 mm)
IP Control	7 (6-7)#	10 (9-10)	17 (16-17)
IP FGF-2	7 (6-7)	10 (9-10)	15 (14-15)
IPantiFGF-2	7 (6-7)	10 (9-10)	19 (18-19)
IL Control	7 (6-7)	10 (9-10)	17 (16-17)
IL FGF-2	7 (6-7)	10 (9-10)	15 (14-15)
IL antiFGF-2	7 (6-7)	10 (9-10)	19 (18-19)

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
IP Control	1 475 ± 0.041@	0 912 ± 0.024	-	-
IP FGF-2	1 682 ± 0 082*↑	0 925 ± 0 020	14↑*	1↑
IPantiFGF-2	1 322 ± 0 039*↓	0 925 ± 0 020	10↓	1↑
IL Control	1 439 ± 0.058	0 925 ± 0 020	-	-
IL FGF-2	1 682 ± 0 082	0 938 ± 0 013	17↑	1↑
IL antiFGF-2	1 258 ± 0 045*↓	0 927 ± 0 029	13↓	0 24↑

@ 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

Figure 1 1 Rate of growth of regenerate from 2-12 mm injected before amputation (Fig 1 1a) , at WE stage (Fig 1 1b) and at BL stage (Fig 1 1c)

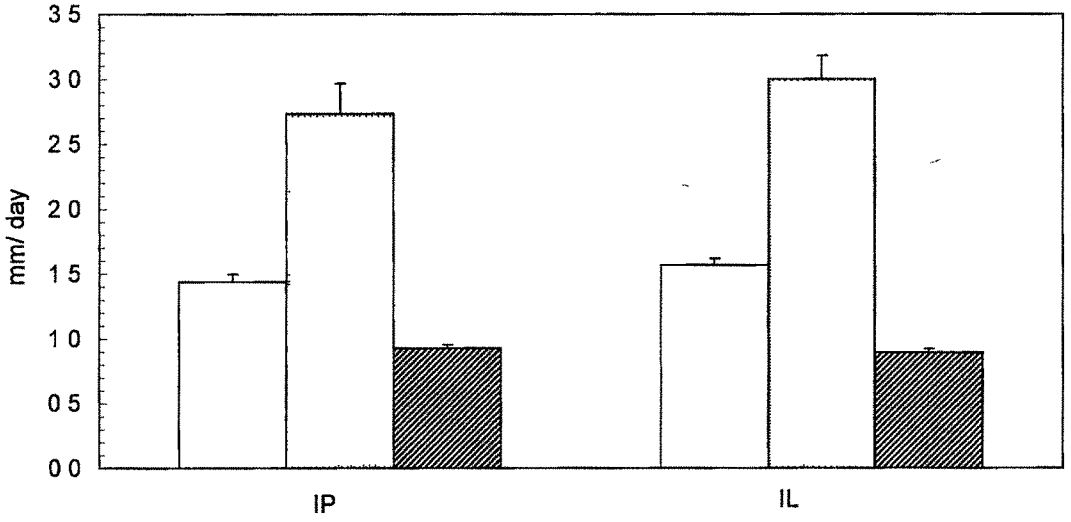

Figure 1 1 a

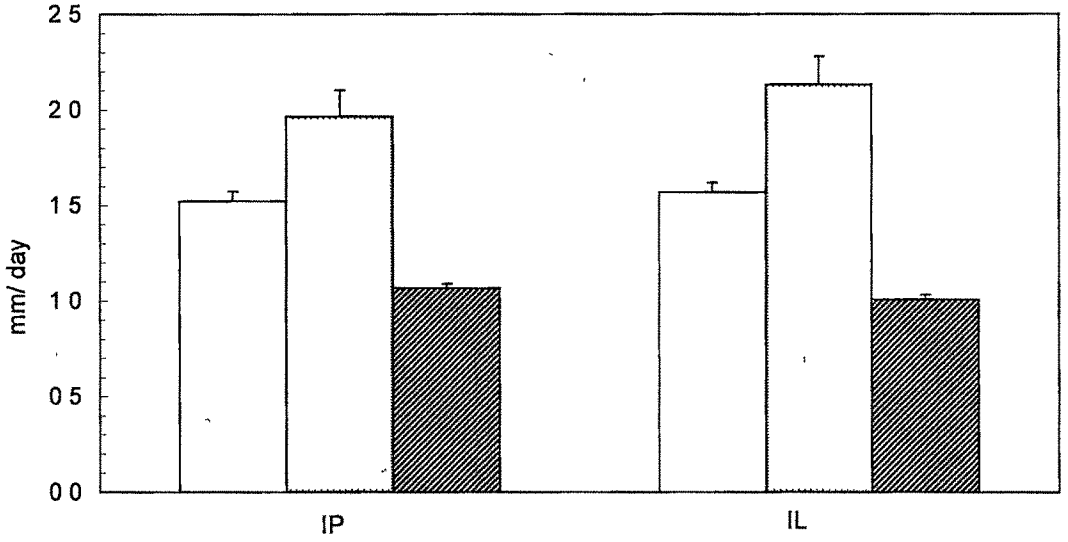

Figure 1 1 b

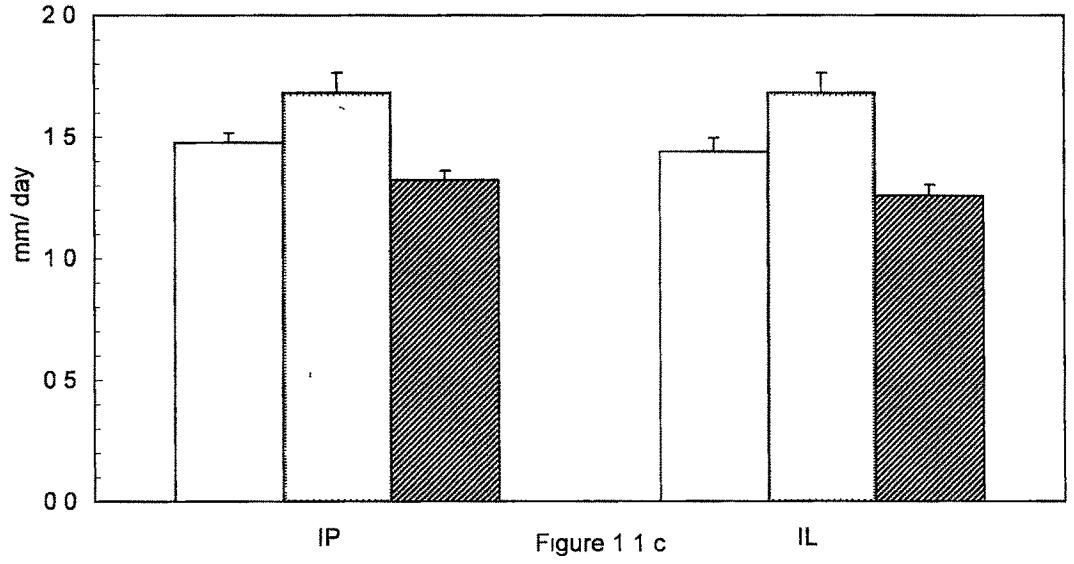

Figure 1 1 c

Figure 1 2 Rate of growth of regenerate from 12-24 mm injected before amputation (Fig 1 2a) , at WE stage (Fig 1 2b) and at BL stage (Fig 1 2c)

