The role of retinoids in dioxin toxicity

Helen Håkansson, Institute of Environmental Medicine, Karolinska institutet, Stockholm, Sweden

Several lines of evidence suggest that retinoids are involved in the toxicity of dioxin like compounds. First, altered retinoid levels in the organism is a very sensitive response following dioxin exposure, that has been observed in all examined species both in experimental situations and in the environment. Secondly, many physiological processes, including growth, maintenance of epithelial tissues, and reproduction, are similarly affected by exposure to dioxins and deficiency of retinoids. Thirdly, TCDD and retinoic acid elicit a number of common responses both *in vivo* and *in vitro*. Finally, there are interactions between the retinoic acid and aryl hydrocarbon receptor signalling pathways.

Existing data support the notion that dioxins exert their toxicity through a common mechanism of action, and that binding to the *Ah-receptor* is the first step in this process. The subsequent steps are less well known, but it is believed that binding of the liganded receptor to DNA may affect a large number of biochemical processes, and that this could be responsible for the different types of effects that have been observed. Such a mechanism of action is compatible with the observed diverse pattern of toxic effects, the species-, strain- and tissue-specific responses, and the lack of toxicity in most cell systems *in vitro*.

Until recently, the effects of most concern for the general population were cancer, reproductive failure and immune deficiency. More recent data suggest that prenatal and perhaps also early postnatal exposure may induce severe developmental consequences at even lower exposure levels. Resulting behavioural and learning deficits as well as reproductive problems seem to persist also later in life.

Retinoids, or vitamin A, play a central and continuing role in many aspects of life. During embryogenesis and foetal development, retinoids have a central role in the patterning of cells along the anterior-posterior axis, and later in the anterior/posterior specification of the limb.[1)] In adult life, retinoids support normal growth and metabolism, vision, maintenance of numerous epithelial tissues, reproduction and overall survival.[2)] It has been known for a long time that there is a complex metabolic mashinery for the transport, storage and metabolism of retinoids, but it was not until 1987 that it became clear that effects of retinoids are mediated via nuclear hormone receptors.[3, 4)] Following that finding, intense research during the last decade has demonstrated an enormous complexity in retinoid-signalling at the molecular level. It is now known that effects of retinoids are mediated via two families of nuclear retinoic acid receptors, i.e. RARs and RXRs, each composed of three subtypes. Multiple forms of each of the receptor subtypes, receptor ligands (all-trans retinoic acid and 9-cis retinoic acid), response elements (RAREs), and intracellular binding proteins (CRBP and CRABP) add to the complexity. Furthermore, in order to bind to DNA, RARs and several other members of the nuclear receptor family require heterodimerization with RXR. Thus, by acting through RXRs, retinoids are critically involved in additional signalling pathways, including the thyroid hormones and vitamin D3. Furthermore, through their receptors, retinoids act in concert with many other signalling systems including growth factors, growth factor receptors, and oncogenes. So, beyond the classical action of retinol

and its metabolites in nutrition and vision, retinoids are fundamental regulators of gene expression in most vertebrate groups.

Already in the seventies it was observed that symptoms of persistent organohalogen exposure are similar to those in vitamin A deficient animals.[5, 6] The most prominent similarities include impaired growth, defective reproduction, developmental abnormalities, impaired immune function, and lesions of epithelial linings.[5-7] Experimental studies have since confirmed that dioxin like compounds are very potent modifiers of retinoid processing.

In contrast to the classical hormones, which are synthesized, processed, and/or released in response to physiological cues, vitamin A is a nutrient and most, if not all, organisms are dependent on its dietary intake, either as carotenoids from plants or as retinyl esters from animals. Dietary sources of vitamin A are absorbed and packaged as retinyl esters of chylomicrons for secretion into lymph (Figure 1A). Most of the newly absorbed chylomicron retinyl esters are cleared from plasma and are taken up by liver hepatocytes (Figure 1B). After hydrolysis and reesterification, retinyl esters are stored in lipid droplets in both hepatocytes and liver stellate cells. Stored retinyl esters are mobilised and delivered to target tissues as retinol bound to retinol-binding protein in serum. The signal triggering the release of stored retinyl esters is not known. In target tissues, retinol could be converted into storage forms, active metabolites, or catabolic products (Figure 1C). Lecitin:retinol acyl transferase (LRAT) and retinyl ester hydrolases (REH) are the two enzyme systems involved in retinoid storage processes. Retinoid-specific alcohol and aldehyde dehydrogenases catalyze the reversible conversion of retinol into retinal, and the irreversible conversion of retinal into retinoic acid, respectively. Both retinol and retinoic acid undergo reversible and irreversible interconversions to more polar metabolites, some of which are functional, and some which are inactive catabolic products. The cytochrome P450 system is involved in these reactions and intense research is ongoing to define retinoid-specific cytochrome P450 isozymes and reactions in various tissues. In addition to the retinoid-specific enzymes, there are several intracellular retinoid-binding proteins which are likely to be important in regulating the level of the ligand-binding metabolites, all-trans and 9-cis retinoic acid.

Under normal dietary conditions retinoids are present at relatively high concentrations and are therefore potentially available to all cells all the time. However, the efficient retinoid machinery ensure proper uptake, storage, transport and conversion into the right concentration of active metabolite both on a temporal and local scale.

