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Alterations in vitamin A metabolism by polyhalogenated aromatic hydrocarbons*)

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Summary: Adequate stores and adequate tissue levels of vitamin A are maintained by a balance of tissue demands and dietary intake of the vitamin and are modified by many factors, including xenobiotics. It is well established that exposure to polyhalogenated aromatic hydrocarbons (PHAH) decreases hepatic content of vitamin A. Recent findings indicate that hepatic depletion of vitamin A is accompanied by an increase in serum and renal vitamin A content and enhanced excretion of vitamin A metabolites in urine and feces. Examination of tissue retinoid profiles reveals that PHAH exposure causes the generation of increased amounts of polar retinoids. It is very likely that PHAH affect enzymes crucial for regulation of vitamin A storage as well as enhance activities of specific enzymes in vitamin A metabolic pathway.

Zusammenfassung: Ausreichende Vitamin-A-Speicher und -Gewebslevel werden durch ein Gleichgewicht von Nachfrage der Gewebe nach Vitamin A und der Vitamin-A-Aufnahme durch die Nahrung aufrechterhalten und durch viele Faktoren beeinflußt.

Es ist hinreichend bekannt, daß polyhalogenierte aromatische Kohlenwasserstoffe (PHAH) die Vitamin-A-Speicher der Leber erniedrigen. Neuere Untersuchungen deuten darauf hin, daß die Erniedrigung der Vitamin-A-Leberspeicher von einem Anstieg des Vitamin-A-Gehaltes in Serum und Niere sowie von einer erhöhten Abgabe von Vitamin-A-Metaboliten in Urin und Fäzes begleitet sind. Die Untersuchung der Vitamin-A-Verteilung in verschiedenen Geweben zeigte, daß die Zufuhr von PHAH zu einem verstärkten Auftreten polarer Vitamin-A-Metaboliten führt. Es ist wahrscheinlich, daß die PHAH Enzyme beeinflussen, die entscheidend für die Regulation von Vitamin-A-Speichern sowie der Aktivität von für den Vitamin-A-Stoffwechsel wichtigen Enzymen sind.

Schlüsselwörter: Vitamin A-Status; polyhalogenierte aromatische Kohlenwasserstoffe; Metabolismus; polare Retinoide

Key words: vitamin A status; polyhalogenated aromatic hydrocarbons; metabolism; polar retinoids

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Introduction

Polyhalogenated aromatic hydrocarbons (PHAH) are widely used in industrialized countries. Many of these PHAH are either toxic or generate toxic derivatives during manufacturing operations or pyrolysis. Among the most toxic is 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD); however, the enormous production and global industrial use of polychlorinated biphenyls (PCBs) (world production 91 million kg in 1970), place them among the major environmental pollutants (15). Concern over xenobiotics and their deleterious effects on health is worldwide because of constant public exposure to these chemicals (15). A classic example is the accidental introduction of polybrominated biphenyls (PBBs) into the food chain of Michigan residents in 1973 (14, 42). Other documented sources of exposure include the atmospheric transport of PCBs, chlorinated dibenzofurans and dioxins (11), and the recurring accidental public exposure to PHAH (26).

The sustained pleiotropic responses that result from PHAH exposure include increased acitivites of mixed function oxidases and of uridine diphosphate glucuronosyl transferases (12, 17, 28, 29, 31). These effects are thought to be linked to the binding of PHAH to specific cytosolic receptors and to the interaction of the receptor-PHAH complex with the nucleus (31). The clinical syndrome associated with PHAH exposure is well characterized and includes skin lesions, weight loss, nausea, and hepatic, intestinal and nervous disorders (1, 2, 24, 25, 30). Some of the lesions resemble those of vitamin A deficiency (20, 21, 25, 37, 38, 40) and have led to inquiries into the effect of PHAH on vitamin A nutritional status.

Vitamin A is stored primarily in the liver. Adequate stores of vitamin A in the liver and an adequate supply of vitamin A to tissues is maintained by a balance between tissue demands and dietary intake of the vitamin; the balance may be modified by many factors, including xenobiotics. Administration of certain xenobiotics results in decreased hepatic content of vitamin A. Agents known to cause this effect include polychlorinated biphenyls (PCBs) (18, 21, 23, 32–39, 41) and polybrominated biphenyls (PBBs) (1, 6). Administration of vitamin A concurrently with xenobiotics partially prevents the symptoms (20). Clearly, numerous studies describe the abnormally low hepatic vitamin A levels associated with PHAH toxicosis, however, little is known about the mechanism(s) underlying this interrelationship.