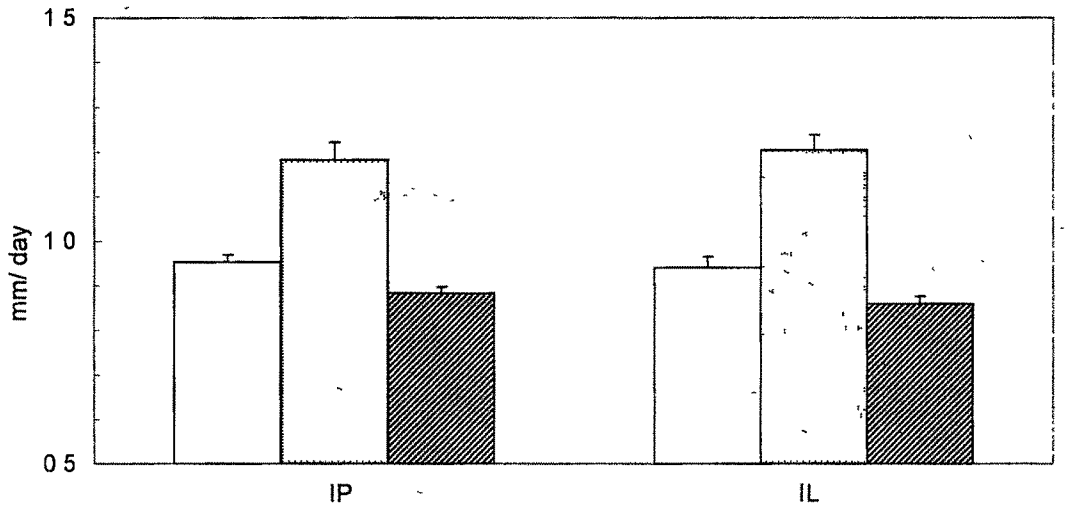

Figure 1 2 a

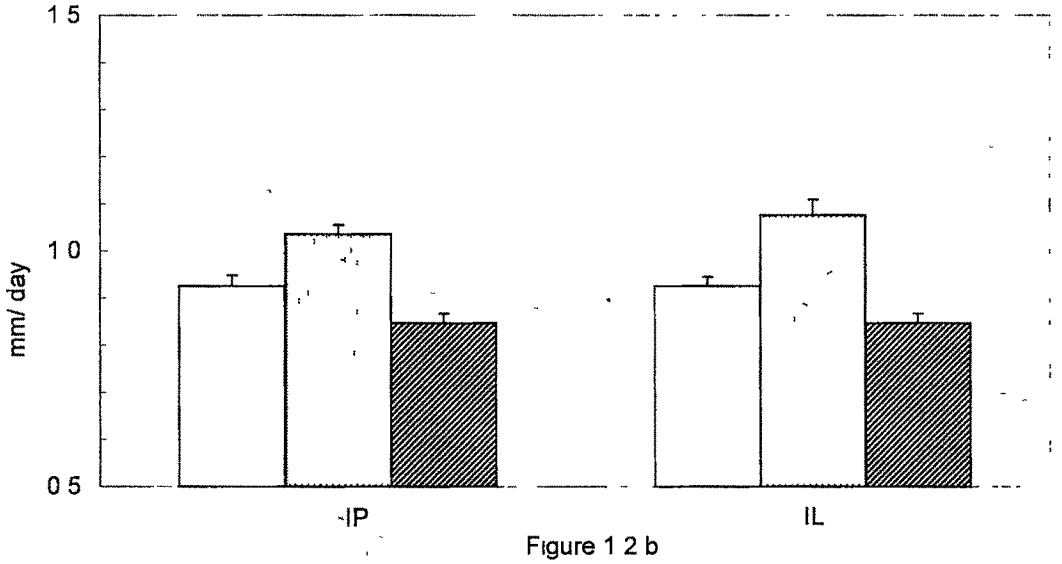

Figure 1 2 b

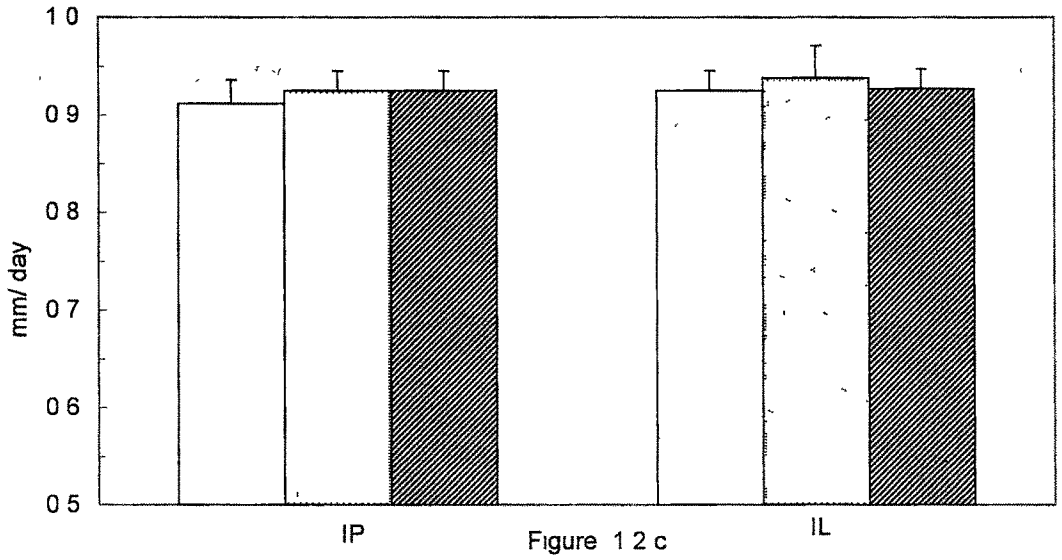

Figure 1 2 c

□ Control ▨ FGF-2 ▨ AntiFGF-2

Figure 1.1. Comparison of early blastema stage in control and experimental lizards

Control

FGF-2 treated

AntiFGF-2 treated