Low serum concentration of all-trans and 13-cis retinoic acids in patients treated with phenytoin, carbamazepine and valproate
Possible relation to teratogenicity

Received: 9 November 1994/Accepted: 13 February 1995

Abstract All-trans retinoic acid deficiency resulting from ethanol's interference with the synthesis of all-trans retinoic acid from retinol was recently suggested to cause the malformations of the fetal alcohol syndrome. Phenytoin, carbamazepine and valproate, might be teratogenic because they lower the concentration of all-trans retinoic acid in serum, by inducing the enzyme systems in the liver responsible for the metabolism of the all-trans retinoic acid, or by other mechanisms. Here we show, that in patients given therapeutic doses of phenytoin, carbamazepine and valproate, serum all-trans and 13-cis retinoic acid concentrations are indeed significantly lowered. We propose that drugs with this ability should be considered as potential teratogens.

Key words Retinoic acid · Phenytoin · Carbamazepine · Valproate · Teratogenicity

Introduction

All-trans retinoic acid is a control molecule in vertebrate morphogenesis (Wagner et al. 1990). High doses of all-trans retinoic acid or other retinoids taken during early gestation result in fetal malformations of the craniofacial type (Lammer et al. 1985; Hathcock et al. 1990). Deficiency of vitamin A in animals is also known to give rise to fetal malformations (Willhite et al. 1989) and it seems reasonable to assume that the same occurs in humans though supporting data are lacking to the best of our knowledge. Thus, both vitamin A deficiency and excess appears to interfere with normal fetal development (Willhite et al. 1989).

It was suggested (Keir 1990) that the fetal alcohol syndrome is caused by a deficiency of all-trans retinoic acid during critical phases of fetal development. The proposed mechanism was that ethanol competitively inhibited the synthesis of all-trans retinoic acid from retinol (Napoli and Race 1987; Keir 1990).

Low concentrations of all-trans retinoic acid in the serum could, however, also result from induction of the activity of the cytochrome P450 enzyme system responsible for the catabolism of all-trans retinoic acid to its 4-oxo derivatives (Martini and Murray 1993).

Among inducers of the activity of the cytochrome P450 system are the anticonvulsants phenytoin and carbamazepine, which are also considered teratogens in man (Briggs et al. 1994) and which induce a pattern of malformations similar to the fetal alcohol syndrome (Keir 1990). We therefore decided to investigate whether those two anticonvulsants could lower the all-trans retinoic acid concentration in serum in humans. We also included valproate which is another widely used anticonvulsant, which does not seem to induce drug metabolism in the liver.

Patients and methods

The patients were epileptic males and females 20-60 years old who were treated only with the anticonvulsant in question, all of whom had been on the drug for more than 3 weeks, in most cases for more than 2 months. Non-fasting venous blood samples were obtained (originally for drug monitoring purposes) in vacuum tubes without anticoagulant and the serum recovered by centrifugation and stored at -20°C in the dark until analysis about 2 weeks later. Eighty healthy blood donors, 40 men and 40 women, 20-60 years old, ten individuals per 10-year period, served as a controls. There was no significant variation in either all-trans or 13-cis retinoic acid concentration among the blood donors with regard to age and sex, so all the data were combined (Table 1).

All-trans and 13-cis retinoic acids were quantitated by high performance liquid chromatography (HPLC) as described by Wyss and Bücheli (1988). The calibration standards, pure all-trans and 13-cis retinoic acid (a gift from Hoffman-LaRoche), were weighed to concentration, dissolved in ethanol and aliquots added to a charcoal-treated serum matrix, devoid of retinoic acid. All handling of samples and retinoids was done under yellow light. The HPLC method
had a total imprecision (CV%) of 5.5% for all-trans retinoic acid and 5.9% for 13-cis retinoic acid, both at the level of 5 nmol/l. The sensitivity of the measurement was better than 0.5 nmol/l for both. The presence of retinol and 4-oxo all-trans and 13-cis retinoic acid was estimated qualitatively from the chromatograms.

**Results and discussion**

Patients with therapeutic serum levels of phenytoin, carbamazepine and valproate had serum concentrations of all-trans retinoic acid and 13-cis retinoic acid that were significantly lower than those in the controls subjects (Table 1). The levels of all-trans and 13-cis retinoic acid in controls were comparable to previous results (Tang and Russell 1991). Serum retinol concentration did not seem to differ between controls and patients; 4-oxo all-trans retinoic acid concentration was too low to be identified with certainty, either in the controls or in the patients, but 4-oxo 13-cis retinoic acid could be identified in all samples. Its concentration was much lower in patients than controls.

