Concentrations of retinoids in early pregnancy and in newborns and their mothers¹⁻³

Maria Berggren Söderlund, Göran A Fex, and Peter Nilsson-Ehle

ABSTRACT

Background: Retinoids are vital for embryonic development; both excesses and deficiencies of vitamin A are known to give similar patterns of birth defects. Concentrations of retinol in newborns and in pregnant women have been investigated, but concentrations of the biologically active metabolite all-trans retinoic acid and its isomer 13-cis retinoic acid have not.

Objective: We measured serum concentrations of these retinoid derivatives in newborns and their mothers and in women in the first trimester of pregnancy, when embryonic differentiation (organogenesis) takes place.

Design: In this descriptive study, 10 newborns from normal deliveries and their mothers and 16 healthy women in their first trimester of pregnancy were studied. Seventeen healthy women served as control subjects. all-trans and 13-cis Retinoic acid and retinol concentrations were measured by HPLC.

Results: The newborns had significantly lower retinol concentrations (1.0 μmol/L) than did their mothers (1.7 μmol/L; P = 0.013). Serum all-trans retinoic acid was also significantly lower in the newborns (3.4 nmol/L) than in their mothers (5.8 nmol/L; P = 0.008). In addition, serum concentrations of 13-cis retinoic acid were significantly lower in the newborns (2.0 nmol/L) than in their mothers (2.6 nmol/L; P = 0.005). The serum concentrations of all-trans retinoic acid and retinol did not correlate in any group.


KEY WORDS 13-cis Retinoic acid, all-trans retinoic acid, retinoids, vitamin A, newborns, pregnancy, mothers, maternal-fetal exchange, teratogens

INTRODUCTION

Vitamin A is a group of substances that have vitamin A activity. The most important of these substances are retinol (the precursor of the metabolically active derivatives) and the active metabolites all-trans retinoic acid, 9-cis retinoic acid, and retinal. The most abundant retinoids in serum are retinol and all-trans, 4-oxo-13-cis and 13-cis (inactive metabolite) retinoic acids. Retinoids are vital for vision, the immune system, reproduction, the differentiation and proliferation of cells, and embryonic development. The mechanism involved in embryonic development probably involves the retinoid nuclear receptors, which bind all-trans retinoic acid, 9-cis retinoic acid, or both retinoic acids. This interaction activates or represses the transcription of retinoic acid responsive genes.

Both excess and deficiency of vitamin A are known to give similar patterns of birth defects (1–4). Evidence suggests that 13-cis retinoic acid is involved in teratogenicity (5), probably by isomerization to all-trans retinoic acid (6). Different species are sensitive in different ways to such teratogenic effects (7), possibly as a result of differences in the detoxication pathways, and to placental transfer (3, 5, 7–9). In insensitive species (rats and mice), placental transfer of 13-cis retinoic acid into embryonic tissues is low, whereas in sensitive species (monkeys) the placental transfer is higher (3, 9). In humans, the transfer is not known (3). The concentrations of retinol in human newborns and in pregnant women were reported (10–13), but information on the physiologic concentrations of all-trans and 13-cis retinoic acids is lacking. Therefore, we investigated these retinoid derivatives in newborns and their mothers and in women in their first trimester of pregnancy, when embryonic differentiation (organogenesis) takes place.

SUBJECTS AND METHODS

Subjects

The investigation included 16 healthy pregnant women (17–39 y old) in week 8–12 of pregnancy (3. week 10), 10 newborns (placental cord blood) from normal deliveries, their mothers (22–39 y old), 1 healthy pregnant woman (34 y old) followed over her entire pregnancy, and 17 healthy women (22–63 y old) who served as control subjects (14). The first group (the pregnant women) was sampled at 2 outpatient maternity clinics in Lund. The second group (the newborns) and the third group (the mothers) were sampled at the Department of Gynecology. All 3 groups were sampled consecutively in the separate clinics after agreement to participate in the study. The control subjects and the pregnant woman were staff members from the laboratory (14). Although the diet histories were not recorded for

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The detection limit of the measurement was

the pregnant women and parturient mothers, pregnant women should not eat liver or consume vitamin A supplements containing >1000 RE/d, according to Swedish recommendations. The project was approved by the ethics committee at Lund University, and informed consent was obtained from all participants.

