University of Groningen

Growth and long-term development after in utero exposure to coumarins

van Driel, Dieneke

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2000

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
van Driel, D. (2000). *Growth and long-term development after in utero exposure to coumarins*. s.n.

Chapter 2

Congenital anomalies after in utero exposure to coumarins:

an overview of cases, follow-up findings, and pathogenesis

D. van Driel, J. Wesseling, P.J.J. Sauer, B.C.L. Touwen,
E. van der Veer, H.S.A. Heymans

Abstract

Coumarin derivatives are thought to be teratogenic when they are administered during pregnancy. We present an overview of the anomalies described in case reports. These include facial and skeletal anomalies (warfarin embryopathy) and major structural malformations of the central nervous system and other tracts. Based on a review of 979 pregnancies in which coumarins were prescribed, the prevalence of warfarin embryopathy is 6% and of the central nervous system anomalies 2%. No cases of warfarin embryopathy are found when coumarins were used from the second trimester of pregnancy onwards. In order to evaluate the long-term implications of coumarin exposure in utero, we review the follow-up findings in children born with coumarin-related anomalies as well as coumarin-exposed children who were born apparently normal. The majority of children with anomalies, both at birth and follow-up, were exposed to coumarins during the first trimester of pregnancy. There is little information about the implications of second and third trimester exposure on growth and long-term development.

Introduction

The report by DiSaia[1], describing a child who was born with congenital anomalies after exposure to warfarin in utero increased awareness of the potential teratogenic effect of coumarins. Coumarin derivatives, i.e. coumadin (warfarin), acenocoumarol, and phenprocoumon, are oral anticoagulants which readily cross the placenta. It had been known for a long time that administration of coumarins to the mother could cause perinatal death due to haemorrhages in the child during delivery[2]. Animal studies showed that foetal haemorrhages and death could be prevented by withdrawal of coumarins a few days before parturition[3]. The congenital anomalies described by DiSaia in 1966 had not been associated with coumarins up to that time. Subsequent to his report, many cases were described with similar foetal abnormalities in relation to coumarin exposure in utero. Although case reports cannot be used to estimate the prevalence of the congenital anomalies associated with coumarin exposure in utero, an overview of cases may provide information about the clinical features and the possible pattern(s) of teratogenicity.

Teratogenic interference in the period of organogenesis, when most major organs and body regions are being established, is related to major structural anomalies. Since the foetal period is characterized by histogenesis and functional maturation, the influence of a teratogen in this period may cause growth restriction or functional disturbances[4]. The latter are usually not evident at birth but become apparent later in childhood during growth and development. When evaluating the implications of in utero exposure to coumarins, follow-up information on children who are prenatally exposed and born apparently normal is essential.

The earliest concepts of the pathogenesis of the congenital anomalies found after in utero exposure to coumarin derivatives were based on the main clinical effect, the prolongation of blood clotting time. It was suggested that deformities in the child were caused by microhaemorrhages and subsequent scarring and calcification[5]. Recently, the results of animal studies brought new insights into the pathogenesis of foetal abnormalities associated with prenatal coumarin exposure. Coumarin derivatives are Vitamin K antagonists; they inhibit the recycling of Vitamin K in the cell. In bone, cartilage, and the developing central nervous system, Vitamin K-dependent proteins have been identified[6,7]. In addition, animal studies confirmed an effect of warfarin on the developing bone and central nervous system[8-10].

The aim of this paper is to give an overview of the clinical features associated with prenatal exposure to coumarins in relation to the exposure period during gestation. In addition to the anomalies found directly post partum, we focus on the long-term effects of coumarin exposure on growth and development both in children born with malformations as well as children born apparently normal. Furthermore, we review the frequency of coumarin-related

anomalies in cohort studies of anticoagulated pregnant women. Finally, the possible pathogenetic mechanisms are summarized.