We have investigated the effect of PHAH on vitamin A metabolism using 3-H-labeled retinoids, and found that a single non-lethal dose of 3,3′, 4,4′, 5,5′-hexabromobiphenyl (HBB) causes a two-fold enhancement in the metabolic output of degraded vitamin A in urine and feces of rats (8). Similar effects were observed using TCDD (18, 19). The potential effect of HBB on absorption of vitamin A was excluded by the experimental design in the above studies (8) and by the finding of Narbonne that polychlorinated biphenyls do not affect vitamin A absorption (27). Subsequent studies with chronic doses of HBB or 3,4,5,3′,4′,5′-hexachlorobiphenyl (HCB) have confirmed the above findings (5, 22). The chronic administration of HBB caused a severe (20-fold) decrease in hepatic retinol and retinyl esters but caused a 6–7-fold increase of retinol and retinyl esters in

the kidneys (22) and a 2-fold increase in serum retinol (23). These alterations are also observed with TCDD and HCB (5, 18, 33).

The available evidence clearly indicates that PHAH significantly alter vitamin A metabolism, perhaps by an effect on the activities of enzymes involved in regulation of vitamin A storage. An examination of hepatic acyl-CoA-retinol acyltransferase (ARAT) and retinyl palmitate hydrolase (RPH) revealed a 50 % and 63 % reduction, respectively, in the activities of these enzymes in HBB treated rats (22). Studies with administered 3-H-labeled-retinyl acetate further confirmed an increased (2-fold) urinary and fecal excretion of radioactive vitamin A degradation products from newly administered vitamin A in HBB intoxicated animals (22), suggesting an enhancement by HBB of vitamin A catabolism.

To obtain additional insights into the PHAH-vitamin A relationship, we examined the alterations in liver, serum, and extrahepatic processing of a physiological form of vitamin A following an exposure to HCB (5). Vitamin A was administered intravenously as the 3-H-retinol-retinol binding protein (RBP)-transthyretin (TTR) complex. The findings from these studies demonstrate that as a result of HCB treatment, there was either a decreased hepatic parenchymal cell uptake of recycled retinol-RBP or an enhanced transfer of retinol to serum. The relative amount of hepatic radioactivity associated with polar retinoid metabolites was increased in these animals, suggesting increased degradation of vitamin A; this was reflected in an enhanced excretion of radiolabel in the urine and feces of HCB treated rats (5).

Vitamin A metabolite profiles in tissues of rats exposed to HBB

In order to gain further understanding of the effect of PHAH on specific vitamin A metabolic pathways, we examined the retinoid metabolite profiles in tissues of rats treated with a single non-anorectic dose of HBB and compared them to untreated controls. The results of these studies are reported here. The experimental model used was the rat with 3-H-labeled vitamin A equilibrated body vitamin A pools, as described in our earlier paper (8).

Weanling male Sprague-Dawley rats were depleted of vitamin A to a weight plateau stage of growth and then repleted with 20 μg of 3-H-labeled retinyl acetate/day for 14 days, at which time the body vitamin A is known to be equilibrated (8), as evidenced by the excretion of a constant amount of radiolabel in urine and feces. Twenty-four hours after the last dose of retinyl acetate the animals were weight matched and one group of them was given orally a single non-anorectic dose of HBB (2 mg/kg body weight); control group received the vehicle (corn oil). The animals were sacrificed in pairs from each group at different times thereafter. Their tissues were rapidly removed, rinsed with saline, weighed, homogenized in presence of antioxidants and lyophilized. The dried tissues were extracted with methanol and hexane, containing antioxidants and internal standards, as described earlier (9, 10). Retinoids were separated and characterized by co-chromatography with authentic retinoids using high pressure liquid chromatography (HPLC) and quantitated by UV and the determination of radioactivity (10). The following authentic retinoids were

