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Serum concentrations of all-*trans* and 13-*cis* retinoic acid and retinol are closely correlated

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*The origin and role of all-*trans *and 13-*cis *retinoic acid in serum is unknown. Certain tissues are known to oxidize retinol via retinaldehyde to retinoic acid. Some p450 isoenzymes are downregulated in inflammation and as retinoic acid is catabolized by members of this enzyme family, we hypothesized that the concentration of all-*trans *and 13-*cis *retinoic acids might be increased in inflammation. Serum retinol, on the other hand, decreases in inflammation.*

We, therefore, measured the serum concentration of the retinoic acids, retinol and the other components of the retinol transport system, the retinol-binding protein, and transthyretin in patients with inflammation. The degree of inflammation was judged from the serum level of C-reactive protein.

*We found that retinol, all-*trans *and 13-*cis *retinoic acid concentrations in serum, were all decreased in inflammation and that they were significantly and positively correlated to each other. The serum concentrations of 13-*cis *retinoic acid, retinol, retinol-binding protein, and transthyretin concentrations, but not all-*trans *retinoic acid, were all significantly and negatively correlated to the concentration of C-reactive protein.*

Retinol concentration in the serum may be one of the determinants of the serum retinoic acid concentration. We speculate that the decrease in serum retinol and retinoic acid concentration, which occurs in inflammation may create an "acute vitamin A deficiency", which may be a factor contributing to the excess mortality associated with measles in children with marginal vitamin A deficiency and in patients with AIDS. (J. Nutr. Biochem. 7:162–165, 1996.)

Keywords: retinol; retinoic acid; inflammation

Introduction

Whereas the concentration of retinol in serum is 1 to 3 μM, the concentration of all-*trans* and 13-*cis* retinoic acid is about 500-fold less, i.e. about 4-6 nM.[1,2] Retinol is secreted from the liver in a 1:1 (mol:mol) complex with its carrier protein, the retinol-binding protein (RBP)[3,4] a low molecular weight protein that, in the blood is complexed (1:1 mol:mol) to another protein, transthyretin (TTR). One of the effects of this complexation is to prevent the RBP:retinol complex from being lost in the urine.[3,4] The retinoic acids are transported bound to serum albumin.[5]

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Many tissues are known to be able to transform retinol to retinoic acid in vitro.[6] The origin and the role of the 13-*cis* retinoic acid is obscure as it does not bind to retinoic acid receptors (RAR:s).[7] 13-*cis* retinoic acid has been suggested to be formed during the absorption of retinoids in the gut[1,2] and it may then be isomerised (at least partly non-enzymatically) to all-*trans* retinoic acid.[8]

The role of retinoic acids in serum is not known, but it may be that the all-*trans* retinoic acid is present in serum to supply all-*trans* retinoic acid to, those tissues, which are not able to synthesize all-*trans* retinoic acid by themselves.[6] All-*trans* and 13-*cis* retinoic acid are catabolized by the enzymes of the liver microsomal p450-dependent enzyme system.[9] It has been reported that these enzymes are down-regulated in inflammation[10] and, consequently, in inflammation, the serum concentration of the retinoic acids might increase due to impairment of their catabolism.

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In preliminary studies in patient samples (in which light protection was not completely controlled) many of the patients with a strong inflammatory reaction with serum C-reactive protein (CRP) level of >50 mg/L (the laboratory's reference interval, for CRP is <5 mg/L) had low serum concentration of both all-*trans* and 13-*cis* retinoic acid and retinol.

We, therefore, decided to measure the concentrations of retinol, all-*trans* and 13-*cis* retinoic acid, RBP, TTR, and CRP in the serum and their interrelation in inflammation (in samples obtained under carefully light-protected conditions) as this might give a clue to the high mortality in children with marginal vitamin A deficiency when infected with measles[11,12] and in patients with AIDS.[13]

Patients

Non-fasting samples from patients (7 males and 26 females aged 14–87 years) most of which with an inflammatory reaction (i.e., CRP >5 mg/L, the reference interval of the laboratory is <5 mg/L) were obtained from the Department of Infectious Diseases. Non-fasting serum samples from subjectively healthy blood donors (40 males and 20 females 20–60 years old) were obtained from the blood bank. All blood samples were obtained in vacuum tubes without anticoagulant. The tubes were immediately wrapped in aluminium foil to protect them from light and sent to the laboratory in light tight containers. Serum was recovered by centrifugation and stored frozen in the dark at 20°C until analyzed (within 2 weeks). All handling of the samples was done in the dark or under yellow light to protect the retinoids from photodestruction and/or isomerization.

