

Faeces

Significant amounts of porphyrins are excreted in faeces (mostly all of the protoporphyrin and 70% of coproporphyrin; Table 1). They may represent pigments that have reached the intestinal tract with the bile, except dicarboxylic porphyrins, which are mainly of dietary origin and may be derived from haem proteins of ingested food or intestinal haemorrhages; they may also be formed by intestinal microorganisms. Because of this, faecal porphyrins should be measured only if the patient's food has been devoid of bleeding or ingestion of meat in the past 3 days.

The mechanism by which protoporphyrin is excreted into bile has been studied mostly after description(s) of fatal liver disease in erythropoietic protoporphyria (see Chapter 16.5). Among haem-forming tissues, the bone marrow is the major source of protoporphyrin, a very poorly water-soluble compound: in the plasma, over 90% of protoporphyrin is bound to albumin with some bound to haemopexin. Hepatic uptake may occur through a process similar to that for other organic anions (such as bilirubin) that are bound to albumin. In the isolated, *in situ*-perfused rat liver, the overall disappearance of protoporphyrin follows first-order kinetics. Within the hepatocyte, protoporphyrin is associated with several proteins, among them one of the Z class of liver cytosolic proteins [10]. The rate-limiting step for the overall transport of protoporphyrin from plasma to bile appeared to be canalicular secretion, as less than 5% of the protoporphyrin extracted by liver was secreted into bile. This secretion should be mediated by the ABCG2/BCRP transporter, a member of the ATP-binding cassette (ABC) family [11]. The basal rate of bile secretion of porphyrins has been studied in healthy humans [12]: the flow of protoporphyrin is slightly higher than the flow of coproporphyrin; the flow of uroporphyrin is the lowest. Hepatic conjugation with glucuronic acid does not occur for protoporphyrin and coproporphyrin. Approximately 85% of hepatic protoporphyrin remains metabolically unaltered before being eliminated by bile secretion; 15% of protoporphyrin extracted by the liver may be converted to bilirubin, with non-haemoglobin haem species as intermediaries, and is also excreted in bile.

Hepatic infusion of micelle-forming bile acids facilitates canalicular protoporphyrin secretion [13]: the micelle-forming taurocholate increased biliary protoporphyrin concentration (by more than six times) and secretion (by more than 12 times) considerably more than dehydrocholate (a non-micelle-forming bile acid). Some bile acids (taurocholate and glycocholate) increase protoporphyrin metabolism 1.7- to 2.7-fold over control values. There are a number of direct and indirect ways in which bile acids might alter the metabolism of protoporphyrin, including either the stimulation of the activity of enzymes such as ferrochelatase and HO or the solubilization of protoporphyrin [14]. Before its final faecal excretion, a significant proportion of protoporphyrin is reabsorbed in the intestine and may circulate through the enterohepatic system [13]. However, it is not yet known how much intestinal microorganisms or food contribute to the total fecal porphyrin excretion.

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2.3.11 Vitamins and the liver (A and D)

Masataka Okuno, Rie Matsushima-Nishiwaki and Soichi Kojima

Summary

The metabolism, pathological relevance and therapeutic applications of retinoids (vitamin A and its derivatives) and vitamin D are reviewed in human hepatic disorders. Both vitamins have profound effects on cell activities, including cell growth, differentiation and apoptosis. Retinoids consist of several molecular species, including retinoic acid (RA), retinol and retinylesters. Dietary retinoids are packed in nascent chylomicrons that are taken up by the liver, in which hepatic stellate cells (HSC) store

the majority of body retinoids as retinylesters. The liver supplies retinoids as retinol to meet the requirements of peripheral tissues through binding to its specific binding protein, retinol-binding protein. RA is biosynthesized from retinol in target cells and exerts its biological functions through two distinct nuclear receptors, RA receptor (RAR) and retinoid X receptor (RXR). An isomer of RA, 9,13-di-*cis*-RA, is involved in the development of liver fibrosis. Retinoids are prime candidates for cancer chemoprevention by reversing the carcinogenic processes through regulating cell proliferation and differentiation. Acyclic retinoid, a synthetic retinoid, successfully suppresses the development of hepatocellular carcinoma (HCC) in cirrhotic patients. Eradication of malignant clones by inducing apoptosis ('clonal deletion') is suggested as a mechanism of the chemopreventive effect. Photolysis of provitamin D3 to previtamin D3 and its thermal isomerization to vitamin D3 take place in the skin. Vitamin D3 is metabolized in the liver to 25-hydroxyvitamin D3, and then in the kidney to its biologically active form, 1,25-dihydroxyvitamin D3 [1,25(OH)₂D3]. As the liver plays a major role in the formation of 1,25(OH)₂D3, osteodystrophy often occurs in patients with chronic liver diseases. 1,25(OH)₂D3 binds to its nuclear receptor, vitamin D receptor (VDR), which forms a heterodimer with RXR and regulates downstream genes mainly related to calcium metabolism. VDR is expressed in non-parenchymal liver cells but not in hepatocytes. Vitamin D also has immunomodulatory effects, and polymorphisms of VDR are implicated in some autoimmune diseases, including autoimmune hepatitis and primary biliary cirrhosis. Use of vitamin D for the treatment of HCC is also suggested.

