Pediatr Surg Int (2008) 24:561–565
DOI 10.1007/s00383-008-2125-9

ORIGINAL ARTICLE

Morphologic study of embryonic development of rat duodenum through a computerized three-dimensional reconstruction: critical analysis of solid core theory

Ivens Baker Méio · Ivonete Siviero · Saulo M. R. Ferrante · Jorge J. Carvalho

Accepted: 27 February 2008 / Published online: 20 March 2008

Abstract To date, intrinsic obstructions of the duodenum have been explained by the "solid core" theory, described by Tandler in 1902 (Morphol Jahrb 29:187–216, 1902). This study aimed to evaluate the epithelial occlusion of rat duodenum during embryonic development, through optical microscopy and computerized three-dimensional reconstruction. The Wistar rat embryos used in this study had 13, 14, 15, and 16 days of gestation. This corresponds to human embryos with 33, 40, 44, and 52 days of development, which is between the fifth and eighth week. The study included 12 embryos studied by optical microscopy, and four by three-dimensional reconstruction (those with 13, 14, 15, and 16 days). Through optical microscopy, an intense epithelial proliferation was observed in the gestation embryo of 13 days, with no occlusion of the opening of the duodenum. In the embryos with 14, 15, and 16 days of gestation, an increase in diameter of the duodenum was observed along with intestinal development. Through three-dimensional reconstruction, it was observed that the opening of the digestive tube of rat embryos with 13–16 days of gestation is never obstructed by epithelial proliferation, although it may follow a sinuous path. This study concludes that the "solid core" phase described by Tandler does not occur in the embryonic development of rat duodenum.

Keywords Duodenum · Embryogenesis · Solid core · Three-dimensional computerized · Wistar rats

I. B. Méio (✉) · I. Siviero · S. M. R. Ferrante
Department of Surgery, Division of Pediatric Surgery,
Medical School of Federal University of Rio de Janeiro,
Instituto de Pediatria e Puericultura Martagão Gesteira,
Av. Brigadeiro Trompovsky s/n, Ilha do Fundão,
Rio de Janeiro CEP: 21941-590, Brazil
e-mail: ivensbm@terra.com.br

I. Siviero
e-mail: ivonetesiviero@terra.com.br

J. J. Carvalho
Department of Histology and Embryology,
Medical School of State University of Rio de Janeiro,
Rio de Janeiro, Brazil

Introduction

Congenital duodenal obstruction is an anomaly of the first trimester of gestation that may be present as complete or partial, intrinsic or extrinsic variety. The intrinsic varieties consist of atresias, stenosis, and diaphragms, whereas the extrinsic varieties may occur on account of intestinal malrotations, intestinal duplications, vascular narrowness, and annular pancreas [1].

The origin of intrinsic duodenal obstructions has so far been correlated with the "solid core" theory proposed by Tandler in 1902 [2]. However, there are several further studies in human embryos in the same stage of duodenal development showing that the total duodenal occlusion is not constantly true. So, this theory has been questioned [3–7].

On experimental basis, only one study was performed in 1998 by Cheng and Tam who studied by themselves, through optical microscopy, the duodenal embryology of female rats between the 9th and 20th days of gestation. They observed that although important epithelial proliferation had happened, total obstruction never existed [8].

The present experimental study is aimed to assess the epithelial duodenal occlusion throughout the embryogenesis, through optical microscopy and three-dimensional computerized reconstruction of duodenum. The Wistar rat

embryos used had 13, 14, 15, and 16 days of gestation, which are equivalent to human embryologic stages of 33, 40, 44, and 52 days, according to the Carnegie Institution [9–11], that is, between the fifth and eighth week of development.

Materials and methods

Animals

Ten females and two males of Wistar lineage at 10–12 weeks of age were eligible for the study. The females had a weight range of 210–245 g and the males had a range of 320–330 g. The rats were kept under controlled conditions of temperature, fed with anima1 food adequate to the race, and received fresh water ad libitum. At the time of estrus, the animals were placed for 1 h in the environment created for them to mate. After this time, the observation of a mucous pad meant that there was copulation. This day was considered the Zero day. Female rats with positive mucous pad were kept in separate cages. From ten females, four fecundated (meeting the criterion of positive mucous pad) females were selected and sacrificed at 13, 14, 15, and 16 days of gestation.