In our experience, light protection, but not low temperature, is important for the protection of the retinoic acids if the time between sampling and freezing is less than a few hours. The presence of erythrocytes protect the retinoic acids from photodestruction and isomerisation. The critical stage is when the blood cells are removed. Serum cannot be exposed to daylight at room temperature for more than 1 hr before the retinoic acids (13-*cis* retinoic acid, in particular) start to decay.

Methods and materials

The retinol was from Janssen Chimica. All-*trans*-, 13-*cis*-, 9-*cis*-retinoic acids, 4-oxo all-*trans* and 4-oxo 13-*cis* retinoic acid and acitretin were gifts from Hoffman LaRoche, Basel. The acetonitrile and the methanol were of HPLC grade. All other chemicals were of pa grade unless otherwise specified.

Retinol concentration was determined in all serum samples using the isocratic HPLC system described by Nierenberg and Lester.[14] The concentration of the retinol calibrator was established by the use of the absorbance coefficient$_{\epsilon 325}$(retinol) = 52.990 M^{-1}, cm^{-1}.[16] The NIST preparation SRM (S) 968 was used to validate the assay for retinol. Retinoic acid (all-*trans* and 13-*cis*) was determined in all serum samples with the gradient HPLC system described by Wyss and Bücheli[15]; the concentration of the calibrators was established by weighing. All handling of samples and retinoids was done in the dark under yellow light.

The total, long-term, analytical errors (CV) were 6% for retinol at 2 μmol/L and 5% at the level of 4–5 nmol/L for retinoic acid (all-*trans* and 13-*cis*). The sensitivity of the retinoic acid method was <0,5 nmol/L, 4-oxo all-*trans* and 4-oxo 13-*cis* retinoic acids were determined qualitatively.

CRP and TTR were determined by immunoturbidimetry using WHO calibrators CRM 470 CAP/IFCC RBP was determined by immunoturbidimetry with a calibrator from Behring (Marburg, Germany). The long-term, total, coefficients of variation (CV:s) of the protein measurements (at the upper levels of the reference intervals) was 5–8%.

Results and discussion

Table 1 shows the concentrations of the serum components in the patient material. It can be seen that many of the patients had retinol values below the laboratory's lower reference limit for retinol in serum (i.e., <1,4-2,2 μmol/L). The serum concentrations of the retinoic acids, RBP and TTR were also low and CRP was increased. 9-*cis* retinoic acid was not observed and the 4-oxo derivatives of all-*trans* and 13-*cis* retinoic acid were not elevated in the samples as judged from visual inspection of the chromatograms (not shown).

Table 2 shows the significant correlations (Spearman's Rho) among the retinoic acids, retinol, the components of the retinoid transport system, and CRP in the patient material and *Figures 1* A–C shows graphically the relationship among the concentrations of retinol, all-*trans* and 13-*cis* retinoic acid in the patients (open symbols) and the blood donors (filled symbols). The correlation between the serum concentrations of all-*trans* and 13-*cis* retinoic acid and retinol was significant only in the patients and not in the blood donors. At higher concentrations, i.e., at "normal" retinol levels the retinoic acid concentration levelled off. This confirms our finding of "normal" levels of all-*trans* and 13-*cis* retinoic acid in uremic patients who have grossly elevated serum retinol concentration (G, Fex et al., unpublished data). The correlation between all-*trans* and 13-*cis* retinoic acids was significant at both low (i.e., in patients) and "normal" (i.e., in blood donors) levels of the retinoic acids and was independent of serum retinol concentration. Though significant, the correlations between retinol and the retinoic acids were not very high. The patient population was, however, heterogenous both with respect to background disease (diseases in addition to the inflammation disease that

Table 1 Mean, SD, and range for all-*trans* and 13-*cis* retinoic acid (atRA and 13cRA), retinol-binding protein (RBP), transthyretin (TTR), and C-reactive protein (CRP) concentration in the patient-material