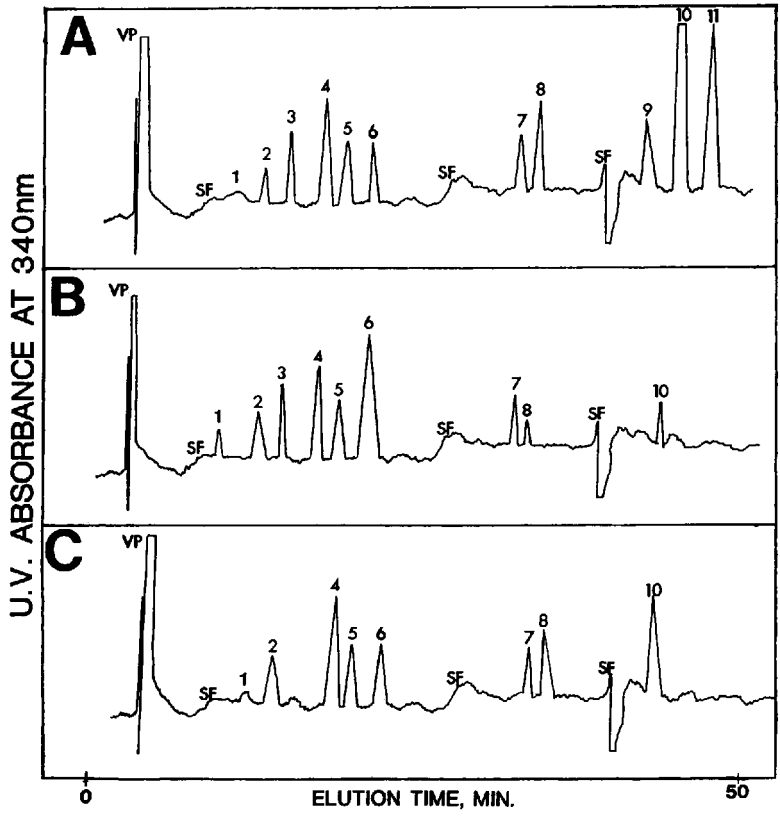

Fig. 1. Separation of vitamin a metabolites by HPLC. Representative HPLC profiles of retinoids in liver (A), kidney (B), and small intestinal mucosa (C) of vitamin A adequate rats. HPLC was on a C-18 reversed phase column. Step gradient elution was with methanol/water (75:25), methanol/water (80:20), methanol/-water (88:12) and methanol/chloroform (83:17). Elution positions of authentic retinoids are indicated, as follows: (1) 4-oxoretinoic acid, (2) retinoyl glucuronide, (3) 13-demethylretinoic acid, (4) 13-cis-retinoic acid, (5) all-trans-retinoic acid, (6) 13-cis-ethylretinamide, (7) 13-cis-retinol, (8) all-trans-retinol, (9) retinyl linoleate, (10) all-trans-retinyl palmitate, (11) all-trans-retinyl stearate; VP, very polar metabolites; SF, solvent front. Detection was by HPLC-UV and radioactivity.

gifts from Hoffmann-La Roche, Nutley, NJ: all-trans-retinol, all-trans-retinyl acetate, all-trans-retinyl palmitate, all-trans- and 13-cis-retinoic acids, 4-oxo-retinoic acid, all-trans-5,6-epoxyretinoic acid and 3-H-retinyl acetate; 13-cis-ethyl retinamide was a gift from the National Cancer Institute; 13-demethylretinoic acid was a gift from Dr. W. Lambert, and HBB was a gift from Dr. S. Aust. Retinoyl glucuronide was generated in vivo as described previously (43).

Figure 1 illustrates representative HPLC profiles of retinoids in liver, kidney and small intestinal mucosa from normal vitamin A adequate rats. Although the relative amounts of the individual vitamin A compounds