Embryos

The embryos were extracted through laparotomy of the pregnant females, with hysterectomy. The number of embryos in each gestation ranged from 6 to 11. Three embryos of each rat, of similar size, were selected to be processed and studied.

After the extirpation of embryonic sac, the embryos were fixed in 10% formaldehyde solution for 24 h. After this period of time, the embryos were washed in running water for 30 min, dehydrated and clarified with increasing concentrations of Xylol, embedded in paraffin twice, and finally blocked with paraffin.

Sections were made with a microtome, with a thickness of 8 μm, always in the Crown Rump (CR) direction. We used slides treated with silane (silicon tetrahydride) placed one by one, always from left to right, numbered in increasing order in the predetermined direction These slides were stained with hematoxylin and eosin (HE).

The slides were examined one by one. The place of the beginning and the end of reconstruction was identified, comprising the foregut and midgut. The foregut and midgut lumen were observed through conventional microscopy, magnified 40×, in the processing sequential order. The specimens with a logical and accurate progression of the intestinal tract, without damaged slides, were studied.

Twelve embryos (three of each pregnant rat) were evaluated under optical microscopy and four, one of each gestational age, that is, 13, 14, 15, and 16 days of gestation corresponding to the embryonic human stages of 33, 40, 44, and 52 days, were processed for the three-dimensional reconstruction.

Three-dimensional reconstruction (3D)

We used a video microscopy system with a BX0 40 microscope connected to an auxiliary video monitor, and an Optronic digital camera, both connected to a Pentium 4 micro computer with a 2.8 GHz, 512 DDR processor. The program used was the Image pro plus (Media Cybernetics). The captured image was stored with a length of 1,280 × 1,024 pixels in the tagged image format (TIF) format, with section position identification. Images were centralized by fixed dots kept in the screen corresponding to embryo notochord and primitive aorta. Starting from the first image and following the dots digitally created for notochord, the remaining images were sequentially captured.

With the 3D reconstruction program, the images were processed to highlight the required anatomic structures. The structures not required were digitally removed, filling the spaces in black, in order to clearly see the lumen of the primitive gut. In order to be seen and to create the 3D model, the lumen spaces were digitalized in a light color that was artificially created and had an invariable tone. This invariable tone was necessary for a capture without noises. The program created three-dimensional images that could be reviewed in the three axes, including animation.

Results

Optical microscopy

Complete closure of the duodenal lumen was not observed, except for images similar to vacuoles, which were not seen in the following observations. An increase of duodenum diameter was seen, but the wall thickness remained practically the same.

In the embryos with an age of 13 days, the rat foregut was differentiated in stomach and duodenum and strong epithelial proliferation without lumen occlusion was seen (Fig. 1)

At 14 days of gestation, larger spaces were seen in duodenum lumen, without apparent occlusion in any slide. A strong increase in the digestive tract diameter and length was seen.

At 15 days, these findings of lumen enlargement and intestinal growth were more evident. Duodenal lumen in

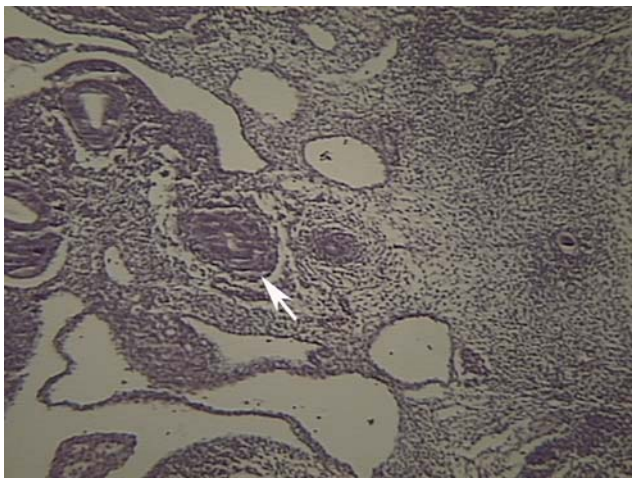

Fig. 1 Cross-section of a rat embryo at 13 days, showing epithelial proliferation without complete lumen occlusion (*arrow*); HE, ×40

the 16-day embryo increased further, and the presence of pancreatic tissue was also observed (Fig. 2)

At this gestational age, it was possible to observe herniated intestinal tracts outside the abdominal cave.