Metabolism and function of retinoids

Vitamin A and its analogues, collectively termed retinoids, have profound effects on cell activities, including cell growth, differentiation, apoptosis, reproduction and morphogenesis [1]. Natural retinoids consist of retinoic acid (RA, an active metabolite that binds to its nuclear receptors), retinol (a transport form in the plasma) and retinylesters (storage forms in the tissues) (Fig. 1) [2]. All natural retinoids originate in the diet as either retinylesters or provitamin A carotenoids. Dietary retinylesters and carotenoids are subjected to a series of metabolic conversions to form retinol in the intestinal mucosa. Retinol is absorbed with other dietary lipids, esterified to retinylesters and packed in nascent chylomicrons. The chylomicrons are secreted into the lymphatic system and then enter into the circulation. Most chylomicron retinylesters are taken up by the liver, the major storage site of body retinoids. The liver stores retinoids in the form of retinylesters and supplies retinoids as retinol after hydrolysis of the esters to meet the requirements of peripheral tissues.

There are specific binding proteins for retinol in the plasma and cells, retinol-binding protein (RBP) and cellular retinol-binding protein (CRBP) respectively [3]. RBP is synthesized in hepatic parenchymal cells (hepatocytes) and secreted into the plasma after binding to retinol. Although RBP has a small molecular weight of 21 kDa, as RBP usually binds to

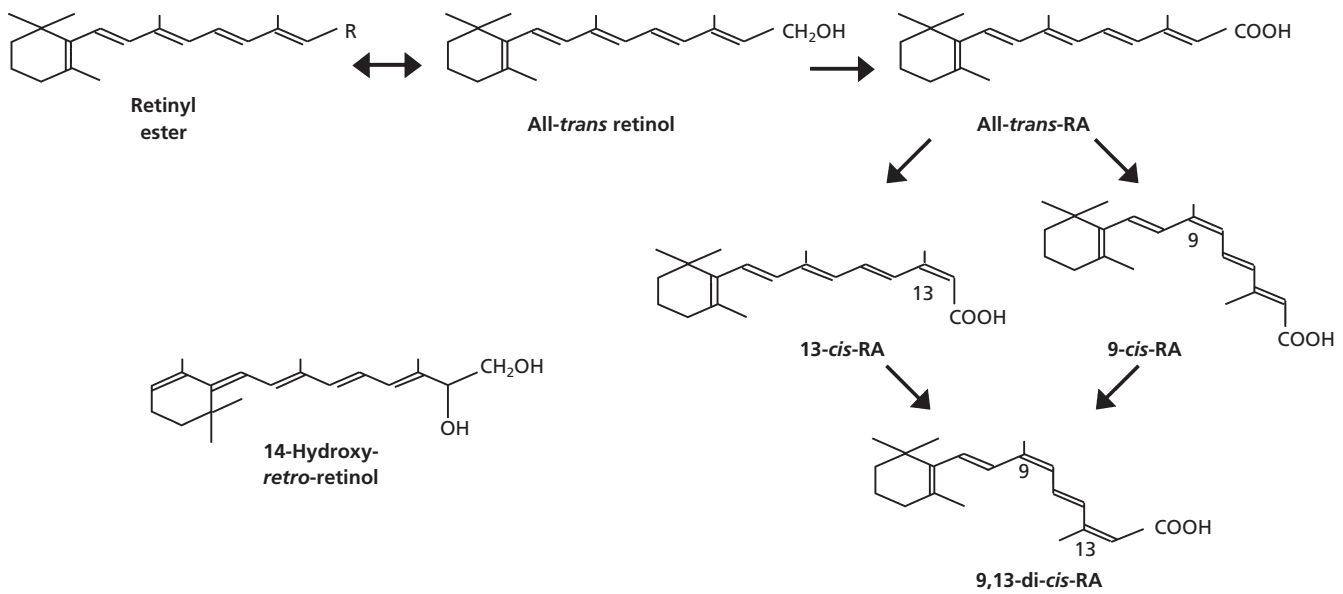
transthyretin (TTR) and forms a RBP-retinol-TTR complex, it can avoid renal glomerular filtration. After delivery of retinol to target tissues, apo-RBP loses the binding to TTR, is rapidly secreted through the glomerulus and is reabsorbed by the renal proximal tubules where RBP is degraded to its constituent amino acids. Because RBP has a short half-life of approximately 12 h, it can be used as a sensitive diagnostic tool, particularly in diseases of the liver and kidney and in malnutrition. Plasma levels of RBP as well as retinol and TTR decrease in patients with acute and chronic liver diseases. RBP and TTR levels are found to correlate highly with the reduction of traditional markers such as prothrombin and albumin, which indicate the severity of liver damage. Two types of hepatic cells are known to participate in retinoid storage and metabolism, hepatocytes and HSCs [4]. HSCs play central roles in the storage of retinoids in the liver (more than 75% of hepatic retinoid), although HSCs account for only around 5% of the total liver population. Hepatocytes take up retinoids from chylomicrons and secrete them as retinol after binding to RBP, which is also synthesized by hepatocytes. The mechanism by which retinoids are transferred between hepatocytes and HSCs remains unsolved, although some pathways are postulated. It is believed that HSCs take up retinol from a retinol-RBP complex in the intercellular space that is secreted by hepatocytes. However, some suggest that intracellular retinol bound to CRBP is transferred directly between hepatocytes and HSCs by means of membrane contacts, including desmosomes and other direct intercellular channels. HSCs esterify retinol into retinylesters (mostly retinyl palmitate) and store the esters in the cytoplasm.