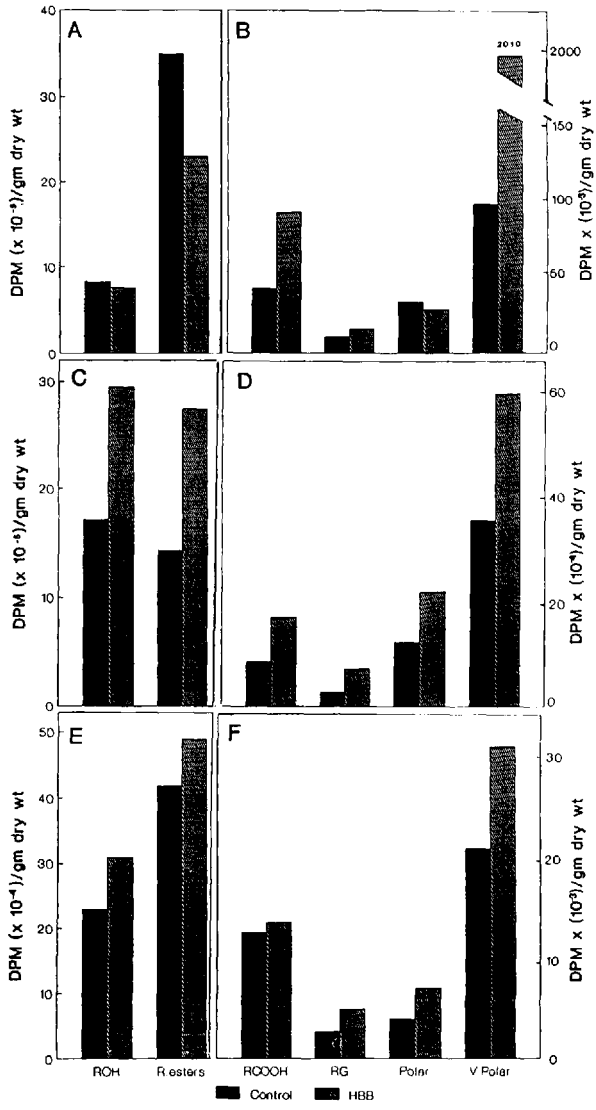

Fig. 2. Effect of HBB exposure on the amount and distribution of vitamin A metabolites in tissues. Retinoids were quantitated by HPLC-UV and radioactivity in liver (A, B), kidneys (C, D), and small intestinal mucosa (E, F) in control and HBB-treated rats 48 h after the administration of HBB. Solid bars, controls; hatched bars, HBB-treated; n = 2. Duplicate analyses were performed for each tissue. Values represent means for two animals; $p \leq 0.15$, except for very polar $p \leq 0.005$. ROH = retinol; R ester = retinyl ester; RCOOH = retinoic acid; RG = retinoyl glucuronide; V polar = very polar.

varied among the different tissues, the same kinds of retinoids were present in all tissues examined; this was also the case with tissues from HBB treated rats (not shown).

The effect of HBB on the amount and distribution of vitamin A metabolites in the different tissues is shown in Fig. 2. Two days after a single non-anorectic dose of HBB there was a significant (30 %) decrease in liver retinyl ester pools (Fig. 2A), while the compartments containing polar retinoids, such as retinoic acid and polar metabolites, were greatly enlarged (Fig. 2B). The kidney responded to HBB treatment by an increased accumulation of retinol and retinyl esters (Fig. 2C), in general agreement with earlier observations (5, 18, 22, 23). In addition, there were increased amounts of retinoic acid and more polar metabolites in the kidneys of HBB treated rats as compared to controls (Fig. 2D).

The effect of HBB on vitamin A metabolism in a target tissue, the small intestinal epithelium, is illustrated in Fig. 2E and F. Since these animals had not received vitamin A in the diet for two days, the metabolic activities associated with absorption and delivery of vitamin A to circulation are not expected to be evident. The retinoids in this tissue are endogenous vitamin A compounds, similar to those reported earlier (9) and are most likely associated with the epithelial maintenance aspects of vitamin A function. The relative amounts of small intestinal mucosal retinoids were shifted towards polar metabolites by HBB treatment, resulting in an increased accumulation of retinoic acid, retinoyl glucuronide and very polar retinoid metabolites (Fig. 2F). Changes in vitamin A metabolite patterns in tissues and an enhanced urinary and fecal excretion of radioactive vitamin A metabolites were evident as early as 12 and 14 h after HBB treatment (not shown).

Discussion

Our experiments with HBB exposed rats confirm a general trend: a shift of vitamin A metabolism towards the more polar forms of vitamin A, such as retinoic acid, its oxidation and conjugation products and additionally, more polar metabolites. We hypothesize that this pattern is indicative of an enhancement by HBB of vitamin A catabolic pathways, most likely involving cytochrome P-450 monooxygenase systems as well as uridine diphosphoglucuronosyl transferase (UDPGT), families of enzymes known to be induced by PHAH (17, 28, 29).