Methods

To identify anomalies in newborns which are associated with in utero exposure to coumarin derivatives, we searched Medline (1966-1998) and Current Contents. In this search, we used the key words 'warfarin', 'coumarin(s)', 'oral anticoagulant(s)', 'congenital anomalies'/'malformations', and 'embryopathy'. In addition, we looked for follow-up findings in children born with coumarin-related congenital anomalies. The following manuscripts were analyzed:

- reports describing infants born after in utero exposure to the coumarin derivatives acenocoumarol, phenprocoumon, or coumadin and
- articles written in English, Dutch, or German and published between 1955 and 1999.

We made a distinction between symptoms that were present between birth and six months of age and findings found at follow-up, from seven months of age onwards. This cut-off point was chosen because many reports describe the clinical situation of the child during the first few months after birth. A longer follow-up period is necessary to gain insight into the implications of coumarin-related anomalies in the long term.

In addition, we performed a second search through Medline (1966-1998) in order to estimate the prevalence of coumarin-related anomalies at birth. In this search the key words 'pregnancy' and 'coumarin' or 'warfarin' were used. The following studies were evaluated:

- cohort studies on treatment with coumarin derivatives during pregnancy,
- articles written and published in English between 1980 and 1999, and
- studies including foetal outcome in combination with a clear description of the foetal exposure.

A third search through Medline (1966-1998) and Current Contents was performed to evaluate the late effects of prenatal coumarin exposure on growth and development of children who were born apparently normal. In this search, key words included 'prenatal exposure', 'warfarin', 'coumarin', and 'follow-up'. Inclusion criteria comprised:

- studies describing the follow-up assessment of cohorts of children exposed to coumarin derivatives during pregnancy and
- articles written and published in English between 1980 and 1999.

Coumarin-related anomalies at birth

We found 56 reports describing 62 cases of congenital anomalies at birth after exposure to coumarin derivatives in utero (Table 1). Forty-eight of these cases were prenatally exposed to warfarin, five to acenocoumarol, eight to phenprocoumon, and one case was partly exposed to phenprocoumon and partly to acenocoumarol. The reports included findings in three elective abortions, five stillbirths, and 54 liveborn children of which ten died neonatally. In three stillborn children a bleeding was established: either an intracranial (n=2) or a general visceral bleeding (n=1). Causes of neonatal death included major structural anomalies incompatible with life (n=3), severe respiratory distress or pneumonia (n=4), prematurity with intrauterine growth restriction (n=1), and intracranial bleeding (n=2).

Of the 62 reported cases, 51 (82%) had skeletal anomalies at birth. Midfacial hypoplasia was the most consistent clinical feature (n=47), including a depressed nasal bridge, underdevelopment or absence of the nasal septum, a small upturned nose with grooves between the tip of the nose and the alae nasi, micrognatia, a prominent forehead, and a flat appearance of the face. Depending on the severity of the nasal hypoplasia, choanal stenosis or atresia was present, resulting in breathing and feeding problems (24 of the 47 children). Another common feature was calcific stippling (n=32), i.e. ectopic calcifications, which were seen on X-rays in the epiphyseal regions of humeri, femora, vertebrae, calcanei, cuboids, or (distal) phalanges. In some reports, skeletal deformities such as rhizomelia, brachydactyly, and hypoplastic nails were described. The above mentioned facial and skeletal abnormalities are rare conditions in the normal population. The combination of these anomalies seen after in utero exposure to coumarins are referred to as 'coumarin or warfarin embryopathy'. In 1980, Hall proposed that this embryopathy is due to coumarin exposure between the 6th and the 9th gestational week[33]. In our review, 48 of the 51 children presenting with skeletal anomalies at birth were exposed during (part of) this window. Of the remaining three children, one had the typical hypoplastic nose, but was exposed until the 5th week of gestation and from the 12th week onwards[54]. The other two children showed bifrontal narrowing and were exposed during the second and third trimester of pregnancy[20,24].