A small portion of dietary retinoids is converted to RA, a bioactive hormone, absorbed through the portal vein and present in the plasma bound to albumin. The majority of RA is biosynthesized in the cells of peripheral tissues and modulates the expression of various target genes [2]. Biosynthesis of RA is mediated by alcohol dehydrogenase and aldehyde dehydrogenase, which convert retinol to retinal and retinal to RA respectively. CRBP transfers retinol to these enzymes and helps in the formation of RA. Excess RA binds to cellular RA-binding protein (CRABP) in the cytoplasm and is further oxidized to an inactive metabolite by cytochrome P450 (CYP). For example, CYPRA1 is a novel P450 that inactivates RA by hydroxylation. CRABP acts as a buffering system to control the intracellular concentration of RA, thereby provoking or inhibiting RA actions. Some enzymes and proteins that modulate biosynthesis or degradation of RA are under the control of RA itself. For example, the expression of alcohol dehydrogenase, CRBP and CRABP is upregulated by RA. Thus, the intracellular concentration of RA is under the positive and negative feedback control of RA itself. These regulatory systems can impose a type of check-and-balance mechanism on RA biosynthesis and thus its function.

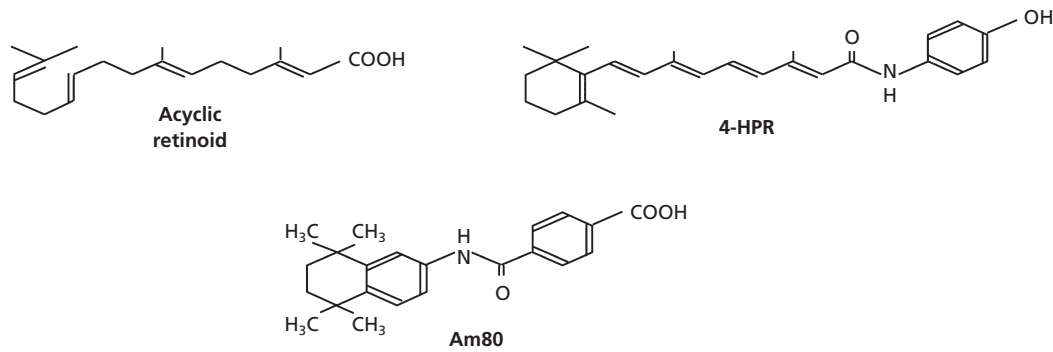
Retinoid receptors

RAs exert their biological functions through two distinct nuclear receptors, RAR and RXR [5] (Fig. 2a). Both RAR and RXR