Studies in the rat has demonstrated that TCDD severely modifies the overall metabolism of vitamin A.[10-12] Hepatic accumulation of dietary vitamin A is inhibited and elimination of endogenous stores of vitamin A is increased. These effects could be related to the severely decreased LRAT-activity in the hepatic stellate cells of TCDD-exposed animals.[13] Studies in the rat also demonstrate a marked increase in renal vitamin A levels and a more moderate increase in serum vitamin A levels of TCDD-exposed animals.[12] The renal vitamin A content is normally very low and increases only when liver vitamin A stores are nearing exhaustion. Serum vitamin A concentrations are normally tightly regulated within a narrow homeostatic range, which is thought to be determined by the release of retinol from the liver via regulation of vitamin A needs in extra hepatic tissues. Thus, even a moderate alteration in serum retinoid concentrations indicates a significant change in the overall dynamics of vitamin A metabolism. In the TCDD-exposed rat, the sharp increase in renal LRAT-activity is correlated, both on a time- and a dose-scale, to the increase in serum levels of retinoic acid.[14] This finding is particularly interesting since it has been suggested that the LRAT-gene contains a retinoic acid response element.[15] Together, these data suggest that TCDD affects the proposed sensitive feedback system between hepatic and renal vitamin A pools that is also linked to the pool of circulating vitamin A.

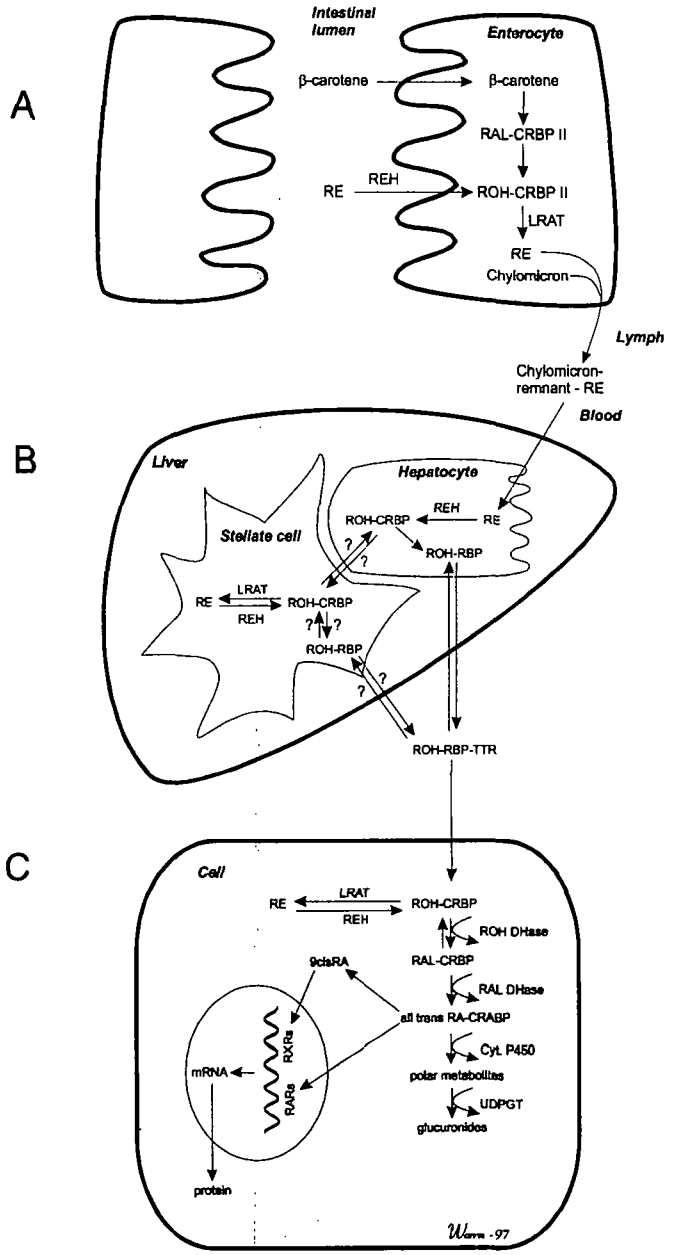

Figure 1. Intestinal absorption of dietary vitamin A (**A**), and hepatic (**B**) and cellular (**C**) metabolism of retinoids. Abbrevations: retinol (ROH), retinal (RAL), retinyl ester (RE), retinoic acid (RA), retinol binding protein (RBP), cellular retinol binding protein (CRBP), cellular retinoic acid binding protein (CRABP), transthyretin (TTR), lecithin:retinol acetyltransferase (LRAT), retinyl ester hydrolase (REH), dehydrogenase (DHase), retinoic acid receptor (RAR), retinoid X receptor (RXR). Modified from Blomhoff *et al*[8] and Giguère[9].

Different kinds of evidence support the notion that disturbed retinoid processing could be involved in dioxin toxicity. First, hepatic retinoid reduction is among the most sensitive responses following subchronic exposure to dioxins in the rat.[16] Furthermore, the decrease in hepatic retinoid levels is seen in all experimentally used rodent species.[17] If equally toxic doses are given to different rodent species, the most pronounced effect on liver vitamin A is observed in the most sensitive species. However, there are differences between species, with regard to the effect on renal vitamin A levels. Finally, there is a good correlation between toxic potency and ability to reduce hepatic vitamin A among PCDD, PCDF and PCB congeners.[18]

In conclusion, TCDD and related compounds alter critical steps in the retinoid metabolism. Possible mechanisms, that may act alone or in concert, behind the modified tissue levels of retinoids include esterification and hydrolyse activities as well as oxidation and glucuronidation reactions. The observed alterations in tissue retinoid levels and metabolism could result in critical changes in cell function due to alterations in the regulation and/or expression of retinoid responsive genes. Altered expression of retinoid responsive genes as a consequence of dioxin exposure could result in a number of the characteristic toxicity symptoms, and these might vary depending on cell type and developmental stage.

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