Effect of intraamniotic vitamin A on palatal closure of fetal rats

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On day 15 of gestation, intraamniotic vitamin A in a dose of 150 IU was administered to the fetal rats to examine its effect on palatal closure. Fetuses subjected to only amniocentesis acted as control for the study. The fetuses were recovered on day 19, 20 and 21, respectively. Vitamin A resulted in poor development of palatine shelves. There was no clear demarcation of the base and the free margins of the shelves were either rounded or blunted with poor attempt towards closure. In the vitamin A group, the incidence of cleft palate were similar in all three days while there was a gradual decline with increasing gestational age in the amniocentesis group. The results suggest that unlike amniocentesis, in vitamin A treated fetuses, there was no attempt towards a delayed closure of the palate.

Palatal clefts are one of the major malformations in rat and mice fetuses following maternal exposure to excess vitamin A. Nanda demonstrated that vitamin A added to the culture medium prevents normal palatal closure in vitro, indicating a direct effect of vitamin A. Nanda and Rome effectively studied the teratogenic effect of vitamin A on rat fetus by intraamniotic administration and noted cleft palate as one of the major anomaly. Besides vitamin A, amniocentesis is also known to produce palatal clefts. However, the exact mechanism responsible for the genesis of cleft palate is still not clear. Some of the possible mechanisms described are the structural abnormality of the shelves and the reduction in the palatal shelf force. In a previous study on amniocentesis induced cleft palate, it was suggested that amniocentesis alone does not interfere with the palatal shelf force. The present study reports the effect of intraamniotic vitamin A on the palatal development of the Charles Foster rat fetus with special reference to the palatal shelf force.

Materials and Methods

Charles Foster rats having an average age of 120 days and weight of about 200 g were used. Females showing proestrus at 1700 hrs were caged overnight with the males of the same stock. When spermatozoa was found in the vaginal smear in the next morning at 0900 hrs, it was taken as the day 0 of gestation. The pregnant animals were kept individually in separate cages.

On gestational day (GD) 15, the rats were anaesthetised with ether inhalation and the uterine horns were exteriorised under aseptic precautions after opening the abdomen by a small ventral midline incision. The uterine horns were placed over a light source so that the amniocentesis as well as the intraamniotic administration of the vitamin A could be carried out under direct vision preventing injury to the fetus. The position and the number of the viable fetuses at the implantation sites were recorded in a diagram. In one group, only amniocentesis was done while in the other group, the fetuses were treated with intraamniotic vitamin A after being subjected to amniocentesis. A microlitre syringe was used for the procedure. The needle was inserted into the amniotic sac through the uterine wall and the amniotic membranes making a zig-zag tract to minimise the leakage of amniotic fluid. Amniocentesis was done by removing 0.04 ml amniotic fluid from each sac. For intraamniotic vitamin A administration, the syringe was disconnected following amniocentesis keeping the needle in place. Vitamin A (Aquasol A, USV India Ltd, 50,000 IU/ml) in a dose of 150 IU diluted in 0.03 ml sterile distilled water loaded in another syringe was fitted to the needle in place and the amount was pushed into the amniotic sac. Following the procedure, the uterine horns were returned to the abdominal cavity and the abdominal wall was closed. The mothers were sacrificed on GD 19, 20 and 21, respectively and the fetuses were recovered after noting the resorption sites and dead fetuses. The live fetuses were cleaned, blotted, weighed and were examined under dissecting microscope for various external malformations and deformities of the palate.

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The fetuses were fixed in Bouin's solution for histological examination. The palates were studied after 5 μm serial sections were stained with haematoxylin and eosin.

**Results**

The percentage of fetal death was higher in vitamin A treated group as compared to the amniocentesis group (Table I). In vitamin A treated rat fetuses, cleft palate was the most prominent congenital malformation observed in about 42% surviving fetuses as compared to 27% in the amniocentesis group. The incidence of cleft palate in two groups of fetuses recovered on GD 19, 20 and 21, respectively are shown in Fig. 1. The vitamin A treated fetuses showed similar incidence in all the three days while there was a gradual decline in the amniocentesis group.

Gross examination of the palatal surface of the specimens in vitamin A treated group revealed extensive and severe palatal clefts with wide gap between the unfused processes (Fig. 2B). The involvement of secondary palate most of the time included both hard and soft palate. Partial clefts were restricted either to the middle region of the hard palate or posterior part of the hard palate with involvement of the soft palate (Fig. 2A). In coronal sections (Fig. 3A-D), the palatal shelves were seen to be poorly developed with wide clefts. The shelves were very short, the free margins were rounded and not clearly demarcated. The base was at times difficult to outline. In some of the specimens (Fig. 3D), the shelves were stubby with blunting of free margins. The nasal septum was short and in most specimens it was not reaching the palatal cleft level. The position of the tongue was below the cleft level and the dorsum had prominent median ridge in some specimens.

