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EFFECT OF VITAMIN A ON THE REGENERATION OF HIND LIMBS IN THE TADPOLES OF RANA BREVICEPS

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ABSTRACT

The last three decades have witnessed an explosion of research on the effect of retinoid (vitamin A derivatives) on the pattern formation in the regenerating amphibian limbs and various other developing systems in vertebrates. The effects of exogenous vitamin A palmitate on limb development and regeneration in anuran tadpoles is very significant as it causes regeneration of complete or nearly complete limbs instead of only the missing distal part altering the proximo-distal pattern of regeneration. Several researchers have reported that vitamin A has an inhibitory effect on tail regeneration from the site of tail amputation but induces the development of ectopic limbs from tail blastema following treatment in the limb bud stage of anuran tadpoles. Such remarkable homeotic transformations have been demonstrated in several anuran species upon treatment with vitamin A. In the present study, investigators have made an attempt to assess the effect of vitamin A on limb regeneration after limb amputation in anuran tadpoles. The mortality was higher in group II than group I. The process of limb regeneration was faster in tadpoles of exposed group in comparison to tadpoles of control group. In group I tadpoles (exposed for 48 hrs) showed normal limb development while in tadpoles of group II (exposed for 96 hrs), majority developed hind limb with thigh, shank and ankle with digits but about 25% of tadpoles showed abnormality in the number of digits. The present findings clearly indicate that vitamin A has a positive effect on the blastema which formed at the site of amputation in replacing the structures appropriate to its proximo-distal position.

KEYWORDS: Regeneration, blastema, homeotic transformation, anuran, ectopic limbs.