Natural retinoid



Synthetic retinoid



Vitamin D

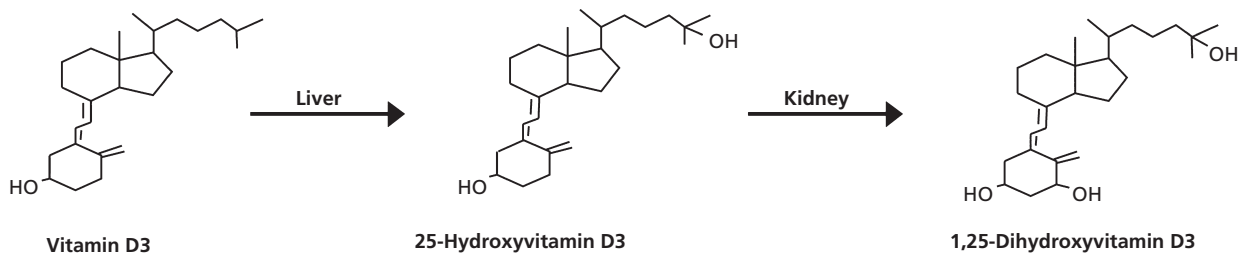


Fig. 1 Chemical structures of retinoids and vitamin D. Retinylesters (R: fatty acid) stored in the liver are hydrolysed to all-*trans*-retinol that is then transported to target cells after binding to RBP in the circulation. RA, a bioactive hormone, is biosynthesized from retinol by alcohol dehydrogenase and aldehyde dehydrogenase in the target cells. This RA-generating process is irreversible (one-way reaction). Two isomers of RA, all-*trans*-RA and 9-*cis*-RA, activate retinoid nuclear receptor, RAR, whereas only 9-*cis* RA activates the other retinoid receptor, RXR. Another isomer of RA, 13-*cis*-RA, is used clinically for the prevention of head and neck cancers, and 9,13-*di-cis*-RA has pathological significance in liver fibrosis. 14-Hydroxy-*retro*-retinol enhances lymphocyte proliferation independently of known retinoid receptors. A number of synthetic retinoids have been developed for pharmacological applications including cancer chemotherapy and chemoprevention. Acyclic retinoid and 4-HPR successfully prevent the development of HCC and breast cancer, respectively, in clinical trials. Am80 is a promising retinoid aiming to induce second remission in relapsed acute promyelocytic leukaemia (APL) patients who have become resistant to RA therapy. Exposure to solar ultraviolet light converts a derivative of cholesterol (7-dehydrocholesterol) to previtamin D3 in the skin, which is rapidly subjected to thermal isomerization to vitamin D3. Vitamin D3 is metabolized in the liver to 25-hydroxyvitamin D3, and then in the kidney to its biologically active form, 1,25-dihydroxyvitamin D3 [1,25(OH)₂D₃].