The possibility of TCDD-induced aryl hydrocarbon hydroxylase (AHH) being responsible for the low hepatic vitamin A levels has been explored by others (6, 39). Brouwer et al. (6) from studies on 3,4,3',4'-tetrachlorobiphenyl (TCB) toxicity in C57BL/Rij and DBA/2 mice, concluded that AHH and related enzymes are not directly involved in the reduction of liver retinoid concentration in these strains of mice. However, these conclusions are restricted to the particular strains of mice used and the effects of TCB, which are different from TCDD (6, 7, 32).

We have tested this hypothesis using 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), since it is a PHAH model compound used for mechanistic studies (31). The administration of a single non-anorectic dose (10 µg/kg body weight) of TCDD caused a 25 % decrease in hepatic vitamin A, a 10-fold increase in renal vitamin A and 2.5-fold increase in serum retinol. Our preliminary work with hepatic and renal microsomes in cytochrome P-450

mediated reactions indicates an enhancement by TCDD of the oxidation of retinoic acid to 4-hydroxy- and 4-oxo-retinoic acids in liver and kidney (3).

Thunberg et al (36, 39) could not demonstrate a correlation between TCDD induced UDPGT activity towards p-nitrophenol (PNP) as substrate and the reduction of hepatic vitamin A stores in the rat. However, the isozyme involved in retinoic acid glucuronidation is not likely to be the same isozyme that catalyzes PNP glucuronidation. This may explain the observation that in the Gunn rat TCDD induces vitamin A depletion even though this rat lacks the PNP-specific UDPGT (36).

In our studies with a single dose of TCDD, measurements of liver and kidney microsomal UDPGT demonstrated that the enzyme activities toward all-trans-retinoic acid were increased 3.7- and 2.7-fold, respectively, 10 days following exposure to TCDD (4). It is thus very likely that TCDD induces a separate isozyme of UDPGT family, involved in retinoic acid glucuronidation.

Normally, retinoyl glucuronide represents only about 10 % of the biliary retinoids (16, 43, 44); however an increased UDPGT activity as the result of TCDD administration may accelerate the loss of vitamin A due to enhanced glucuronidation of retinoic acid and subsequent excretion of the conjugate in bile. Our preliminary work demonstrates an increased amount of retinoyl glucuronide in the bile after PHAH treatment (unpublished).

Our studies strongly suggest that the observed shift towards polar vitamin A metabolites and the enhancement of vitamin A metabolism by retinoid specific cytochrome P-450 and UDPGT enzyme systems may be significant factors in the hepatic depletion of vitamin A and the increased excretion of vitamin A degradation products following PHAH exposure. Other mechanisms contributing to the altered vitamin A homeostasis include the observed decrease in the activities of hepatic acylCoA-retinol acyltransferase and retinol palmitate hydrolase (RPH) in chronically HBB treated rats (22). These enzymes are involved in the dynamic balance between esterification of retinol for storage purposes and hydrolysis of the retinyl esters to provide free retinol for binding to RBP and the subsequent release into circulation. Hepatic RPH activity was also found to be depressed by TCB (32), but this tetrachlorinated biphenyl has effects on vitamin A homeostasis that differ from those of TCDD, HBB and the other hexahalogenated congeners.

Conclusion

We conclude that specific PHAH enhanced enzyme activities cause an accelerated metabolism of vitamin A compounds at vitamin A storage sites and at vitamin A target sites, these events providing a signal for hepatic mobilization of vitamin A, reflected in an increased serum retinol concentration and a depletion of liver vitamin A stores. Also affected are the enzymes involved in regulation of vitamin A storage in liver and kidney. Although the specific mechanistic events remain to be elucidated, it is very likely that altered activities of enzymes associated with vitamin A homeostasis account in great part for the vitamin A depleted nutritional

status of the PHAH exposed animal. Humans continually exposed to environmental pollutants and accumulating critical levels of polyhalogenated aromatic hydrocarbons will be at a risk for marginal vitamin A status, a condition that may predispose these populations to a higher incidence of infectious diseases and cancer.

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