Component	Mean	SD	Range	Reference interval of the laboratory
atRA (nmol/L)				
N = 32)	4,4	0,99	2,8–6,7	3,4–8,9
13 cRA (nmol/L)				
N = 30	4,1	1,54	1–8,9	2,2–8,1
Retinol (μmol/L)				
N = 32	1,71	0,76	0,6–3,2	Males 1,4–3,4 Females 2,2–3,3
RBP (mg/L)				
N = 32	38	20	12–99	40–90
TTR (g/L)				
N = 28	0,27	0,105	0,1–0,51	0,23–.0,45
CRP (mg/L)				
N = 33	46,2	60,2	4–257	<5

Table 2 Significant correlations (Spearman's Rho) between serum retinoid concentrations, C-reactive protein (CRP), and the proteins of the retinol transport system in the patient material

	atRA nmol/L	13 cRA nmol/L	Retinol µmol/L	RBP mg/L	TTR g/L	CRP mg/L
atRA nmol/L		0,57 *N* = 30 *P* = 0,0023	0,37 *N* = 31 *P* = 0,0416	NS	NS	NS
13 cRA nmol/L			0,57 *N* = 29 *P* = 0,0024	NS	0,66 *N* = 25 *P* = 0,0012	−0,43 *N* = 30 *P* = 0,0220
Retinol µmol/L				0,84 *N* = 25 *P* = 0,0001	0,94 *N* = 28 *P* = 0,0001	−0,65 *N* = 32 *P* = 0,0003
RBP mg/L					0,76 *N* = 25 *P* = 0,0002	−0,56 *N* = 25 *P* = 0,0060
TTR g/L						−0,66 *N* = 28 *P* = 0,0006
CRP mg/L						

NS = not significant.
The patients were those described in Table 1. AtRA = all-*trans* retinoic acid, 3cRA = 13-*cis* retinoic acid, RBP = retinol-binding protein, TTR = transthyretin, and CRP = C-reactive protein.

prompted treatment at the clinic for infectious diseases), and pharmacological treatment. It is known that at least some drugs are associated with low concentration of retinoic acids in the serum.[16] Both all-*trans* and 13-*cis* retinoic acids have been suggested to be formed in the gut during absorption of retinyl palmitate.[1,2] If both all-*trans* and 13-*cis* retinoic acid are formed at the same time from retinol, or if one retinoic acid is derived from the other is not known. However, from the halflives of all-*trans* and 13-*cis* retinoic acids in the serum[17,18] it can be calculated that about 95% of the retinoic acid synthetized/day must be all-*trans* and less than 5% 13-*cis* to maintain a serum concentration of 5 nM each for the two retinoic acids. If any, 13-*cis* retinoic acid can play a role in serum as a source of all-*trans* retinoic acid with long halflife (to which it can isomerize).[8] T1/2 for 13-*cis* retinoic acid in serum is about 17 hr[17] and for all-*trans* retinoic acid about 1 hr.[18] In samples obtained immediately before and after UV-irradiation of patients (treatment for psoriasis) the concentration ratio all-*trans*/13-*cis* retinoic acid was unchanged (G Fex et al., unpublished data).

The strongest correlation (*Table 2*) was between the serum concentrations of retinol and the RBP and TTR, as has been shown before.[3]

It is well known that inflammation and fasting decreases the concentration of retinol in the serum.[19,20] Retinol is the substrate for the dehydrogenase(s) that synthetize retinoic acid.[6] Our finding of a correlation between the concentration of serum retinol and the serum retinoic acids, might mean that serum retinol concentration is one of the determinants of the rate of synthesis of retinoic acid in the tissues engaged in that synthesis, and, consequently, serum retinol may be one of the determinants of serum concentration of all-*trans* and 13-*cis* retinoic acid. The availability of retinoic acid to tissues uncapable of all-*trans* retinoic acid synthesis, might, thus, be regulated by retinol availability to those capable.

We checked (not shown) if all-*trans* or 13-*cis* retinoic acid concentrations were more strongly correlated to other possible indices of retinol availability[21,22] like retinol saturation of RBP or the TTR/RBP ratio, but the strongest correlation was with serum retinol concentration.