INTRODUCTION

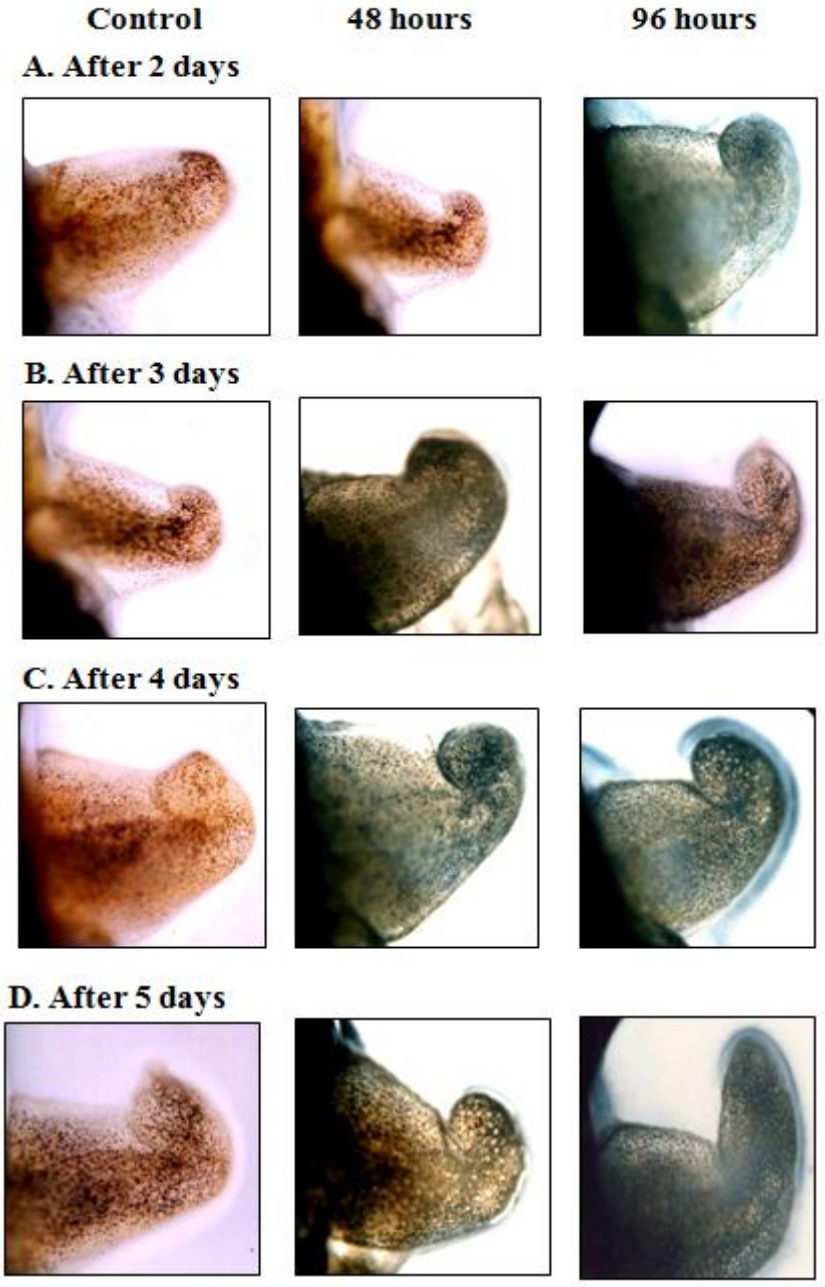
Since the early part of last century, study of vertebrate limb development has been a field of interest as limb provides a classical model for vertebrate pattern formation. It is well known that vitamin A causes severe teratogenesis in animals ever since their discovery in 1909. Niazi and Saxena (1968) investigated the effects of vitamin A (Retinol) on regeneration system (Tail and Limb) in anuran tadpoles for the first time and found that vitamin A inhibits normal tail regeneration in *Bufo andersonii*. Later it was found that anuran limb regeneration and development is also influenced by vitamin A and its derivatives. Retinol induces the duplication of regenerated limbs and at the same time suppresses the normal limb development (Niazi and Saxena, 1968). Later many other researchers worked on this and reported that tail regenerated by amputing at different level (Iten and Bryant, 1976) and also had modifying effect on regeneration of tails and limbs both in amphibians (Niazi and Saxena, 1979; Maden, 1983; Bryant and Gardiner, 1992). In generating amphibian limbs, retinols can lead to pattern duplication in the proximo-distal, antero-posterior and dorso-ventral axis (Niazi and Saxena, 1978; Maden, 1982, 1983; Ludolph et al., 1990). Retinoic acid appears to be critical for the initiation of limb bud outgrowth, since blocking the synthesis of retinoic acid with certain drugs prevents limb bud initiation (Stratford et al., 1996). Bryant and Gardiner (1992) and Scadding and Maden (1994) suggested that a gradient of retinoic acid along the antero-posterior axis might activate certain homeotic genes in particular cells and thereby has significant implications for pattern formation during limb development and regeneration. When the tails of tadpoles were amputed and the stumps exposed to retinoic acid during first days of regeneration, the tadpoles regenerated several legs from tail stumps. It appears that retinoic acid caused a homeotic transformation in the regenerating tail by re-specifying the tail tissues as a limb forming region (Muller et al., 1996). In our investigation we have found the regeneration of amputed hind limbs of anurans. We choose this species because of its prolific reaction to retinoid treatment as previously reported.

MATERIAL AND METHOD

Eggs of *Rana breviceps* were collected and processed in laboratory i.e. hatching of eggs, rearing of tadpoles and feeding. After hatching, emerging tadpoles were distributed into 3 plastic tubs of size 40x30 cm each containing 3 liters of de-chlorinated tap water and were allowed to acclimatize for 7 days. Tadpoles were fed daily with spinach leaves and maize powder. They were reared in 10:14 hours light : dark cycle and room temperature of 30 ± 2℃

throughout the duration of the experiment to mimic the natural condition. Tadpoles of hind limb bud stage were anaesthetized with 1/3000 MS222 and then hind limb buds were amputed in the middle. 30 tadpoles (control group) maintained in one tub in normal de-chlorinated water after amputation of limb. Remaining two tubs were experimental groups containing 30 limb bud amputed tadpoles each and exposed to 20 IU/mL vitamin A for 48 hrs (tub 2) and 96 hrs (tub 3) respectively. After 48 hrs and 96 hrs the equal number of tadpoles were transferred from the treated solution to the normal de-chlorinated water separately and observed daily till the limb is regenerated.