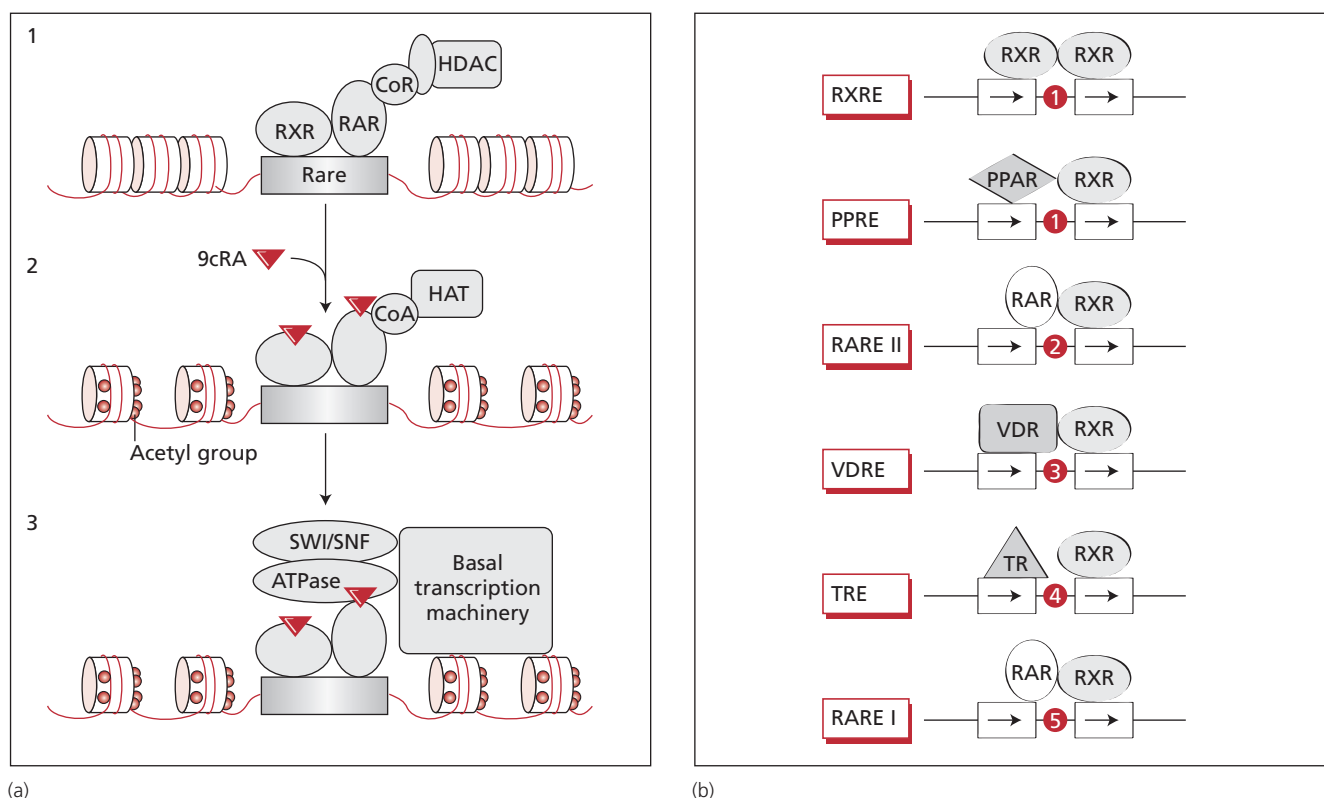


Fig. 2 (a) Transcriptional repression and activation of RAR–RXR heterodimer. In the absence of ligands (RA), RAR–RXR heterodimer is linked to corepressor complexes (CoR) and associated with histone deacetylases (HDACs). HDACs remove acetyl groups from histones, induce chromatin compaction and silence the promoter region of the target genes (repression). Binding of RA to the receptor induces its conformational alteration, which destabilizes the interaction with CoR and, instead, allows a connection to coactivators (CoAs) (derepression). CoAs mediate the association between the heterodimer and histone acetyltransferase (HAT) complexes, which induces acetylation of histones and thus leads to chromatin decondensation. Subsequently, activation of transcription takes place by contact with the basal transcription machinery, ATPase and other related factors (transactivation). (b) Direct repeats serve as hormone-response elements for RAR, RXR, VDR, thyroid receptor (TR) and peroxisome proliferator activator receptor (PPAR). The elements consist of direct repeats of core sequence AGGTCA (arrows in boxes) separated by defined numbers of nucleotides (the nucleotide number is shown between the two boxes). RXR functions as a master regulator, forming homo- and heterodimers with RXR, PPAR, RAR, VDR and TR.

consist of three subtypes, α , β and γ , characterized by a modular domain structure. The RA molecule contains four coupled double bonds and thus has several stereoisomers, including all-*trans*-RA (atRA) and 9-*cis*-RA (9cRA), 13-*cis*-RA (13cRA) and 9,13-di-*cis*-RA (dcRA) (Fig. 1). RAR interacts with both atRA and 9cRA, whereas RXR binds only to 9cRA. 13cRA and dcRA are relatively weak ligands for RAR and do not bind to RXR. AtRA and 13cRA are used for the treatment and prevention of cancer, as discussed later. DcRA is involved in the development of liver fibrosis by inducing tissue plasminogen activator in HSCs and subsequently activating transforming growth factor- β , the most potent fibrogenic cytokine [6]. Both RAR and RXR have a DNA-binding domain called C-domain and a ligand-binding domain (E-domain). RXR forms a homodimer as well as heterodimers with RAR and several other nuclear receptors. These dimers bind to their respective response elements and subsequently activate or inhibit the expression of target genes. RAR and RXR bind to an RA response element (RARE) and an

RXR response element (RXRE) respectively. These elements consist of direct repeats of the core sequence AGGTCA separated by a defined number of nucleotides. RARE has direct repeat spacers of two or five nucleotides (DR-2 and DR-5 respectively), and RXRE has a spacer of one nucleotide (DR-1) (Fig. 2b). The tissue-specific expression patterns of the receptors suggest that distinct functions of each subtype and functional redundancy make the retinoid signalling highly complex. The detailed mechanism of transactivation via RAR–RXR heterodimers has been revealed recently [7] (Fig. 2a). In the absence of RA, the RAR–RXR heterodimer binds to corepressor complexes that link between the heterodimer and histone deacetylases (HDACs). HDACs induce chromatin condensation and gene silencing by removing acetyl groups from nucleosomal histones. The binding of RA to ligand-binding domains of RAR and RXR induces the conformational changes in the domain, which allows the interaction between RAR/RXR and coactivators. Coactivators recruit histone acetyltransferases (HATs) such as

CREB-binding proteins or p300 that induce the acetylation of histone amino-terminal tails, resulting in nucleosomal repulsion and chromatin decondensation. On the other hand, a novel retinol (but not RA) metabolite, 14-hydroxy-*retro*-retinol (Fig. 1), induces lymphocyte proliferation, the activity of which cannot be substituted by any isomers of RA, implying the presence of other orphan receptors and/or other retinoid signalling pathways independent of nuclear receptors.

In HCC, both local deficiency of retinoids in the tumour tissues and unresponsiveness of the cancer cells to retinoids lead to loss of retinoid signalling and normal cell function, which seems to be linked to the development of cancer (see also Chapter 18.2, Malignant tumours) [8,9]. Alcohol consumption accelerates retinoid depletion in the cirrhotic liver associated with hepatitis virus infection, which may be related to its enhanced carcinogenic state [10]. RXR α is phosphorylated in HCC cells by extracellular signal-regulated kinase (Erk) 1/2 (also called mitogen-activated protein kinase), loses its function and is accumulated in the cancer cells [11,12], leading to their enhanced proliferation. In addition, RAR β is suggested to be a tumour suppressor gene in some tumours such as head and neck cancer [7,13]. As the RAR β -RXR heterodimer is more activated by retinoids than the RAR α -RXR heterodimer, expression of RAR β as well as RXR α may be advantageous in suppressing tumour cell growth. Cells with normal retinoid signalling would be deleted and, as a result, only the remaining cells with impaired response might survive during the carcinogenic process.