Sampling

The subjects were sitting or lying for 15 min before sampling. Venous blood samples were taken with the women in a nonfasting state and collected in vacuum tubes without anticoagulant. The pregnant women were sampled at their first visit to the maternity clinic (in the first trimester). Placental cord samples were obtained after delivery and represented mixed blood. The sampling of the cords and the parturient women was performed within a few hours after delivery. The pregnant woman was followed with 1 sample each month during a year, including 3 samples collected before her pregnancy. Serum was recovered by centrifugation at 3000 × g for 10 min at 4 °C and stored at −70 °C. Samples taken for retinoid analysis were protected against light.

Analytic methods

all-trans and 13-cis Retinoic acids and retinol were quantitated by HPLC (Kontron 420 HPLC-pump, Kontron Instrument Milano, Italy; and Shimadzu SPD-10AV UV-detector; Kyoto, Japan) as described by Wyss and Bucheli (15), with pure substances from Hoffman-La Roche (Basel, Switzerland) and Aldrich (Milwaukee) as calibrators. The calibrators were dissolved in ethanol, and aliquots were added to a charcoal-treated serum matrix, devoid of retinoic acid, their 4-oxo-derivatives, and retinol. The retinoids were extracted and concentrated on a precol

TABLE 1

<table>
<thead>
<tr>
<th>Age</th>
<th>all-trans RA</th>
<th>13-cis RA</th>
<th>Retinol</th>
<th>all-trans RA corrected for albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>y</td>
<td>nmol/L</td>
<td>nmol/L</td>
<td>μmol/L</td>
<td>nmol/g</td>
</tr>
<tr>
<td>Pregnant women (n = 16)</td>
<td>28 (17–39)</td>
<td>5.4 (4.7–6.2)</td>
<td>4.4 (3.6–4.8)</td>
<td>1.6 (1.4–1.8)</td>
</tr>
<tr>
<td>Parturient mothers (n = 10)</td>
<td>30 (22–39)</td>
<td>5.8 (5.2–6.9)</td>
<td>2.6 (2.2–2.9)</td>
<td>1.7 (1.5–1.8)</td>
</tr>
<tr>
<td>Newborns (n = 10)</td>
<td>—</td>
<td>3.4 (3.2–4.0)</td>
<td>2.0 (1.5–2.2)</td>
<td>1.0 (0.8–1.5)</td>
</tr>
<tr>
<td>Control subjects (n = 17)</td>
<td>36 (22–63)</td>
<td>5.2 (4.5–5.6)</td>
<td>4.5 (4.1–5.0)</td>
<td>2.1 (1.7–2.5)</td>
</tr>
</tbody>
</table>

1 Values are means; ranges in parentheses.
2 Values are medians; interquartile intervals in parentheses.
3,4 Significantly different from control subjects (Mann-Whitney U test): 3 P < 0.005, 4 P < 0.05.
5 Significantly different from control subjects (P < 0.005) and pregnant women (P < 0.001) (Mann-Whitney U test).
6 Concentrations measured in placental cord blood.
7,8 Significantly different from parturient mothers (Wilcoxon's signed-rank test): 7 P < 0.01, 8 P < 0.05.

The concentrations of retinol in the sense that they contained several extra peaks. The separation of peaks was best at 40 °C. The concentrations of albumin were determined by 2 accredited methods (photometric brom cresol purple, Hitachi Modular-P800, Tokyo; and photometric brom cresol green, Kodak Ektachem 700XR-C, Rochester, NY) at the Department of Clinical Chemistry.