Three-dimensional reconstruction

Serially captured images demonstrated that the intestinal tract lumen of rat embryos at 13, 14, 15, and 16 days, although sometimes has a sinuous path, is never obstructed by cell proliferation at any time. With bi-dimensional images, the intestinal lumen, examined through optical microscopy enhanced 40×, appears to be filled with a mass of epithelial cells. However, in the following sections, this aspect disappears. Images in motion, with rotation and through scanning, clearly demonstrated the perviousness of the whole digestive tract, although sometimes the path is sinuous (Figs. 3, 4, 5)

Because of animation, the figures only show a yellow sinuous path representing the duodenal lumen, encircled by gray tones around its walls, and the suppression of the remaining structures for better visualization. The duodenal patency becomes evident through the three-dimensional visualization and animation.

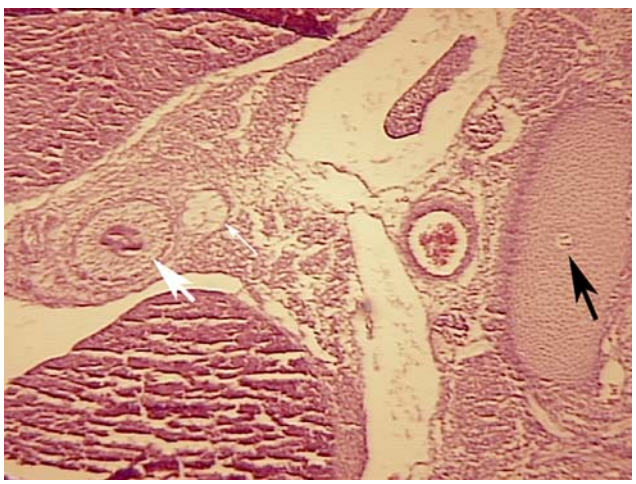

Fig. 2 Cross-section of a 16-day rat embryo showing pancreatic tissue (*arrow*) and enlargement of duodenum lumen (*arrow large*) and notochord (*black arrow*); HE, ×40

Discussion

According to the current embryologic thoughts, between the sixth and eighth weeks, the digestive tract of the embryo (stages 17–19 or 15–20 mm) undergoes intense epithelial proliferation with complete lumen obstruction that gradually disappears. This epithelial proliferation is particularly intense in duodenum, mainly in the vicinity of papilla, with complete lumen occlusion (solid core). Tandler made this observation in the beginning of the nineteenth century [2] and so far it has been thought to be the cause of intrinsic duodenum obstruction.

Other congenital intestinal malformations, such as esophagus atresia, jejuno-ileal atresia, and anorectal anomalies, which were explained by solid core theory, have gradually been questioned.

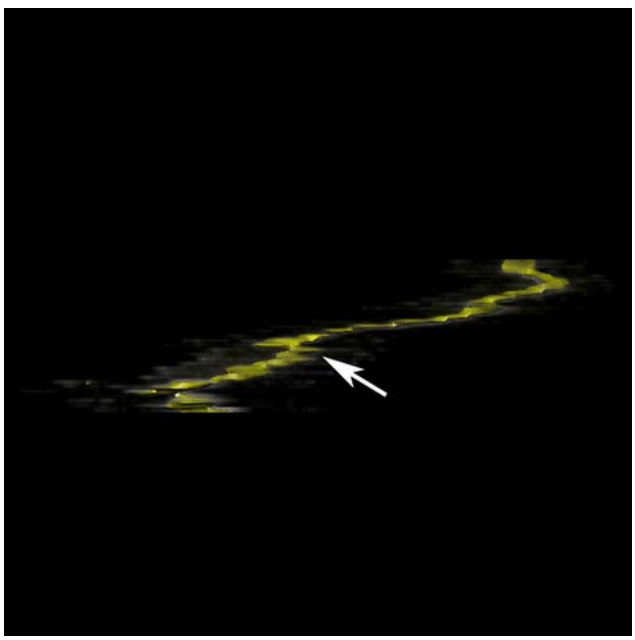

Fig. 3 Three-dimensional reconstruction profile of a 14-day embryo. The structures not required were digitally removed, filling the spaces in *black*, in order to clearly see the lumen of primitive gut. The duodenum is visible in *gray* tone with the lumen in *yellow*. Sinuous appearance of intestinal lumen (*arrow*)