Chemoprevention of HCC (see also Chapter 18.2)

Retinoids inhibit carcinogenesis at several steps (i.e. initiation, promotion and progression) and are thus prime candidates for cancer chemoprevention [14]. Recent advances in understanding the molecular mechanisms of carcinogenesis and the parallel progress in molecular targeting have stimulated the development of novel synthetic retinoids for cancer chemotherapy and chemoprevention. Some retinoids work as agonists that enhance the transactivation via RARE or RXRE, whereas others function as antagonists that inhibit the transcription induced by natural RA. In addition, novel mechanisms of retinoid receptor-independent induction of apoptosis have been reported recently with some synthetic retinoids such as 4-hydroxyphenyl retinamide (4-HPR, fenretinide) [7] (Fig. 1). In clinical studies, striking successes have been achieved in the therapy of acute promyelocytic leukaemia (APL) as well as the prevention of several malignancies, including cancers of the oral cavity, head and neck, breast, skin and liver [7,13,15]. Now, differentiation induction therapy with RA and a synthetic RAR ligand, Am80 (Fig. 1), has become standard in the treatment of APL. 13cRA is used clinically for the prevention of cancers in the oral cavity, head and neck region and the skin [7,13].

HCC, one of the most frequent cancers in the world, is an important target of cancer prevention by retinoid. HCC is closely linked to hepatitis viral infection and commonly arises

in livers with chronic inflammation. The annual incidence of HCC reaches approximately as high as 3–7% in hepatitis virus-infected cirrhotic patients [16]. Moreover, the annual incidence rises to approximately 20–25% after the curative removal of the primary HCC [17,18]. Such a high carcinogenic state of the cirrhotic liver is a major cause of the limited 5-year survival rate (approximately 40%) even after the curative treatment [19]. Therefore, a new strategy to prevent post-therapeutic recurrence of HCC is required to improve further the therapeutic outcome of HCC. A number of clinical studies have attempted different strategies to suppress the development of HCC. For example, interferon (IFN) suppresses hepatic necroinflammation and thus serves to reduce the incidence of HCC [20,21]. IFN may belong to a category of biopreventive (or immunopreventive) agents, functioning as a biological response modifier. On the other hand, retinoids are chemopreventive agents, as the retinoid seems to act directly on (pre)malignant cells without modulating hepatic necroinflammation. We have developed a synthetic acyclic retinoid (all-*trans*-3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentanoic acid or polyprenoic acid), aiming for the chemoprevention of HCC (Fig. 1). A double-blind and placebo-controlled clinical study [22,23] has shown that oral administration of acyclic retinoid for 12 months significantly reduced the incidence of post-therapeutic recurrence and subsequently improved survival. In that clinical trial, serum lectin-reactive α -fetoprotein (AFP-L3), which indicates the presence of unrecognizable cancer cells in the remnant liver, disappeared in the acyclic retinoid group after administration [24]. These findings await validation in independent studies. This observation suggests a new concept in cancer chemoprevention, ‘clonal deletion’, the removal of latent malignant (or premalignant) cells that are invisible by diagnostic images from the organ with a hypercarcinogenic state such as cirrhosis-HCC sequence [25,26]. This concept may explain the reason why only a short-term administration (12 months) of acyclic retinoid has brought about a long-term suppressive effect on the development of HCC for several years. Once (pre)malignant clones are deleted, it would take at least several years for the development of *de novo* cancer in the cirrhotic liver. Acyclic retinoid not only functions as a RXR α ligand but also suppresses phosphorylation of RXR α by inactivating the Ras/Erk system, and thereby restores the function of RXR α [27]. Restoration of the function of RXR α leads to apoptosis induction of the cancer cells, which is a mechanism of clonal deletion. The detailed underlying molecular mechanisms of retinoid-induced apoptosis are discussed elsewhere [26]. Acyclic retinoid is now being tested in a clinical trial in Japan, aimed at chemoprevention of HCC.