Our data show that low levels of serum retinol occurs together with low serum levels of retinoic acid (all-*trans* and 13-*cis*). Since all-*trans* retinoic acid has a halflife in the serum of about 1 hr[17] while that of serum retinol is about 12 hr (3) and 13-*cis* retinoic acid 17 hr[18] the serum concentration of all-*trans* retinoic acid will rapidly adjust to decreased rate of its production. Our finding of normal levels of the retinoic acids and no correlation between the retinoic acids and retinol in the blood donors and "normal" levels of all-*trans* and 13-*cis* retinoic acids in hemodialysis patients who have elevated serum retinol levels (G Fex et al. unpublished data) shows that serum retinol levels are not correlated to the concentrations of the retinoic acids in serum at all retinol concentrations. This may indicate that the enzymes oxidizing retinol to retinoic acid are saturated already at "normal" serum retinol levels though their K_m do not indicate this,[23] or that other retinol pools than serum retinol contribute to retinoic acid synthesis. This association of low levels of retinol and all-*trans* and 13-*cis* retinoic acid in serum might allow the speculation that the precipitation by measles, of acute symptoms of vitamin A-deficiency and high mortality in children with "marginal vitamin A deficiency"[11,12] and in patients with AIDS[13] is mediated by the inflammation-induced lowering of serum retinol concentration. This, in turn, may diminish the synthesis of retinoic acids in tissues capable of that, which, may lower the concentration of all-*trans* and 13-*cis* retinoic acid in serum and, thus, the supply of retinoic acid to tissues with no capability of retinoic acid synthesis.

As retinol concentration is depressed in inflammation in general, a similar series of events occurs in, and may com-

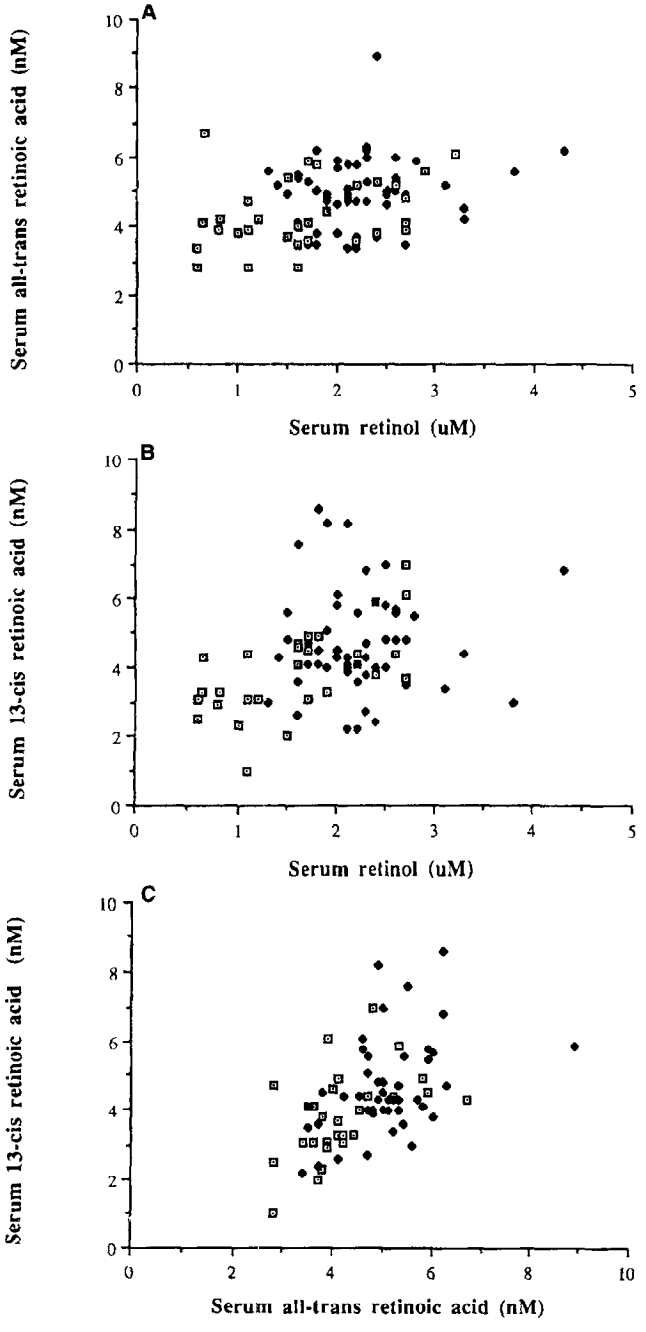

Figure 1 Diagram showing the relation between retinol, atra and 13cra in the patient materiel and the blood donors. A. Serum retinol and all-trans retinoic acid. B. Serum retinol and 13-cis retinoic acid. C. all *trans* and 13-*cis* retinoic acid. Filled symbols: blood donors, Open symbols: patients.

plicate, several other inflammatory diseases such as AIDS.[15]

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