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Mechanism of Bone and Cartilage Maldevelopment in the Warfarin Embryopathy

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Introduction

Teratogenicity in humans of warfarin and related vitamin K antagonist anticoagulants was first clearly recognized about 12 years ago [1–3]. Coumarin derivative exposure can result in four distinct untoward effects: hemorrhagic manifestations in the newborn if it is used shortly before delivery; a nonspecific increased rate of spontaneous abortion; central nervous system disruptive effects presumably secondary to intrauterine hemorrhage; and the warfarin embryopathy [4]. The last is characterized by abnormalities of cartilaginous and bony development [4]. The principal clinical marker is rather marked nasal hypoplasia with underdevelopment of the nasal cartilage resulting in a flat, upturned nose with or without a deep groove between the alae nasi and nasal tip [4]. This hypoplasia of the nose, with or without true choanal stenosis, often results in neonatal respiratory distress [4]. The other major manifestation of the warfarin embryopathy is a characteristic radiologic abnormality: punctate radiologic densities (stippling) in uncalcified periepiphyseal regions, most often primarily in the axial skeleton, at the proximal femora and in the calcanei [4]. Additional abnormalities of the digits – most often brachydactyly secondary to distal digital hypoplasia – are common [4]. These limb abnormalities together with the nasal hypoplasia and stippling seem to indicate a generalized effect of coumarin derivatives on bone and/or cartilage development resulting in both premature, excess or aberrant mineralization and abnormal growth.

The warfarin embryopathy occurs only after exposure to oral anticoagulants during a critical period in the first trimester between approximately 6 and 9 postconceptual weeks [4]. Yet relatively few embryos at risk because of appropriately timed exposure show these characteristics. In fact, for pregnancies in which exposure occurs in the 6–9 week the risk that the warfarin embryopathy will result is probably in the vicinity of 4–6% [4, Pauli, unpubl. obs. 1987].

The warfarin embryopathy may serve as an interesting model, not only of human teratogenesis but also for a series of disorders which are characterized principally by stippling – the various forms of chondrodysplasia punctata [5] and related disorders (fig. 1). Unfortunately no animal model for

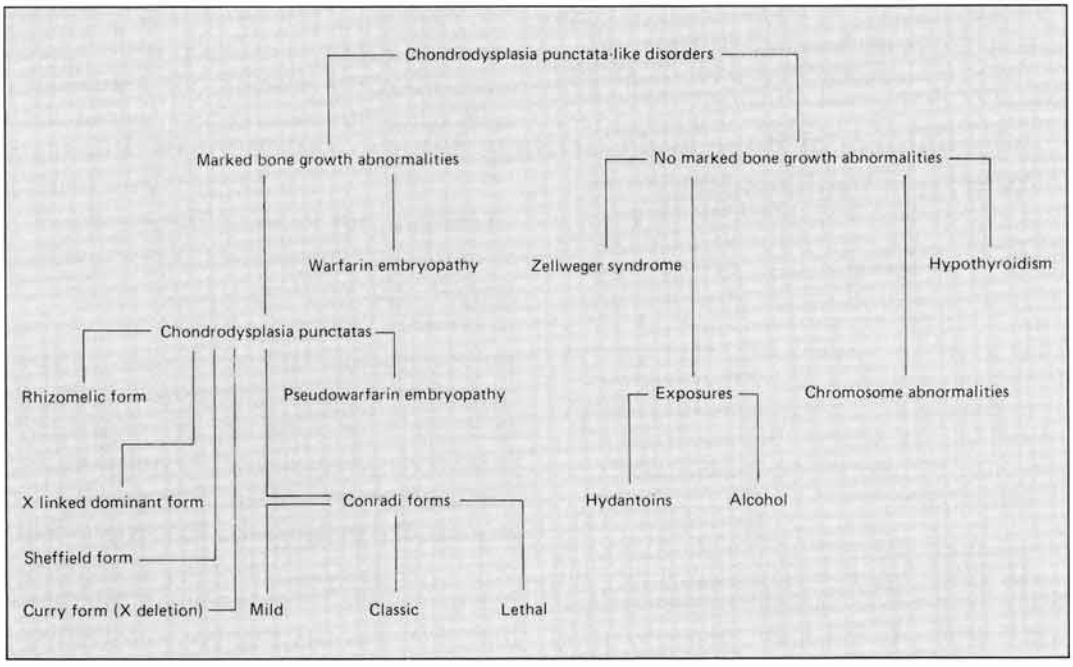

Fig. 1. Outline tree of the various syndromes and disorders which are related through the presence of stippled epiphyses.

the warfarin embryopathy has been generated. Furthermore, while it has been postulated that the features of the warfarin embryopathy result from the primary pharmacologic effects of coumarin derivatives [4, 6], neither direct nor indirect proof of this hypothesis has been previously obtained. This paper records the clinical and biochemical features of two boys whose inborn error of metabolism sheds light on this question [7, Leonard, personal commun. 1987].