RESULT AND DISCUSSION



E. After 6 days

G. After 8 days

H. After 9 days

I. After 10 days

J. After 11 days

In previous years, most of the research has been done on homeotic transformation i.e. development of ectopic limbs from tail region. As mentioned, the Hox genes are likely to be

components of homeotic transformation of the tail into legs (Maden, 1993). The genes that play important roles in establishment of axes and limb regeneration are the Hox-4, Hox-3, and Hox-1 cluster (Simon and Tabin, 1993). The Hox-3 cluster is responsible specifically for the expression of normal and regenerating posterior appendages (hind limbs and tail). Simon and Tabin (1993), demonstrated the effect of retinoid on Hox-4, 5 gene expressions in newt blastema and therefore vitamin A treatment may influence the Hox gene level, leading to suppression of limb development (Mahapatra and Mohanty-Hejmadi, 1994).

Dose and exposure-time related suppressions of limb development by retinol palmitate have also been observed in the axolotl *Ambtsoma mexicanum* and larvae of *Xenopus laevis* by Scadding and Maden (1986a, 1986b). According to Bryant and Gardiner (1992), the application of retinoic acid to developing vertebrate limb buds causes all cells to be reprogrammed towards uniform positional values. Apparently, the lack of positional diversity in the progress zone leads to failure of growth and pattern formation and to the formation of reduced or truncated limbs. This reprogramming on vitamin A treatment leads to the suppression of limb development. The suppression of limb development is due to increase in the level of retinoic acid above the threshold level because of the additive effects of endogenous and exogenous retinoic acid (Mahapatra and Mohanty-Hejmadi, 1994). However, in present study effect of vitamin A delays the expression of hind limb regeneration rather than suppressing it. The process of re-differentiation and morphogenesis were delayed at shank level amputation and completely suppressed at thigh level amputation on treatment with 15 IU/ml of vitamin A solution (Saxena, 1973; Saxena and Niazi, 1977).

Vitamin A produces proximalizing effect on regeneration cells during their dedifferentiation and blastema formation but inhibits regeneration if given once re-differentiation begins. Shank-level blastema from treated tadpoles grafted into orbits of previously treated/untreated host tadpoles formed complete limbs. Proximalizing effect is proportionate to vitamin A concentration, duration of treatment, amputational level and stage of tadpoles. The present findings clearly indicated that vitamin A has a positive effect on the blastema which formed at the site of amputation in replacing the structures appropriate to its proximo-distal position. Proximalizing effect is correlated with natural ability in limbs to regenerate. Vitamin A improves regenerative ability and can induce it to some extent in non-regenerating limbs. Vitamin A excess retards limb development and produces stage dependent teratogenic effects. Findings of Niazi, 1996 supports the present result, they demonstrated that: (i) The

retinoid treatment does not inhibit but actually promotes the process of blastema formation; (ii) if the treatment continues beyond blastema stage it inhibits its re-differentiation and morphogenesis; and (iii) although the blastema of treated cases appears similar to that of controls it is very different from the latter in respect of its morphogenetic potency which may be even equal to that of the original limb bud capable of forming a complete limb. The effect of vitamin A also depends on the stage at which the treatment has been given. Retinoic acid produced proximalizing effects on regenerates when given up to late blastula stage but it became inhibitory when given with the beginning of re-differentiation (Niazi et al., 1985).

The conclusion made from the above discussion is that the inducing or inhibitory effect of vitamin A and its derivatives is depend on various factors- concentration of test solution, duration of exposure, stage of exposure and stage of amputation.

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