Phenytoin, carbamazepine and valproate are all considered teratogens in man (Briggs et al. 1994). For phenytoin, a recognizable pattern of malformations, the "fetal hydantoin syndrome", with craniofacial abnormalities and variable degrees of hypoplasia and ossification of the distal phalanges, was described in 1968 (Briggs et al. 1994). Carbamazepine and valproate exposure have been associated with congenital malformations consisting of craniofacial, heart and CNS defects and mild fingernail hypoplasia, i.e. a pattern similar to the "fetal hydantoin syndrome" (Briggs et al. 1994) and the fetal alcohol syndrome (Keir 1991). The latter consists of craniofacial defects, heart and limb malformations, neural tube defects and microcephaly.

The malformations reported in vitamin A deficiency in animals (Willhite et al. 1989) involve various craniofacial abnormalities including malformed external ears, cleft palate, anophthalmus/microphthalmus and abnormalities of the heart and the aortic arch (Willhite et al. 1989). Vitamin A excess, finally, is associated with craniofacial malformations, abnormalities involving the external ears, the maxilla and palate, and with defects of the heart and aortic trunk, the thymus, the retina and optic nerve and CNS (Lammer et al. 1985; Hathcock et al. 1990; Wagner et al. 1990) suggests that drugs which affect retinoic acid metabolism should be regarded as potential teratogens by virtue of the lowered all-trans retinoic acid levels, which may disrupt the specific role of retinoic acid in normal morphogenesis.

**Table 1 Serum concentration**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug conc* (µmol/l)</th>
<th>allRA (nmol/l)</th>
<th>13cRA (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin</td>
<td>40.2 ± 26.5 (5)</td>
<td>1.60 ± 0.60*** (7)</td>
<td>1.27 ± 0.30* (6)</td>
</tr>
<tr>
<td>Carbamazepin</td>
<td>27.3 ± 6.70 (9)</td>
<td>2.12 ± 0.47*** (9)</td>
<td>1.69 ± 0.40** (9)</td>
</tr>
<tr>
<td>Valproate</td>
<td>470 ± 149 (10)</td>
<td>3.69 ± 0.85* (10)</td>
<td>2.67 ± 0.61*** (10)</td>
</tr>
<tr>
<td>Blood donors</td>
<td>5.13 ± 0.99 (80)</td>
<td>4.79 ± 1.35 (80)</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.025, ***p < 0.01 (Wilcoxon signed rank test) patient groups compared to blood donors

*Therapeutic concentrations for the anticonvulsants are 40–80, 20–40 and 350–700 µmol/l, respectively.

There are thus several similarities between the patterns of malformations associated with the anticonvulsants, ethanol, vitamin A deficiency and vitamin A excess which may indicate a common cause.

The fact that we detected a decrease of all-trans and 13-cis retinoic acids in the serum of adult patients treated with phenytoin and carbamazepine does not, however, prove that this would also occur in a fetus. Likewise, the association of decreased concentration of all-trans retinoic acid in serum with a drug does not prove that the malformations associated with the drug are related to all-trans retinoic acid. More work is obviously needed to establish such a relationship.

The mechanism by which serum concentrations of retinoic acids is lowered by these drugs is also unclear. Increased metabolism of retinoic acids by drug-induced hepatic enzymes (phenytoin, carbamazepine) is one possibility, which apparently is not valid for valproate (Briggs et al. 1994). Evidently, this problem deserves more study.

However, the apparent importance of low levels of endogenous all-trans retinoic acid acting as a morphogen in the process of embryogenesis (Lammer et al. 1985; Hathcock et al. 1990; Wagner et al. 1990) suggests that drugs which affect retinoic acid metabolism should be regarded as potential teratogens by virtue of the lowered all-trans retinoic acid levels, which may disrupt the specific role of retinoic acid in normal morphogenesis.

**Acknowledgements** This study was supported by grants from The Swedish Medical Research Council (03X-03364), the Gyllenstierna Foundation, The Lunds Sjukvårdsdistrikt Foundations, The Medical Faculty, University of Lund and The Albert Påhlsson Foundation.

**References**