Case Histories, Results and Discussion

The first boy [7] (proband 1) was born at term following an essentially uncomplicated pregnancy during which there was no exposure to anticoagulants or antiepileptics. During his vaginal delivery he incurred a 3-cm scalp laceration when an episiotomy was performed. Despite suturing, the wound continued to bleed. Because of abnormal coagulation measures and clinical evidence for decreased intravascular blood volume, fresh blood products were administered following which bleeding did not recur. He bruised easily throughout infancy and had three significant, difficult to control episodes of bleeding in his first $3^1/_2$ years of life. An uncontrollable nosebleed at that age led to coagulation studies. Then, and subsequently these have shown markedly prolonged prothrombin times and selective deficiency of vitamin K dependent factors (II, VII, IX, X). An inborn error of vitamin K utilization was

assumed and oral vitamin K therapy initiated which resulted in resolution of clinical symptoms and partial correction of vitamin K dependent clotting parameters. From these data it seems likely that this boy had a combined coagulopathy secondary to abnormalities of vitamin K handling as has been previously reported in 6 other individuals [8–12].

In addition to these hematologic problems he was said to have a small nose and 'stubby' fingers. The only additional medical problem that has developed is mild conductive hearing loss. I evaluated him when he was $7^1/_2$ years of age because of parental concerns about the implications of his mildly dysmorphic features. Positive physical features were limited to the face and hands. He has persistent flattening of the nasal base and a small anteverted nose with mild septation between the alae nasi and nasal tip. The upper limbs showed brachydactyly secondary to distal digital hypoplasia. Both infant and current radiographs confirm the clinical impression of hypoplasia of the terminal phalanges. In addition, radiographs taken in the first day of life show increased irregularity and mild stippling in the perilumbar and perisacral region.

Subsequent to my evaluation of this proband, a second child [Leonard, personal commun. 1987] (proband 2) has been recognized who shares all of the clinical and hematologic features including easy bleeding in the neonatal period, evidence for a combined coagulopathy involving all vitamin K dependent factors, nasal hypoplasia, short fingers with hypoplastic distal phalanges and clear stippling on neonatal radiographs.

Therefore it appears that both boys have not only a combined vitamin K-dependent coagulopathy, but also show all of the usual clinical and radiographic features of the warfarin embryopathy. How can this be explained and what does that explanation teach us about the teratogenic mechanism of action of warfarin?

Vitamin K is a necessary cofactor for γ-carboxylation of glutamyl residues of a variety of precursor proteins. This vitamin K-dependent posttranslational modification creates calcium-phospholipid binding sites which are necessary for enzymatic activity of these proteins [13]. Coagulation factor measures in both probands include selective deficiency of this group of factors. Furthermore, various nonphysiologic and antigenic assays of prothrombin demonstrate a pattern entirely analogous to that seen after warfarin therapy, including the presence of significant amounts of des-γ-carboxy prothrombin. Direct assays using antibodies specific for carboxylated and des-γ-carboxylated prothrombin [14] confirmed that only about a third of the prothrombin in each child is normally modified [carboxylated prothrombin = 39 μg/ml in proband 1 and 36 μg/ml in proband 2 (normal ~ 98 μg/ml); descarboxylated prothrombin = 48 μg/ml in the first proband (normal undetected)].

Two distinct enzymatic deficiencies would most likely cause generalized undercarboxylation of vitamin K dependent proteins seen in the individuals described here and in the six previously reported individuals with similar coagulopathies. Transformation of vitamin K to its active form is cyclic and dependent upon the warfarin inhibitable enzyme, vitamin K epoxide reductase [13]. Abnormality of this enzyme could block vitamin K regeneration and result in undercarboxylation. Alternatively, undercarboxylation could be secondary to abnormal function of the protein carboxylases per se. These

Table I. Comparison of clinical features of the warfarin embryopathy and the described probands

Feature	Warfarin	Proband 1	Proband 2
Warfarin exposure	+++	–	–
Nasal hypoplasia	+++	++	++
Stippled epiphyses	+++	+	++
Brachydactyly	+	+	+
Distal digital hypoplasia	+	+	+
Conductive hearing loss	+/–	+	–
Intracranial hemorrhage	+/–	–	+
Upper airway obstruction	+/–	–	+
Persistent coagulopathy	–	++	++