**Discussion**

The palate is normally fused throughout its entire length by GD 17 in rats. However, the events leading to the elevation of palatal shelves from a vertical position lateral to the tongue to a horizontal position above the tongue is critical for palatogenesis. Before GD 14, the palatal shelves are not visualised in rats. On GD 14, the palatal shelves are seen as vertical growths from the maxillary process on either side of the tongue. Between GD 15 and 16, the shelves elongate in size and still hang vertically by the side of the tongue. The shelves elevate from a vertical to

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of fetuses</th>
<th>Fetal death and resorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD 19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>36</td>
<td>27 (75.0)*</td>
</tr>
<tr>
<td>Amniocentesis</td>
<td>75</td>
<td>43 (57.3)</td>
</tr>
<tr>
<td>GD 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>37</td>
<td>23 (62.1)</td>
</tr>
<tr>
<td>Amniocentesis</td>
<td>65</td>
<td>30 (46.1)</td>
</tr>
<tr>
<td>GD 21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>28</td>
<td>20 (71.4)**</td>
</tr>
<tr>
<td>Amniocentesis</td>
<td>40</td>
<td>17 (42.5)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>101</td>
<td>70 (69.3)**</td>
</tr>
<tr>
<td>Amniocentesis</td>
<td>180</td>
<td>90 (50.0)</td>
</tr>
</tbody>
</table>

Statistical significance (Z test) values: *<0.05; **<0.01; ***<0.001
shelf force is not interfered with, the palatal closure is possible till the last day of gestation.

In the present study, vitamin A was administered intraamniotically to observe its effect on the palatal closure process and the delayed palatal closure was evaluated by collecting the fetuses on GD 19, 20 and 21. However, there was no evidence of delayed palatal closure as the incidence of cleft palate were similar in the above gestational days. This was in contrast to the observations in amniocentesis group in

![Graph showing incidence of cleft palate in amniocentesis and vitamin A treated rat fetuses recovered on GD 19, 20 and 21, respectively.]

![Images of palates viewed from the ventral surface showing various types of clefts in vitamin A treated rat fetus. (A) Partial cleft involving the posterior part of secondary palate (arrow). (B) Complete cleft with wide apart free margins (arrow).]
Fig. 3—Coronal section of the palate on day 21 in vitamin A treated rat fetus HE stain x 42. (A) The palatine shelves are poorly developed. The base is not clearly demarcated and the free margins (arrow) are rounded. The gap between the two shelves is wide leading to a wide cleft. The tongue is situated much below the shelf level. (B) The palatine shelves (arrow) are poorly developed. Left side shelf is very small in size. The cleft is wide. (C) Left side shelf is poorly developed with a wide gap (arrow) between the shelf and the nasal septum. (D) The palatine shelves (arrow) are short, stubby and deformed with blunting of free margins. The nasal septum is quite high up from the shelf level. The tongue is not seen in the picture indicating a much lower level.

which the incidence of cleft palate gradually declined with increasing gestational age and the shelf elevation and fusion continued till the last day gestation. As the intact intrinsic shelf force is essential for the palatal closure, the absence of delayed closure in vitamin A treated rat fetus could be due to a sustained interference with the palatal shelf force. Walker and Crain also demonstrated a retardation of palatine shelf movement in hypervitaminosis A treated litters of mice.

For normal palatogenesis, proper anatomy of the palatine shelves is also essential. The shelves should have a proper base as it acts as a fulcrum for the upward rotation of the shelves. Therefore, teratogen induced alterations in the external and internal structures of the shelves would interfere with the palatal closure. In the present study, there was poor development of palatine shelves in vitamin A treated rat fetuses. The shelves were short, distorted and the base of the shelves were not clearly demarcated. Other workers also showed that hypervitaminosis A in rat embryos produce shelves that had reduced amount of mesenchyme and were either rounded, reduced in size or even absent at the posterior ends. Abbott et al suggested that the retinoic acid induced hypoplastic shelves were due to reduced extracellular matrix production as well as hydration because the rapid increase in shelf size before palatal closure is mostly attributed to increase in synthesis of hyaluronic acid and hydration of the extracellular matrix. It seems that a single basic mechanism is responsible for the proper anatomy as well as the physiology of the shelves. So it is not unreasonable to believe that the hypoplastic or the distorted shelves would have a reduced shelf force.

In conclusion, it may be said that vitamin A induced cleft palate is possibly due to formation of abnormal palatine shelves with reduced shelf force. However, it is often not possible to identify the precise means by which a particular teratogen acts to produce a given malformation. So more studies on vitamin A induced biochemical changes are required.
so that it could be conclusively linked with the morphogenetic process.

References