In 28 reported cases, central nervous system malformations were present at birth. Anomalies of midline structures, e.g. agenesis of the corpus callosum, schizencephaly, meningocele, Dandy Walker malformation, and optic atrophy, as well as more general conditions, such as microcephaly, cerebral atrophy, hydrocephalus or ventriculomegaly, hearing loss, and retardation or slow development were described. In five children an intracranial haemorrhage was present at birth. Usually a combination of nervous system malformations were reported, many of them reflecting a disorder in the migration of neu ronal cells. All children with anomalies of midline structures were at least exposed to coumarins during organogenesis (first weeks of pregnancy), except two children with optic atrophy who were

Table 1. Congenital anomalies associated with in utero exposure to coumarins described in case reports (N=62).

Reference	Anticoagulant daily dose (mg)	Period	Partus (wk)*	Face and skeleton**	Central Nervous System	Other
Quenneville[11],1959	warfarin	24-40			optic atrophy/microcephaly	
DiSaia[1], 1966	warfarin	0-26/28-36	39	nose/stippling	bilateral optic atrophy	
Kerber[12], 1968	warfarin 7.5	0-31	39	nose/breathing problems	occasional seizure / slow development (2.5 mns)	
Ikonen[13], 1970	warfarin	0-35	36/ ND	nose/breathing problems	-	died 3rd postnatal day: cerebral and pulmonary hemorrhage, S. Aureus pneumonia, sepsis
Tejani[14], 1973	warfarin 5-10	0-35	36	nose/occipital bone defects	occ. meningocele/microphthalmia/ hydrocephalus/ bulge subocc. region	low ears/high palate/ persist truncus arteriosus
Becker[5], 1975	warfarin 7.5	0-35	35/ ND	nose/stippling/rhizomelia	-	died after few minutes: viscera confom 29 wks gestation/poor developed ears/opacity left lens
Fourie[15], 1975	warfarin 5	0-37	38	nose/breathing problems/stippling/ short fingers/dystrophic nails	-	
Pettifor[16], 1975 (I)	warfarin	0-36	38	nose/breathing problems/stippling /brachydactyly/dysplastic nails		
Pettifor[16], 1975 (II)	warfarin	0-38	41	nose (slight)		
Shaul[17], 1975	warfarin 2.5-5	0-35	35	nose/breathing problems/stippling	hypotonia	downward slanting palpebral fissures
Warkany[18], 1975(I)	warfarin 5	6-16	ND	-	hydrocephalus/blood in cranial cavity	
Warkany[18], 1975(II)	warfarin 10	0-35		-	parieto-occipital cephalocele / Dandy Walker malformation / hydrocephalus	urinary tract anomaly

Reference	Anticoagulant daily dose (mg)	Period	Partus (wk)*	Face and skeleton**	Central Nervous System	Other
Barr[19], 1976	warfarin 7.5	0-16	17/ EA	midfacial hypoplasia/brachydactyly /disordered chondrogenesis (PA)		
Carson[20], 1976	warfarin 3-4.5	12-36	38	bifrontal narrowing	microcephalus	
Holzgreve[21], 1976	warfarin 10	0-12		nose (slight)	agenesis corpus callosum/hypotonia/ delay psychomot development (5mns)	
Pauli[22], 1976	warfarin 7.5-10	6-32	32	nose/breathing problems/stippling /frontal bossing		syst. ejection murmur
Richman[23], 1976	warfarin	0-28	36	nose/stippling	optic atrophy	
Sherman[24], 1976	warfarin	2nd/3rd trim		bifrontal narrowing	microcephaly/hypotonia/ developmental retardation	
Abbott[25], 1977	warfarin 6-7	0-24	29	nose/stippling/laryngeal calcifications/ coronal clefts vertebrae	-	died in 3rd week of severe hyaline membrane disease
Cox[26], 1977	warfarin	0-6	39/ND	short broad distal phalanges/ hypoplasia nails		asplenia syndrome (heart and abdominal anomalies)
Raivio[27], 1977	warfarin	0-35	37/ ND	nose/breathing problems/stippling	-	died in 3rd week of pneumonia
Vanlaeys[28], 1977	acenocoumarol	0-26	term	nose/stippling		
Robinson[29], 1978	warfarin 6-8	0-38	38	nose/breathing problems/stippling	-	
Smith[30], 1979	warfarin	0-33	33 (ND)	nose	widely separated sutures/bilateral subdural hemorrhages	hypertelorism
Baillie[31],1980	warfarin	0-35	39	nose/breathing problems/stippling /abnormal vertebral body S4/5	-	
Curtin[32], 1980	warfarin 3-4	0-40	42	nose/breathing problems/stippling		
Hall[33], 1980	warfarin 10-12.5	16-24		-	cerebral and cerebellar atrophy/ ventriculomegaly/optic atrophy	