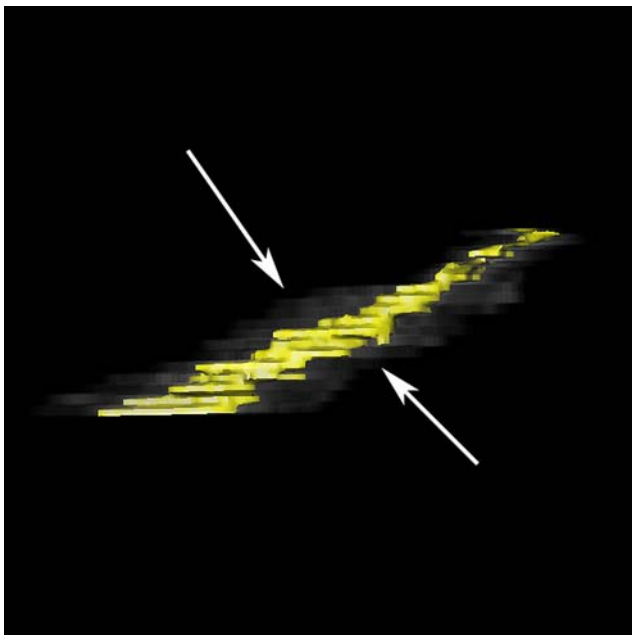

Fig. 4 Three-dimensional reconstruction profile of a 15-day embryo. Intestinal lumen in *white*. See the increased diameter (*arrow*)

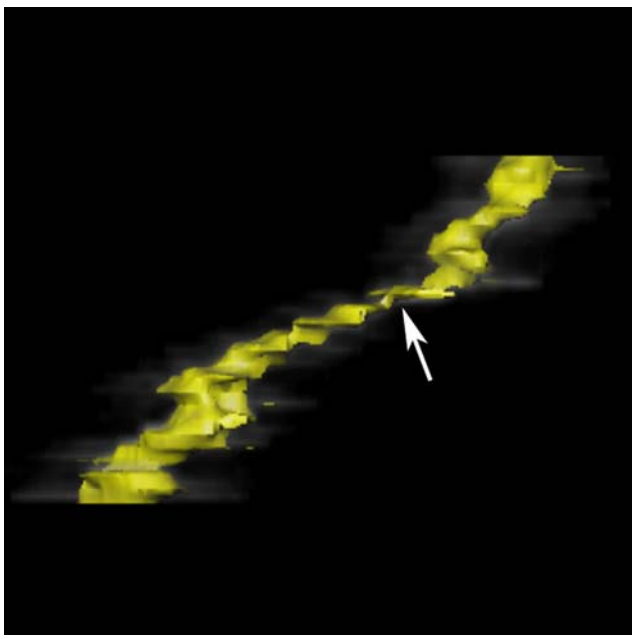

Fig. 5 Three-dimensional reconstruction profile of a 16-day embryo. Intestinal lumen in *yellow*. Exponential increase of intestinal lumen with sinuous aspect (*arrow*)

Reinforcing Tandler's hypothesis, in 1967, Boyden et al. analyzed four human embryos of 9.5, 11, 18, and 25 mm, and performed duodenum graphic reconstruction. The authors found epithelial proliferation and spaces in the duodenum of the most developed embryos (18–25 mm). This fact was explained by vacuole formation. They concluded that the stage of epithelial proliferation, which might be responsible for the duodenal lumen obstruction, occurs between 30 and 50 days of gestation and this phenomenon is caused by epithelial proliferation without a proportional mesenchymal growth [5].

Therefore, the complete duodenum occlusion between the fifth and the tenth week is not a constant fact and has been questioned. In 1966, Moutsouris [6] analyzed 70 human embryos with serial sections and found proliferation in the duodenum with patent lumen in 50% of embryos between 9 and 24 mm CR.

In 1977, Méio IB developed a project of a 21 mm embryo (CR) reconstruction corresponding to the seventh week of gestation. Through conventional microscopy, intense epithelial proliferation in the primitive duodenum was found. In two sections, vacuoles with foregut lumen occlusion were seen. The embryo reconstruction was performed by hand and at the end, the vacuoles seen through the microscope were actually part of the same sinuous path with a patent lumen observed in different sections [12].

