Early Morphological Changes Leading to Central Polydactyly, Syndactyly, and Central Deficiencies: An Experimental Study in Rats

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Purpose: Various combinations of central polydactyly, syndactyly, and cleft hand have been frequently observed in the individual hands and feet in the same patients. Little is known, however, about the early changes of abnormal induction of digital rays during limb development. To determine the early changes and process of formation of central polydactyly, syndactyly, and cleft hand, we experimentally induced these anomalies in the hind limbs of rat embryos and discussed the relationship among these abnormalities.

Methods: Inbred WKAH/Hkm rats were used for this study. Pregnant females were treated with busulfan at embryonic day (E) 11. The embryos were removed at E12 to E21 and stained with alcian blue and alizarin red S. The abnormal changes in the treated embryos' hind limbs were observed with a microscope.

Results: The edges of the footplates were irregular, and their growth was reduced at E14. By E15, abnormal clefts in the distal edge were present that disrupted the central digits (2 to 4) of the footplates. Because of these abnormal clefts, the digital rays were bent or branched, and the neighboring interdigital spaces were narrowed. These changes led to central polydactyly, syndactyly, and central deficiencies.

Conclusions: These findings show that central polydactyly, syndactyly, and central deficiencies have the same early morphological changes: abnormal clefts in the central part of the footplate. (J Hand Surg 2007;32A:1413–1417. Copyright © 2007 by the American Society for Surgery of the Hand.)

Key words: Polydactyly, syndactyly, cleft hand/foot, limb morphogenesis, busulfan.

The appearance of central polydactyly, syndactyly, and cleft hand are quite different from each other, and each of these anomalies has been placed into a separate category in Swanson's classification.[1] Complicated combinations of central polydactyly, syndactyly, and cleft hand, however, are frequently observed in both individual and bilateral hands and feet in the same patients. In addition, experimental studies have shown that central polydactyly, syndactyly, cleft hand, and their various combinations can be induced simultaneously in rat embryos whose pregnant mothers have been treated with a teratogen at a critical time.[2–4] Based on these clinical and experimental observations, several authors have proposed that central polydactyly, syndactyly, and cleft hand should be classified into a single category.[2–10]

Little is known, however, about the process through which differences in phenotype between central polydactyly, syndactyly, and cleft hand can develop. There has been no experimental study to determine the early changes leading to these limb anomalies. The present study was done to determine the early changes and process of formation of central polydactyly, syndactyly, and cleft hand, and to address the classification of these conditions.

The hind limbs are formed later than the forelimbs in the development. Therefore, with the larger size embryo, it is easy to manage these specimens and also to observe the abnormalities in hind limbs com-

pared to the forelimbs. Moreover, the results from this study can be compared with those of other types of longitudinal deficiencies, in which hind limbs were used as a model of longitudinal deficiencies. Therefore, in this study hind limbs with central deficiency were used as a model of cleft hand (central deficiency of the hand).

Materials and Methods

Inbred WKAH/Hkm rats were used for this study. Nulliparous females and males were mated overnight. The morning when spermatozoa were found in vaginal smears by microscope was designated embryonic day (E) 0. A total of 49 pregnant females at E11 were given a single oral dose of 20 mg/kg busulfan. Busulfan is used to treat chronic myelocytic leukemia. The treated embryos were then removed at E12, E13, E14, E15, E16, E18, and E21. Numbers of treated embryos were as follows at each day: 18 embryos at E12, 18 embryos at E13, 35 embryos at E14, 51 embryos at E15, 63 embryos at E16, 29 embryos at E18, and 56 embryos at E21. Embryos of treated females were compared to a group of control animals that did not receive the busulfan. Numbers of control embryos were as follows at each day: 21 embryos at E12, 19 embryos at E13, 21 embryos at E14, 18 embryos at E15, 42 embryos at E16, 18 embryos at E18, and 21 embryos at E21.

Cartilaginous and Skeletal Staining

To identify the anomalies induced in these hind limbs, these embryos were whole mounted and fixed in 95% ethanol for 4 days. The cartilage and bone in these whole embryos were stained with alcian blue and alizarin red S as described.[11] Embryos that were not exposed to busulfan were processed in the same way as controls.

The abnormal changes in the treated embryos' hind limbs were observed with a Leica MZ-8 microscope (Leica Microsystems GmbH, Wetzlar, Germany). A total of 160 control embryos and 270 treated embryos were used in this study. In all, 315 hind limbs of control embryos and 523 hind limbs of treated embryos were observed in this study. The residual 5 hind limbs of control embryos and 17 hind limbs of treated embryos were inadvertently destroyed through the process of the staining. Animal care was carried out with the prior approval of the animal experimental guidelines of our university.

Results

Compared with the embryo hind limbs in the control group, which showed no unusual changes, morphological abnormalities in the treated group were initially detected at E14. At this time, growth of the central parts of treated footplates was reduced, and the edges were irregular (Figs. 1B, 1C) compared with the control group (Fig. 1A). By E15, abnormal clefts in the central edges of the footplates were evident. Beginning at E15, two patterns were evident: in some cases, an abnormal cleft was present at the level of an interdigital web space and in other cases, an abnormal cleft was noted in line with one of the central digital rays. When an abnormal cleft was present at the level of an interdigital web space, the neighboring digital rays were bent, and the interdigital space was widened (Fig. 2B). When an abnormal cleft was present in line with one of the central digital rays, the ray was widened (Fig. 2C). With association of this bending or widening of the digital rays, the surrounding interdigital spaces were widened or nar-

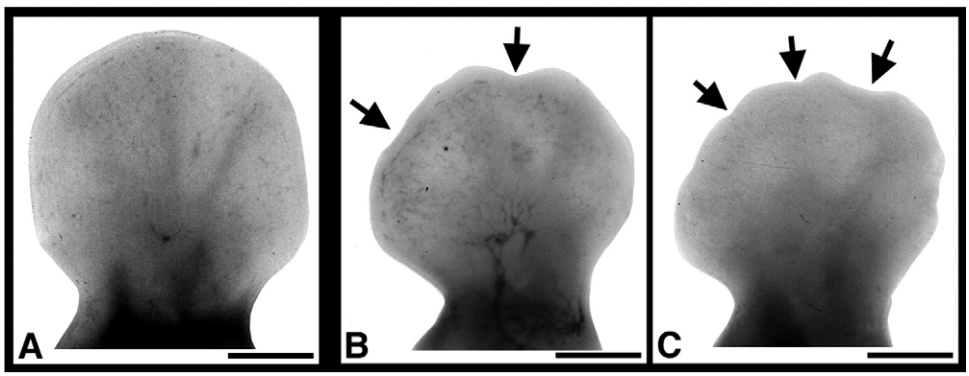

Figure 1. Dorsal views of right hind limbs stained with alcian blue and alizarin red S at E14. (A) Hind limb of control embryo at E14. (B, C) Hind limbs of treated embryos at E14. The growth of central parts of footplates were reduced and the edges were irregular (arrows). Bar=500 μm.