Reference	Anticoagulant daily dose (mg)	Period	Partus (wk)*	Face and skeleton**	Central Nervous System	Other
Stevenson[34], 1980	warfarin 5	0 - not clear	31	nose/breathing problems/stippling	bilateral optic atrophy/macrocephaly	mild pectum carinatum
Whitfield[35], 1980	warfarin 10	0-20	33	nose/breathing problems/stippling /brachydactyly/disproport. stature		
Harrod[36], 1981 (I)	warfarin 12.5	6-24		nose/breathing problems/stippling		mesodermal dysgenesis right eye/wide nipples
Harrod[36], 1981 (II)	warfarin	9-30		nose		
Weenink[37], 1981(I)	phenprocoumon /acenocoumarol	0-13/ 13-38	40	nose		
Weenink[37],1981(II)	acenocoumarol	0-36		nose/stippling		
Kaplan[38], 1982	warfarin 10	0-16; 18-33	34	-	Dandy Walker cyst/dilated ventricular system/absence septum pellucidum	
Lamontagne[39], 1983	warfarin 7.5	0-27	40/ND	nose/breathing problems/stippling /toe deformities	-	severe respiratory distress, died at 8 wks cardioresp. arrest
Struwe[40], 1984	phenprocoumon 18 tap.down	8-22	39	nose/breathing problems/stippling rhizomelia/hypoplasia pelvic bones		low ears/antimongoloid eyes
Kaplan[41], 1985	warfarin	8-12		-	agenesis corp. callosum /Dandy Walker cyst/ hydrocephalus	goniodysgenesis right eye (central cataract/ microphthalmia)
Pawlow[42], 1985 (I)	phenprocoumon	0-12	term	nose/breathing problems/stippling /short, broad hand and feet	macrocephalus	hypertelorism/high palate/ exophthalmus/ macroglossia
Pawlow[42], 1985 (II)	phenprocoumon	0-11	34	nose/breathing problems/stippling /short extremities/hypoplastic phalanges/broad hand and feet	macrocephalus/hydrocephalus/ agenesis corpus callosum/ intra-ventricular bleeding/abnormal EEG	hypertelorism/ exophthalmus /macroglossia
Lapiedra[43], 1986	acenocoumarol	0-6	22 / EA		hydrocephalus/scoliosis+tethered skin in sacrococcygeal region	