In 1998, Cheng and Tam studied rat embryos between the 9th and 20th days of gestation, observing that although there was important epithelial proliferation, there was not, at any time, complete occlusion of the duodenal lumen [8].

These authors also studied duodenal apoptosis of rat embryos. They observed embryos at 13–20 days of gestation and evaluated the number of apoptotic cells. They concluded that the number of 7–8 cells per field was not compatible with the duodenum "opening" phenomenon. They also saw that, whereas the increase of the duodenum diameter was intense between 17–19 days, the wall thickness remained practically the same. So, the authors concluded that the duodenal lumen grows according to the growth of the duodenal diameter [13].

Besides the morphologic study of the embryonic development of rat duodenum under microscopic optics, this study aimed at providing additional evidences that the complete obliteration of duodenal lumen does not exist. Further studies employing more sophisticated techniques such as magnetic resonance, and using human embryos, may provide additional evidence.

Therefore, this experimental study, although performed in rats, suggests that Tandler's theory has no scientific support. Such assumption is also further corroborated by the reconstruction performed in one human embryo by Méio [12], the morphogenic genes' participation in digestive tract anomalies using experimental models like in humans [14], and the lack of intense apoptosis in duodenum embryological development in experimental animals [13]. All these arguments suggest that duodenal obstructions might be secondary to changes in embryo organogenesis, making the stage of epithelial proliferation, a normal, non-obstructive occasion of the intestinal embryonic development.

Conclusion

In the present study, we concluded that throughout the embryonic development of the rat duodenum, analyzed through optical microscopy and three-dimensional computerized reconstruction, the solid core stage described by Tandler does not occur.

References

1. Puri P, Sweed Y (1996) Duodenal obstructions. In: Puri P (ed) Newborn surgery 1. Butterworth-Heinemann, Cambridge, pp 290–297
2. Tandler J (1902) Zur entwicklungsgeschichte des menschlichen duodenum in frühen Embryonalstadien. Morphol Jahrb 29:187–216
3. O'Rahilly R, Müller F (1984) Respiratory and alimentary relations in staged human embryos. New embryological data and congenital anomalies. Ann Otol Rhinol Laryngol 93:421–429
4. Kluth D, Steding G, Seidl W (1987) The embryology of foregut malformations. J Pediatr Surg 22:389–393
5. Boyden EA, Cope JG, Bill AH Jr (1967) Anatomy and embryology of congenital intrinsic obstruction of the duodenum. Am J Surg 114:190–202
6. Moutsouris C (1966) The "solid stage" and congenital intestinal atresia. J Pediatr Surg 1:446–450
7. Madden JL, McCann WJ (1956) Congenital diaphragmatic occlusion of the duodenum, with report of three cases. Surg Gynecol Obstet 103:1–15
8. Cheng W, Tam PK (1998) Murine duodenum does not go through a "solid core" stage in its embryological development. Eur J Pediatr Surg 8:212–215
9. Streeter GL (1987) Developmental horizons in human embryos. Description of age groups XIX, XX, XXI, XXII, and XXIII, being the fifth issue of a survey of the Carnegie collection (prepared for publication by C. H. Heuser and G. W. Corner). Carnegie Institution, Washington, Publ 592, Contrib Embryol 34:165–196, 1951. In: O'Rahilly R, Müller F (eds) Developmental stages in human embryos. Carnegie Institution, Washington
10. Hill M: Rat development. Available in http://anatomy.med.unsw.edu.au/cbl/embryo/OtherEmb/Rat.htm
11. Hill M: Mouse development–estrus cycle. Available in http://embryology.med.unsw.edu.au/OtherEmb/mouse2.htm
12. Irving IM (1990) Duodenal atresia and stenosis: an annular pancreas. In: Lister J, Irving IM (eds) Neonatal surgery, 3rd edn. Butterworth's, London, pp 424–441. Cit. personal, Méio IB
13. Cheng W, Tam PK (2000) Apoptosis in murine duodenum during embryonic development. Pediatr Surg Int 16:485–487
14. Mo R, Kim JH, Zhang J et al (2001) Anorectal malformations caused by defects in sonic hedgehog signaling. Am J Pathol 159:765–774