The Influence of the Genetic Background on the Interaction of Retinoic Acid with *Lx* Mutation of the Rat

(retinoic acid – genome interaction / mutant major gene *Lx* / polymorphism of modifying genes)

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Abstract. The teratogenic effect of RA was found to be significantly influenced both by genetic background and by the genotype of malformation mutation *Lx*. The presence of the *Lx* mutation and BN genetic background strongly increases the teratogenic effect of RA. On the contrary, the SHR genetic background was shown to protect foetuses from RA teratogenic affliction. Recombinant inbred strain BXH2 is endowed with a specific combination of BN and SHR genes, and following RA administration it exhibits the same embryolethal effect as the BN genetic background alone.

Without the *Lx* mutation there was no effect of RA on hind limbs in SHR/SHR or SHR/BN progeny whilst there was a significantly higher occurence of oligodactyly in SHR/BN on forelimbs as compared to SHR/SHR (92.2% vs 11.5%). In +/*Lx* progeny, forelimbs were significantly more afflicted with oligodactyly in SHR/BN +/*Lx* in comparison with both SHR/SHR and SHR/BXH2 foetuses, which indicates that BN modifiers responsible for oligodactyly were not passed to the BXH2 strain. On the contrary, hind limbs of SHR/BXH2, +/*Lx* progeny exhibited the highest affliction (62% of polydactyly and/or oligodactyly). In homozygous *Lx*/*Lx* progeny, polydactyly prevailed in forelimbs of SHR/BXH2 following RA administration, whilst in BN/BN progeny oligodactyly was the most frequent affliction. On the hind limbs, the highest reduction of toe number after RA treatment was connected with BN modifiers.

The polymorphism of normal morphogenetic factors was shown to be responsible not only for *Lx* phenotypic manifestation, but also for the variability in the response to RA teratogenic action.

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Abbreviations: CRABPs – cellular RA-binding proteins, ND – normodactyly/normodactylous, OD – oligodactyly/oligodactylous, PD – polydactyly/polydactylous, PLS – polydactyly-luxate syndrome, RA – retinoic acid, RAR(s) – retinoic acid receptor(s), RAREs – retinoic acid response elements, RI – recombinant inbred, SD – syndactyly.

Endogenous retinoic acid (RA) is necessary for morphogenetic processes. Its concentration is regulated by cellular RA-binding proteins (CRABPs) and its effect is mediated by ligand-activated transcription factors, nuclear receptors (retinoic acid receptors, RARs and RXRs). The receptors activated by RA binding form dimers, enter the nucleus, bind to specific DNA sequences (retinoic acid response elements, RAREs) of target genes, and control their transcription in this way (for review see Chambon, 1996; Underhill and Weston, 1998).

Early events in limb development are RA dependent, as it was confirmed recently (Vermot et al., 2000). A number of genes participating in various steps of limb morphogenesis are regulated by RA directly or indirectly (for review see Cohn and Bright, 1999).

Exogenous RA in high doses acts as a teratogen. In mice and rats, RA treatment in adequate time period causes predominantly reductional limb defects (phocomelia, micromelia, reduced number of digits). The affected limbs display bone deformities, suggesting RA interference with chondrogenesis and skeletogenesis. The resulting affliction depends strongly on the time of treatment, as limb susceptibility manifests cephalocaudal and proximodistal gradients (Kochhar, 1973; Kwasigroch and Kochhar, 1980; Kistler, 1981).

The mechanisms of RA teratogenic influence are not yet completely understood; however, it is clear that RA interferes with some basic morphogenetic events. Apoptosis, the crucial event in morphogenesis, was found to be induced by RA (Sulik and Dehart, 1988; Alles and Sulik, 1989; Jiang and Kochhar, 1992; Lee et al., 1994; Ahuja et al., 1997). The interference of RA with chondrogenesis was ascertained to be caused by inhibition of differentiation of chondroprogenitor cells (Lau et al., 1993; Jiang et al., 1995). However, it was demonstrated recently that RA may interfere with chondrogenesis by inhibition of proliferation in distal limb bud mesenchyme, decreasing the number of cells procurable for chondrogenesis (Tsuiki and Kishi, 1999a, b).

There is a number of indications that RA exerts its teratogenic effect via modulation of expression of various genes. The aim of our study was to demonstrate a model which possibly enables identification of genes responsible for limb development and for reaction to RA teratogenic influence. Our experiments utilize an inbred, congenic and recombinant inbred (RI) strain system of