Metabolism and function of vitamin D

Vitamin D is known as a ‘sunshine vitamin’ [28] because of its dependency on sunlight for conversion to an active metabolite. There are six vitamin D compounds, vitamin D2–D7, sharing a common basal structure with different side-chains. Among these,

vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol) exert high biological activities. In particular, vitamin D₃ is the major vitamin D species in man. The first step in vitamin D₃ production normally takes place in the skin. Exposure to solar ultraviolet light converts a derivative of cholesterol, 7-dehydrocholesterol (provitamin D₃), to previtamin D₃ in the skin via a photolysis reaction. Previtamin D₃ is then rapidly subjected to thermal isomerization to vitamin D₃ in the skin. Vitamin D₃ originating from either the skin or the diet is transported to the liver microsome via the circulation, where it is converted by vitamin D 25-hydroxylase to 25-hydroxyvitamin D₃ [25(OH)D₃], the major circulating form of vitamin D₃ (Fig. 1). The 25-hydroxylation reaction is the prerequisite step for the subsequent 1 α -hydroxylation and 24-hydroxylation reactions in the kidney. Namely, 25(OH)D₃ enters the circulation again and, in the renal mitochondria, it is converted to 1 α ,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], the active form of vitamin D₃, by 25(OH)D₃ 1 α -hydroxylase, whereas the 24-hydroxylation reaction leads to inactivation and disposal of vitamin D₃. Both reactions are strictly controlled by serum phosphorus as well as by parathyroid hormone, calcium and 1,25(OH)₂D₃.

Presently, six cytochrome P450s (CYP2C11, 27A1, 2D25, 2R1, 3A4 and 2J3) are found to exhibit vitamin D 25-hydroxylation activities, and CYP27B1 and CYP24 have been established as 1 α -hydroxylase and 24-hydroxylase respectively [29]. CYP24 metabolizes vitamin D and 1,25(OH)₂D₃ to their excretion products [30]. As 24-hydroxylase is induced by 1,25(OH)₂D₃ itself, the vitamin has a negative feedback system. Thus, vitamin D, like retinoids, is really more of a hormone than a vitamin.

Vitamin D plays a central role in calcium and phosphate homeostasis and is essential for the proper development and maintenance of bone, thus acting on the major target organs, bone and intestine, through a vitamin D receptor (VDR), which belongs to the class II steroid hormone superfamily and is closely related to RAR and RXR [30] (Fig. 2b). VDR forms a heterodimer with RXR and regulates the downstream genes via vitamin D-responsive element (VDRE), consisting of a direct repeat of consensus AGGTCA separated by three nucleotides (DR-3) (Fig. 2b). Among such genes, as described above, CYP24 (24-hydroxylase) is the most inducible gene, and participates in the degradation of vitamin D. When VDR interacts with 1,25(OH)₂D₃, VDR moves away from the corepressor and acquires the ability to recruit coactivators after forming a heterodimer with RXR at VDRE, which is similar to the regulation of RAR/RXR (Fig. 1a). VDR is expressed not only in the well-known target cells such as osteoblasts and renal tubule cells, but also in a variety of cells including colon cells, lymphocytes and promyelocytes, suggesting novel functions for the hormone beyond osseous tissues [30]. Indeed, 1,25(OH)₂D₃ is a potent regulator of cell growth and differentiation, with recent evidence showing inhibition of tumour invasion, angiogenesis and tumour cell death [31]. For example, 1,25(OH)₂D₃ has been shown to induce terminal differentiation of promyelocytes to monocytes [32].

The liver is generally considered to be negative for VDR, although it is obviously a direct target organ of 1,25(OH)₂D₃ [33]. This may be explained by the low expression of VDR in hepatic parenchymal cells but significant expression in non-parenchymal cells including sinusoidal endothelial, Kupffer, stellate and biliary epithelial cells [34]. VDR expression is also positive in HCC, and thus experimental and clinical trials to use 1,25(OH)₂D₃ for the treatment of HCC have been suggested [31,35].

Osteodystrophy (osteomalacia and osteoporosis) is often seen in patients with advanced chronic liver diseases [36]. Serum 25(OH)D₃ concentrations of < 80 nmol/L are associated with reduced calcium absorption, osteoporosis and increased fracture risk [37]. However, although serum levels of 1,25(OH)₂D₃ are low in cirrhotic patients, this does not correlate with the bone formation rates [38]. Thus, the pathogenesis of the bone disease is multifactorial, including not only altered vitamin D metabolism, but also other factors such as impaired vitamin K activity, malnutrition and hypogonadism.

A potential link between vitamin D and the immune system has emerged as an interesting area of investigation [39]. Vitamin D interacts with helper T lymphocytes and thereby suppresses the inflammatory responses. For example, inflammatory bowel diseases (IBDs), including ulcerative colitis and Crohn's disease, are closely related to vitamin D deficiency [40]. In addition, VDR deficiency has been shown to exacerbate IBD in experimental animals. Vitamin D-deficient mice on low-calcium diets developed severe IBD, and 1,25(OH)₂D₃ treatment of mice improved IBD symptoms [39]. Thus, the idea of using vitamin D for the suppression of IBD has been proposed. The link between VDR polymorphisms and two autoimmune-related liver diseases, autoimmune hepatitis and primary biliary cirrhosis, has also been suggested, although the underlying mechanism has not yet been clarified [41,42].