Statistics

Differences between groups were evaluated by the Mann-Whitney U test with the use of Bonferroni correction for multiple tests. Wilcoxon’s signed-rank test was used to compare paired data in the mother-child pairs. Correlations between variables were analyzed by Spearman’s rank correlation test. The probability level of P < 0.05 was set for statistical significance. Data were analyzed with STATVIEW STUDENT (Abacus Concepts Inc, Berkeley, CA).

RESULTS

Pregnant women and parturient mothers had significantly lower concentrations of retinol than control subjects (14). The concentration of all-trans retinoic acid was higher in the parturient mothers than in the control subjects. In contrast, the concentrations of 13-cis retinoic acid were lower in parturient mothers than in the control subjects. No difference was observed in the concentrations of all-trans and 13-cis retinoic acid between pregnant women and control women (Table 1). Pregnant women had significantly higher concentration of 13-cis retinoic acid than did the parturient mothers.

One pregnant woman was followed with 1 sample a month during a year, including 3 prepregnant samples (Figure 1). Serum retinol and serum 13-cis retinoic acid were almost constant during pregnancy. In contrast, serum all-trans retinoic acid increased by ≈40% in the second trimester, and this level persisted throughout the pregnancy. When considering the decrease in the concentration of the binder protein albumin during pregnancy and correcting for the concomitant changes in serum albumin, the increase of serum all-trans retinoic acid was even more pronounced (≈100%), as seen in Figure 1.

When calculating the data from the single pregnancy as all-trans retinoic acid-to-albumin ratio, there is an increase from 0.14 (first month) to 0.19 (third month) and 0.28 (ninth month). This is consistent with the significant difference in all-trans retinoic acid:albumin for pregnant women in the third month (0.13) and for parturient mothers (0.23).
and retinol, respectively. The ratio for 13-cis retinoic acid was higher in the parturient mothers than in newborns. The active derivative of retinoic acid has a slower metabolism to retinol, which also has a higher degree of placental transfer (3, 16). Humans belong to the sensitive species. In one study a low dose of 13-cis retinoic acid medication induced teratogenic effects in humans; the dose was 100 times lower (in mg/kg body weight) than the doses in mice and rats that induce teratogenic response (7). As shown in the present study, the placental transfer efficiency of 13-cis retinoic acid under physiologic conditions in humans seems to be higher (0.7) than in other species, such as monkeys (0.4; another sensitive species) and mice and rats (<0.1; insensitive species) (3). The biological mechanism behind the high concentrations of 13-cis retinoic acid remains to be elucidated. In addition, interpretation of our data suggests that there may be a tendency for the equilibrium of cis and trans isomers to be shifted toward higher concentrations of 13-cis retinoic acid in newborns than their mothers under physiologic conditions. If extrapolated to pharmacologic conditions, our results would be consistent with empirical data and lend mechanistic support for the clinical observation that humans are sensitive to the teratogenic effects of 13-cis retinoic acid.

DISCUSSION

The serum concentrations of all-trans retinoic acid and retinol did not correlate among the newborns or among the pregnant women. Thus, retinol concentrations did not accurately reflect the concentrations of the active derivative all-trans retinoic acid.

Serum retinol was essentially constant during pregnancy. Serum retinol concentrations were presented with and without correction for albumin. The corrected values for all-trans retinoic acid were divided by a correction factor for concomitant changes in albumin concentration. Serum all-trans retinoic acid concentrations were not significantly different from that of their mothers. When considering the concentration of binding protein albumin the difference was slightly more pronounced for the mother and newborn pairs. All-trans retinoic acid:albumin ratio was significantly higher for mothers than for newborns. Serum 13-cis retinoic acid demonstrated a similar pattern, with lower concentrations in newborns than in their mothers.