Plus and minus signs indicate subjective assessment of presence and severity of or absence of listed features.

alternatives were assessed by measuring vitamin K and K epoxide levels in plasma. While receiving large doses of oral vitamin K supplementation proband 1 showed the expected excesses of circulating vitamin K (42 ng/ml; normal ~ 1–5 ng/ml). However he also demonstrated large amounts of circulating vitamin K epoxide (50 ng/ml; normally undetectable even with oral administration of vitamin K). A completely analogous pattern was demonstrated in proband 2. The presence of large amounts of vitamin K epoxide is consistent with an abnormality of vitamin K epoxide reductase and would be unexpected if the protein carboxylase was the site of dysfunction. This is analogous to the findings in normal human subjects maintained on warfarin [15]. A similar situation is seen in the warfarin-resistant rat which has a high vitamin K requirement, low K epoxide reductase activity and increased tissue K epoxide levels [16].

Features of the boys described here include irregular ossification (stippling) in radiographs when they were newborns which disappeared with maturation, nasal hypoplasia, and distal digital hypoplasia. The first two are characteristics uniformly present in individuals with the warfarin embryopathy while the third is more variable but consistent with this disorder (table I). While it is conceivable that both of these children have a mild form of chondrodysplasia punctata which closely resembles the warfarin embryopathy *and* an inborn error of vitamin K epoxide reductase *by chance* a more parsimonious and reasonable explanation is that all of these abnormalities are explicable on the basis of the demonstrated enzymatic defect. We would suggest that one such possible explanation is generalized undercarboxylation of vitamin K dependent proteins including the bone and cartilage K dependent proteins, osteocalcin [17] and matrix Gla protein [18]. Decreased levels of carboxylated glutamic acid excretion (13–18 μg/g creatinine in proband 1 versus age-matched control values of 56–63 μg/g creatinine) is what one would expect if there is, in fact, a generalized defect of posttranslational modi-

fication of glutamyl residues which is not completely corrected by oral administration of vitamin K.

Plasma osteocalcin assay in both boys demonstrates higher than normal levels (47.5 and 35.9 μg/ml respectively, approximately threefold normal values for age); similar compensatory increases of osteocalcin levels are seen in individuals maintained on coumarin derivative anticoagulants [19]. Measurements of matrix Gla protein have not been done.

Since its initial description, the warfarin embryopathy has been assumed to result from some primary pharmacologic action of coumarin derivatives. Initially the phenotypic features were postulated to result from hemorrhage secondary to inhibition of fetal coagulation factors [2]. However, given the demonstration of a critical period of exposure between 6 and 9 weeks gestation and since vitamin K-dependent coagulation proteins do not appear until 12–14 weeks gestation [20], the warfarin embryopathy must result either from inhibition of some other vitamin K-dependent protein or proteins or is secondary to some action completely unrelated to the pharmacologic action of warfarin. Both osteocalcin and matrix Gla protein are vitamin K-dependent, γ-carboxy glutamyl residue containing, warfarin-inhibitable proteins. Both have been postulated to be critical in embryonic cartilage and bone differentiation at least in part through modulation of calcium deposition. Since the warfarin embryopathy is principally characterized by abnormalities of calcium deposition (stippled epiphyses) and bone and cartilage development (nasal hypoplasia, distal digital hypoplasia), these teratogenic effects may result from warfarin's pharmacologic inhibition of posttranslational carboxylation of one or the other of these proteins. If this mechanism is true, then if there were an inborn error of metabolism which resulted in inhibition of all vitamin K-dependent function, it should result in a 'genocopy' of the warfarin embryopathy as well as abnormalities of the vitamin K-dependent coagulation factors.

The combined coagulopathy in the two probands is secondary to undercarboxylation of vitamin K-dependent factors. It appears to be secondary to an inborn deficiency of vitamin K epoxide reductase. This inborn error of metabolism also has produced a virtual carbon copy of the warfarin embryopathy. Therefore, by inference, the warfarin embryopathy is almost certainly secondary to a primary pharmacologic effect of warfarin – inhibition of vitamin K epoxide reductase.

Whether other stippling disorders (fig. 1) also result from abnormalities of vitamin K epoxide reductase or from structural defects of vitamin K-dependent bone and cartilage proteins is currently unknown. Likewise unassessed is the possibility that susceptibility to in utero effects of warfarin may depend on maternal and/or fetal variation of vitamin K epoxide reductase activity. Nor, for that matter, am I aware of any studies of bone and cartilage morphogenesis in the warfarin-resistant rat.

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