Because both retinoids and vitamin D are fat-soluble vitamins and can thus accumulate when intake is excessive, less toxic synthetic analogues have been developed for use as clinical therapeutics (for detailed descriptions of their toxic effects, see Chapter 14.2, Toxic liver injury, and Chapter 14.4, Hepatic toxicity induced by herbal medicines).

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2.3.12 Normal iron metabolism

Kyle E. Brown

Introduction

Iron is an essential nutrient, but one with considerable potential for toxicity. It is therefore understandable that the uptake and disposition of iron are controlled by elaborate physiological mechanisms. Although the highly regulated nature of iron metabolism has been recognized for decades, the mechanisms governing its regulation have only recently been elucidated. This has been made possible by the discovery of a variety of proteins involved in iron transport, as well as the iron-regulatory hormone, hepcidin. The aim of this chapter is to provide an overview of iron metabolism with an emphasis on these new discoveries, particularly as they relate to the liver.

Overview of iron metabolism

Before describing these discoveries, a brief review of iron metabolism is necessary. Iron metabolism is a highly conservative process characterized by recycling. The body of the average adult male contains approximately 5 g of iron, of which the single largest component is the haemoglobin contained in the erythrocytes. At the end of their relatively short lifespan, these cells are destroyed, their haemoglobin catabolized and the resulting iron made available for reuse in the synthesis of haemoglobin, myoglobin or any of a number of iron-requiring enzymes, including the cytochrome P450 system, ribonucleotide reductase and the prolyl hydroxylases. In an iron-replete individual, some iron is stored, primarily in the liver, spleen and bone marrow. Hence, once absorbed, iron is conserved. This observation is underscored by the fact that there is no regulated pathway for the excretion of iron, and daily iron loss is negligible, resulting mostly from desquamation of cells.

Given the lack of a regulated means of excreting iron, the control of iron uptake is clearly of paramount importance. The duodenum and proximal jejunum are the main sites of absorption of dietary iron. Haem iron is absorbed more efficiently than non-haem iron, apparently by endocytosis of the intact iron–protoporphyrin complex at the enterocyte brush border.

Iron is then liberated from the haem moiety by the action of haem oxygenase and enters the intracellular iron pool from which it can be transferred across the basolateral membrane, bind to transferrin and enter the circulation. In contrast, the absorption of non-haem iron is more limited, in part as a result of its more complex uptake. As detailed below, absorption of non-haem iron requires reduction of ferric iron at the brush border membrane, followed by internalization by a proton-coupled transporter. Presumably, once iron derived from non-haem sources enters the intracellular iron pool within the enterocyte, its fate is similar to that of haem-derived iron. It is worth noting that, although a great deal has been learned about the mechanisms controlling iron absorption, iron stores exert a major influence on this process under physiological conditions but the means by which iron stores are sensed remains unclear.

Iron transport and uptake mechanisms

Because of the ability of iron to catalyse the production of reactive intermediates, its uptake and transit through the body require mechanisms to diminish its reactivity and thus prevent free radical generation. One of the means by which this is accomplished is by the binding of iron to proteins for transportation and storage. Thus, iron is transported in the blood bound to transferrin. Each molecule of transferrin can bind two atoms of ferric iron. Transferrin-bound iron is taken up at the cell membrane by the interaction of transferrin with the extracellular ligand-binding domain of the transferrin receptor 1 (TfR1). Upon binding of transferrin to TfR1, the entire complex is internalized by receptor-mediated endocytosis. Iron dissociates from transferrin in the acidic milieu of the endosome and then enters the intracellular iron pool, from which it is incorporated into iron-containing proteins, while apotransferrin and TfR1 are recycled to the cell membrane.

The abundance of TfR1 is regulated by cellular iron status, while the identical mechanism controls expression of the iron-storage protein ferritin in an inverse manner. Cellular iron content determines the composition of a cytosolic protein termed the iron regulatory protein 1 (IRP1). Under iron-replete conditions, IRP1 contains a 4Fe–4S cluster that is unable to bind to iron-responsive elements (IRE) in the mRNAs of TfR1 and ferritin. When cellular iron content is low, the iron–sulphur cluster is disassembled, liberating an apo-IRP that binds to specific stem–loop structures in the 3′ or 5′ untranslated regions (UTRs) of the mRNAs encoding these proteins. In the case of TfR1, the IREs are located in the 3′ UTR, and binding of IRP1 increases the stability of the message and enhances the synthesis of TfR1. Conversely, binding of IRP1 to the IREs in the 5′ UTR of ferritin mRNA mediates translation repression. Thus, under iron-replete conditions, there is more rapid turnover of TfR1 mRNA, leading to diminished translation and cell-surface expression of TfR1, reduced uptake of transferrin-bound iron and an expanded capacity for iron storage through increased synthesis