Reference	Anticoagulant daily dose (mg)	Period	Partus (wk)*	Face and skeleton**	Central Nervous System	Other
Zakzouk[44], 1986	warfarin	unclear	38	nose/breathing problems/stippling	-	
Ruthnum[45], 1987	warfarin	0-6	39	broad forehead/small narrow jaw/ hypoplasia distal phalanges + nails		high palate/microglossia
Tamburrini[46], 1987	warfarin	whole		nose/stippling		hypertelorism/little orbital arches/oropalpebral syncinesia /palpebral ptosis left
Zipprich[47], 1987	phenprocoumon	0-11	23 / SB	nose/stippling (PA)/ hypoplasia femurhead and phalanges		intra uterine growth retardation /bleeding organs
Balde[48], 1988	phenprocoumon 1.5	0-8	41	-		Tetralogy of Fallot/high palate/hypertelorism/low ears/antimongoloid eyes
Hall[49], 1989	warfarin 5	0-8	31/ND	nose/breathing problems/bell shaped chest/club foot left		absent left and hypoplastic right kidney/ macrophallus/ anterior placed anus/low ears
Hosenfeld[50], 1989	phenprocoumon 2.5-15	6-13	36	nose/breathing problems/stippling /rhizomelia/tapering + clefts vertebrae		low ears
Kreyberg-Normann[51], 1989	warfarin	0-14	term/ ND			hypoplastic lungs/ agenesis left diaphragma
Freude[52], 1991	phenprocoumon 3	0-42	42	nose	hypotonia /strong Moro/normal development (4 mns)	
Mason[53], 1992	warfarin	unclear	37	nose/breathing problems/stippling /rhizomelia	macrocephalus	
De Vries[54], 1993	acenocoumarol	0-5; 12-32	32	nose/stippling/rhizomelia	hydrocephalus	intra uterine growth retardation
Gartner[55], 1993	phenprocoumon 3.4	0-28		nose/stippling/hypoplasia distal phalanges +12th ribs/naildystrophy	macrocephalus/bilateral hearing loss (40dB)	atrial septal defect type II

Reference	Anticoagulant daily dose (mg)	Period	Partus (wk)*	Face and skeleton**	Central Nervous System	Other
Ville[56], 1993 (I)	warfarin 3-6	26-36	36 / SB		intracranial hemorrhage/cerebral atrophy	depressed fetal coagulation factors
Ville[56], 1993 (II)	warfarin 5-6	15-29	29 / SB		intraventricular hemorrhage	
Barker[57], 1994	warfarin	0-13	42	nose/breathing problems/hypoplastic nails	-	dextro cardia/abdominal situs inversus
Pati[58], 1994	warfarin	0-24	35	midfacial hypoplasia/arthrogryposis legs	microcephaly/schizencephaly/ dysfunction thermoregulation/ developmental delay	
Howe[59], 1997	warfarin 6-11	0-38	40	nose/breathing problems/stippling /hypo-plasia distal phalanges + cerv. vertebral bodies /loss cerv. lordosis		ptosis left eyelid
Takano[60], 1998	warfarin	1st trim	35	nose/stippling/hypoplasia dist. phalanges		hypospadia/atrial flutter
Wellesley[61], 1998 (I)	warfarin 10-12	0-24	24 / EA	nose/stippling/rhizomelia/brachydactyly/ hypoplasia nails/short fingers +toes	ventriculomegaly/macrocephalus	
Wellesley[61],1998 (II)	warfarin 9-10	0-18	18 / SB	nose/rhizomelia/short fingers+toes		
Tongsong[62], 1999	warfarin 10	0-26	26 / SB	nose/stippling/rhizomelia	hydrocephalus	intra uterine growth retardation
Van Driel[63], 2000	acenocoumarol	8-12	41	nose/breathing problems	-	

*EA = elective abortion; SB=stillbirth; ND=neonatal death / **nose= nasal hypoplasia

exposed in the second and third trimester of pregnancy. Four other children were not exposed during the first trimester of gestation. Two of them displayed a combination of microcephaly, cerebral atrophy or ventriculomegaly, the other two had an intracranial haemorrhage.

In 14 cases, anomalies of other tracts were described including dysgenesis of part of the eye (n=3), tetralogy of Fallot, persistent truncus arteriosus, atrial septal defect, undefined urinary tract anomaly, absence of the left and hypoplasia of the right kidney, hypospadia, hypoplasia of the lungs with agenesis of the left diaphragma, dextrocardia and abdominal situs inversus, and asplenia syndrome. These cases were all exposed early in pregnancy during organogenesis.

