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Review

Retinoic acid in developmental toxicology: Teratogen, morphogen and biomarker

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ABSTRACT

This review explores the usefulness retinoic acid (RA) related physiological factors as possible biomarkers of embryotoxicity. RA is involved in the morphogenesis of the early embryo as well as in the development and maturation of a wide variety of organ anlagen. The region-specific homeostasis of RA in the embryo is in many ways the driving force determining developmental cell proliferation versus differentiation. As a consequence, RA concentrations are carefully controlled in time and space in the developing embryo. RA deficiency and overdosing both result in characteristic patterns of malformations that may involve many different organ systems. The central role of RA in embryo development provides us with a set of sensitive biomarkers that may be employed in developmental toxicity testing. This includes the synthesizing and metabolizing enzymes of RA, but also a myriad of related morphogenetic factors and their genes, of which the expression may be affected by changes in RA balance. Several examples of embryotoxicants interfering with the homeostasis of RA and related parameters have been described. A preliminary adverse outcome pathway framework for RA mediated malformations has been published. Expansion of this framework and its application in developmental toxicity testing may allow the detection of a large variety of embryotoxicants with diverse modes of action. RA homeostasis therefore provides a promising set of molecular tools that may be employed in the advancement of mode of action driven animal-free developmental toxicity testing.

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Abbreviations: AER, apical ectodermal ridge; AOP, adverse outcome pathway; Bmp, bone morphogenic protein; Col, collagen; CRABP, cellular retinoic acid binding protein; Cyp, cytochrome p450; Dhrs, dehydrogenase/reductase; En, engrailed; Fgf, fibroblast growth factor; Gata, transcription factors binding to the DNA sequence "GATA"; Hox, homeobox; Irx, iroquois homeobox; Isl, leptin serum levels; Krox, zinc finger transcription factor; Meis, myeloid ecotropic viral integration homeobox; Nkx, NK transcription factor homeobox; Nppa, natriuretic peptide A; Olig, oligodendrocyte transcription factor; Otx, othrodenticle homeobox; Pax, paired homeobox; Pbx, pre-B-cell leukemia homeobox; Pitx, paired-like homeodomain transcription; RA, retinoic acid; Rax, retina and anterior neural fold homeobox; RAR, retinoic acid receptor; Rdh, retinol dehydrogenase; Rldh, retinal dehydrogenase; Shh, sonic hedgehog; Sox, sex determinig region Y box; Stra, stimulated by retinoic acid; Tbx, T-box transcription factor; TGF, transforming growth factor; Vegf, vascular endothelial growth factor; Wnt, wingless; ZPA, zone of polarizing activity.

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1. The physiology of retinoic acid

All-trans retinoic acid (RA) is the active metabolite of vitamin A (retinol) [1]. The compound has been widely studied for its essential physiological functions throughout the life cycle. In adult life, vitamin A is essential for various body functions, such as for vision [2], for the continuous renewal of skin epithelium [3], for spermatogenesis [4], and for the functioning of the immune system [5]. Prenatally, vitamin A is an important regulator of the generation of the vertebrate body plan, as will be detailed extensively below. The effects of RA are highly concentration dependent. Both deficiency and overload can lead to severe adverse health effects in the embryo/fetus, the child and the adult. Deficiency affects vision, and can result in anemia and weakened resistance to infection [6]. Overdosing may cause headaches, nausea, dizziness, fatigue, and may affect eyes, skin and skeleton [7]. Moreover, overdosing may cause severe malformations in the unborn child, as illustrated later in this review.

The intake from external sources of vitamin A is advised between relatively narrow boundaries. RA is generated by the body from provitamin A (beta-carotene) in the diet in vegetables and fruit, but also from retinyl esters in dietary liver, meat, fish and eggs. Various agencies advise intakes around 300–400 μg vitamin A per day for children, 700 and 900 μg for women and men, respectively, and a maximum intake of 3000 μg for all groups, to prevent adverse health effects of overdosing [8]. Especially liver may contain high levels of vitamin A, therefore it is advised that children and pregnant women limit liver consumption. Given its various physiological effects, RA and structurally related retinoids are being applied extensively as pharmaceuticals. They are used in the treatment of cancer [9], skin disorders [10] and multiple sclerosis [11], to name just a few.

2. Retinoic acid function in embryogenesis

The function of retinol as a vitamin was discovered early in the twentieth century [12]. Its crucial role in embryo development became known only much later, secondary to the causation of malformations by high doses of the same molecule. We now know that RA levels are carefully controlled by a complex balance among synthesizing and metabolizing enzymes (Fig. 1), regulated in time and space in the embryo. Rdh10 (retinol dehydrogenase) produces retinal from retinol, and Rldh2 (retinal dehydrogenase) is the main enzyme producing RA from retinal in the embryo. Dhrs3 (dehydrogenase/reductase) provides a feedback loop from retinal to retinol. The Cyp26 (cytochrome p450) enzyme family, with individual members distributed differently in the embryo, metabolizes RA [13]. RA has a paracrine function, traversing the cell bound to the Stra8 (Stimulated by retinoic acid) protein, and acts through two families of nuclear retinoid receptors [14,15]. These include RA receptors and retinoid X receptors, the latter binding the RA isomer 13-*cis*-RA [16]. These receptors function as heterodimers in multiple conformations, and show relative redundancy. It would seem that the local RA concentration determined by the balance between RA synthesis and metabolism, but not RA receptor availability, is rate-limiting for RA activity. Moreover, RA competes with a host of physiological signaling molecules supporting pattern formation in the vertebrate embryo.

The current extensive knowledge base on the subject allows RA and its physiological partners to be considered for use as biomarkers of developmental toxicity, which can e.g. be applied in non-animal test systems for chemical hazard and risk assessment. The following paragraphs will briefly review the roles of RA, discovered subsequently as teratogen, morphogen and a source of identifying biomarkers, with the aim to discuss implications for their potential use in mode of action based detection of developmental toxicants.

3. Teratogenicity of retinoids

Fetal malformations due to vitamin A deficiency were first described in the 1930's [17] and 1940's [18,19]. Wilson, Warkany and others reported on congenital defects of the skeleton, eye, urogenital tract, diaphragm, heart and aortic arches following maternal vitamin A deficiency. In 1954, Cohlan reported on the teratogenic effects of high doses of vitamin A during pregnancy in the rat [20]. These included exencephaly, eye malformations, cleft palate, shortening of the mandible and maxilla, spina bifida with meningocele and hydrocephalus. In 1979, Geelen produced an extensive overview of hypervitaminosis A related teratogenicity in animals [21]. After years of suspicion [22], the teratogenicity of vitamin A overdosing was established unequivocally also in man [23]. In addition to defects of the body axis and neural tube formation [24], vitamin A overdosing may affect facial structures [25], the thymus [26], the heart [27], the urogenital system [28], and melanocyte differentiation [29]. The wide variety of structural malformations induced by vitamin A deficiency or overdose suggest that the vitamin plays a major role in pattern formation in the embryo.

In the 1980's, synthetic retinoids, such as those used for treating skin conditions, were shown to have teratogenic properties as well [30–32]. The human teratogenicity of isotretinoin (13-*cis*-RA) was reported in an epidemiological study [33]. The pattern of malformations observed appears to be very similar to that of vitamin A overdosing. Typical malformations included craniofacial anomalies [34] and thymic hypoplasia [35], reminiscent of deficiencies of neural crest cell migration into these tissue anlagen. A host of structural analogous retinoids have been developed showing varying potencies regarding inhibition of chondrogenesis in an *in vitro* assay, which proved a useful model for predicting their relative teratogenic potency *in vivo* [36].