Journal of Pediatric Surgery (2011) **46**, 1400–1405

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Journal of Pediatric Surgery

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The effect of vitamin A deficiency during pregnancy on anorectal malformations

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Received 8 June 2010; revised 10 January 2011; accepted 2 February 2011

Key words:
Anorectal malformation;
Enteric nervous system;
Vitamin A;
PGP9.5;
S-100

Abstract

Objective: The aim of this study was to study the effect of vitamin A deficiency (VAD) on the embryological development of anorectal malformations (ARMs) and the enteric nervous system.

Materials and Methods: Female Sprague-Dawley rats were divided into 3 groups: VAD group, normal group (negative control), and ethylene thiourea (ETU) group (positive control) with a normal diet. On day 20 of pregnancy, cesarean section was performed on all rats. The incidence of ARMs in the fetal rats and Protein gene product 9.5 (PGP9.5) and S-100 protein expression by immunohistochemistry were determined.

Results: The incidence of ARMs in VAD and ETU groups was 64.8% (59/91) and 45.9% (61/133), respectively ($P > .05$). Anorectal malformations were not found in the normal group. Protein gene product 9.5 and S-100 protein expression in the non-ARM rectums of the VAD group was lower than the ETU ($P = .0156$ vs $P = .0105$) and normal groups ($P = .0091$ vs $P = .0024$). There was no significant difference in PGP9.5 and S-100 protein expression between ETU and normal groups. In the ARM rectums, PGP9.5 and S-100 protein expression in the VAD group was lower than the ETU group ($P < .0001$). Protein gene product 9.5 and S-100 protein expression was also lower in ARM than non-ARM rectums in the VAD and ETU groups ($P < .0001$, $P = .0203$, and $P = .0122$, respectively).

Conclusion: Vitamin A deficiency during pregnancy may result in the embryological development of ARMs. Enteric nervous system development may be related to ARMs.

Anorectal malformations (ARMs) are congenital malformations that occur during hindgut development. Improved knowledge and surgical preservation of the anorectal muscles have decreased the incidence of incontinence. Unfortunately, postoperative bowel dysfunction, such as fecal soiling and constipation, significantly affect quality of life. Prior research has begun to elucidate the effect of the enteric nervous system (ENS) on normal bowel function and that abnormal ENS development is present in the rectal pouch of approximately 60% of all anal atresia cases [1]. Protein gene product 9.5 (PGP9.5) and S-100 antibodies are expressed in the ENS with S-100 staining, highlighting negatively stained ganglion cells. These antibodies, if used together, are complementary and enhance the sensitivity and specificity for ARM diagnosis in the ENS of the rectal pouch.

The etiology and the pathologic mechanism of ARMs have not been fully elucidated. It is generally accepted that ARMs may be associated with viral [2], chemical [3], environmental [4], nutritional [5], and other factors [2,6],

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doi:10.1016/j.jpedsurg.2011.02.042

during pregnancy. Vitamin A (VA), or retinol, is a fat-soluble vitamin and a necessary micronutrient for human growth and development. Its metabolic products are involved in many physiologic processes, including vision, reproduction, growth, cellular differentiation, immune response, and embryonic development. Dietary VA deficiency (VAD) can cause metabolic abnormalities that may lead to various pathologic states. In this study, we evaluated the effects of VAD during pregnancy on the occurrence of ARMs in fetal rats and the development of ENS in the rectum.

1. Materials and methods

All experimental protocols were compliant with the Helsinki Act and approved by our institutional ethics committee.

1.1. Materials

1. Animals: adult female nonparous Sprague-Dawley (SD) rats aged 3 months (weight, 200-250 g) were provided by the Animal Center of Fudan University School of Medicine.
2. Diet: a VAD diet was prepared according to a formula developed by the Association of the United States Official Analytical Chemists.

1.2. Methods

1.2.1. Experimental groups and the establishment of the fetal rat model of ARMs

Sixty adult female SD rats were randomly divided into 3 groups and fed with a normal diet. Different doses of dietary VA were administered from 2 weeks before pregnancy to the end of pregnancy. (1) The VAD group (n = 20) was fed a diet without VA, (2) the normal control group (negative control, n = 20) was fed a normal diet, and (3) the ethylene thiourea (ETU) group (positive control, n = 20) was fed a normal diet.

After being fed for 2 weeks, 5 female rats and 1 adult male SD rat (fed with a normal diet) were placed in the same cage at 20:00 hours. At 09:00 hours, vaginal secretion smears were observed under a microscope. If sperm or vaginal suppositories were found, this was considered gestational day 0. Rate of pregnancy was calculated based on the ratio of rats that did or did not become pregnant within 1 week. At gestational day 10, ETU group rats were fed with 1% ETU (125 mg/kg) via nasogastric tubes. Vitamin A deficiency and normal control group rats were administered saline. All fetal rats were delivered by cesarean section on gestational day 20 (full term).

1.2.2. Outcome indexes

1.2.2.1. Serum VA content in pregnant rats. Serum samples (2 mL) were collected from the orbital vein 2 weeks before pregnancy and at gestational day 0.

1.2.2.2. Incidence of live fetal rats with ARMs. An ARM was diagnosed if there was no anus visible in the normal location of the anal orifice by microscopic evaluation.

1.2.2.3. Immunohistochemistry

1.2.2.3.1. Specimen preparation. Ten fetal rats with or without ARMs were randomly selected from each group. The anorectal area with the surrounding tissues were excised, fixed with 4% paraformaldehyde, paraffin embedded, and cut into 4- to 5-μm coronal serial sections. One slice was prepared with hematoxylin-eosin staining to identify the histologic location.

1.2.2.3.2. Immunohistochemical staining. The Envision 2-step method was used. Rabbit antirat PGP9.5 (PGP9.5, Dako Co, Shanghai, China) and S-100 protein polyclonal antibodies (S-100, Dako Co) were used. By qualitative judgment, positive results were recorded if they appeared brown-yellow. Protein gene product 9.5 was routinely expressed in the nucleus and cytoplasm of glia and neurons. S-100 was expressed in neuronal membranes.

1.2.3. Imaging and statistical analysis

Five high-power fields (20 × 10) with good expression characteristics were randomly selected from each slice. QWIN3000 bio-image analysis software (Zhuhai, China) was used to determine the average percentage of positive areas within each field. SPSS software version 14.0 (Chicago, IL) was used for statistical analysis. All data are shown as mean ± SD. The χ^2 test or 1-way analysis of variance were used to evaluate differences between groups. $P < .05$ was considered statistically significant.

2. Results

2.1. Serum VA contents in pregnant rats

Two weeks before pregnancy, serum VA levels were not significantly different between groups. After being fed a VAD diet for 2 weeks (at gestatinal day 0), serum VA concentrations in the VAD group were significantly lower than those in the normal control (0.56 ± 0.03 vs 0.76 ± 0.03 μmol/L; $P = .0310$) and ETU groups (0.56 ± 0.03 vs 0.72 ± 0.03 μmol/L; $P = .0401$). Thus, it appears that rats fed a VAD diet were indeed VA deficient.

2.2. The incidence of ARMs in fetal rats

The number of surviving fetal rats in the VAD, ETU, and normal control groups was 91, 133, and 124, respectively. Anorectal malformations rates in surviving fetal rats in the VAD and ETU groups were 64.8% (59/91) and 45.9% (61/133), respectively ($P > .05$). No ARMs were found in the normal control group. For fetal rats with ARMs (Fig. 1A-C), the incidence of no tail and short tail deformities in the VAD group were 89.8% (53/59) and 10.2% (6/59), respectively;