The placental transfer of the different retinoids was calculated as the ratio of the concentrations in newborns and mothers. The mean ratios were 0.60 (range: 0.44-1.00), 0.74 (0.54-0.95), and 0.65 (0.21-1.22) for all-trans retinoic acid, 13-cis retinoic acid, and retinol, respectively. The ratio for 13-cis retinoic acid was significantly different from that of all-trans retinoic acid (P < 0.05). Serum all-trans retinoic acid and serum retinol concentrations did not correlate significantly in any group, nor did we find any correlations between the concentrations of the retinoic acids.

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State at ≈40% above the initial concentration. When all-trans retinoic acid concentrations were corrected for changes in binder protein concentrations, ie, albumin concentrations, this tendency was more obvious, with about a 2-fold increase in the second and third trimesters. This finding was confirmed by comparison of the all-trans retinoic acid:albumin for pregnant women in their third month and parturient mothers, which demonstrated significantly higher values for the parturient mothers. A possible explanation is that the selective rise in serum all-trans retinoic acid represents a physiologic adjustment to meeting increasing demands on the availability of this biologically active compound in the fetus, resulting in a favorable gradient of serum all-trans retinoic acid over the placental barrier.

Placental transfer was calculated as the ratio of newborn to maternal serum concentration. Newborns had significantly lower serum retinol concentrations than their mothers. This finding is consistent with earlier investigations that reported concentrations in newborns at ≈50–60% of the concentrations of their mothers (11–13) (≈65% in our study). The active metabolite all-trans retinoic acid showed a similar pattern, with placental cord serum concentrations ≈60% of the concentrations in pregnant women. In contrast, 13-cis retinoic acid had higher placental cord serum concentrations, ≈75% of maternal serum concentrations.

Retinoids exhibit more or less pronounced teratogenic actions in all species investigated. Probably, the teratogenic effects are exerted mainly by way of all-trans retinoic acid (9), although 13-cis retinoic acid and its metabolite 4-oxo-13-cis retinoic acid also may have teratogenic effects (5, 16). The exact roles of the various derivatives are difficult to evaluate, because all-trans retinoic acid can isomerize to 13-cis retinoic acid, and this reaction is reversible (3). For example, exposure to high doses of 13-cis retinoic acid is well known to induce birth defects.

The teratogenicity of 13-cis retinoic acid has considerable species variation (3, 4, 7, 9), which has been hypothetically ascribed to different detoxification pathways (3, 5, 9). In insensitive species, retinoids are glucuronidated to retinoyl-β-glucuronide (8, 16). This process leads to low placental transfer of 13-cis retinoic acid into embryonic tissues, because retinoyl-β-glucuronide shows limited placental transfer (5). However, in sensitive species, 13-cis retinoic acid has a slower metabolism to the active 4-oxo-13-cis retinoic acid, which also has a higher degree of placental transfer (3, 16). Humans belong to the sensitive species. In one study a low dose of 13-cis retinoic acid medication induced teratogenic effects in humans; the dose was 100 times lower (in mg/kg body weight) than the doses in mice and rats that induce teratogenic response (7). As shown in the present study, the placental transfer efficiency of 13-cis retinoic acid under physiologic conditions in humans seems to be higher (0.7) than in other species, such as monkeys (0.4; another sensitive species) and mice and rats (<0.1; insensitive species) (3). The biological mechanism behind the high concentrations of 13-cis retinoic acid remains to be elucidated. In addition, interpretation of our data suggests that there may be a tendency for the equilibrium of cis and trans isomers to be shifted toward higher concentrations of 13-cis retinoic acid in newborns than their mothers under physiologic conditions. If extrapolated to pharmacologic conditions, our results would be consistent with empirical data and lend mechanistic support for the clinical observation that humans are sensitive to the teratogenic effects of 13-cis retinoic acid.
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GAF and PN-E conceived and designed the study. MBS collected samples and analyzed the data with advice from PN-E. MBS wrote the manuscript and interpreted the results. PN-E made critical revisions to the paper. None of the authors had any conflict of interest.

REFERENCES