In many reports a combination of malformations affecting different tracts were described. In 17 children, a combination of skeletal and central nervous system anomalies were present. The extent of the abnormalities ranged form mild to severe. Anomalies of other tracts were seen in combination with both skeletal deformities (n=8) or central nervous system malformations (n=2). In two children skeletal, nervous system, and anomalies of the heart were reported. In addition, minor physical anomalies including low-set ears, poorly developed ears, high palate, hypertelorism, antimongoloid eyelids, and widely spaced nipples were described in 13 cases.

Follow-up of children born with coumarin-related anomalies

Of the 62 reported cases with coumarin-related anomalies at birth, 44 children survived after the neonatal period and follow-up information could be found on 20 of them (Table 2). Twelve of these children displayed skeletal deformities at birth, one showed central nervous system anomalies and seven cases were born with a combination of both. The duration of follow-up varied between seven months and 32 years; in eight children a long-term follow-up (3½ years or more) was described. One child died of aspiration pneumonia following a generalized seizure at ten years of age.

Of the 17 cases with midfacial hypoplasia at birth, in seven children marked nasal hypoplasia was reported at follow-up. The nose of one child was described to be normal and in another case nasal hypoplasia was reported to be less severe. No description of the midfacial region was given at follow-up in the other eight children. In addition, in seven children deformities of the spine were reported at follow-up, five of whom displayed calcific stippling of the spine at birth. Takano et al[60] described 17 years of follow-up in a child with diffuse calcific stippling in the cervical and sacral spine at birth. At two years of age, the patient experienced neck pain and intermittent loss of strength in his legs. Radiologic examination revealed abnormal ossification of the cervical vertebral bodies with atlanto-axial instability. Over time,

Table 2. Case reports with follow-up data of children who were born with coumarin-related anomalies (N=20).

Reference	Exposure drug (weeks)	‡	Follow-up	Face and Skeleton at follow-up	Central Nervous System at follow-up
DiSaia[1], 1966	warfarin (0-26 and 28-36)	C	5 yrs	weight P25/height P25 (15mns)/ kyphoscoliosis (3yrs)	mild retardation (5yrs)/blind
Kerber[12], 1968	warfarin 7.5mg (0-31)	C	10 yrs (died)	prognatism/subluxation C1-2/progressive platyspondylisis/ posterior wedging thoracic vertebrae/shallow acetabula	cerebral asymmetry/focal cerebral atrophy/shortening olfactory tracts/focal disorientation gyral pattern (postmortem exam)
Pettifor[16], 1975	warfarin (0-36)	S	7 mos	not reported	normal milestones/normal vision/alert
Shaul[17], 1975	warfarin 2.5-5mg (0-35)	C	8 mos	normal growth	normal development
Carson[20], 1976	warfarin 3-4.5mg (12-36)	C	unclear	not reported	microcephaly/spasticity/blind/retardation (cared for in long-stay accommodation)
Stevenson[34], 1980	warfarin 5mg (0- unclear)	C	16 mos	midfacial hypoplasia less severe/most stippling incorporated/calcified hyoid and thyroid cartilage/ height 67cm (<P3)/weight 6.8kg (<P3)	bilateral optic atrophy/delayed motor development/normal language skills
Whitfield[35], 1980	warfarin 10mg (0-20)	S	2 yrs	nose not abnormal/disproportional stature/atlanto-axial instability	slow motor development (7mos)/slight hemiparesis/normal intellect (2yrs)
Harrod[36], 1981 (I)	warfarin 12.5mg (6-24)	S	15 mos	striking nasal hypoplasia/stippling/butterfly thoracic vertebrae/hypoplasia distal phalanges/ normal growth	abnormal right pupil/normal development
Harrod[36], 1981 (II)	warfarin (9-30)	S	3.5 yrs	marked nasal hypoplasia	mild high frequency hearing loss/delayed language skills/ behavioral problems
Weenink[37], 1981	phen (0-13)/ aceno (13-38)	S	1 yr	not reported	normal development
Struwe[40], 1984	phenprocoumon 18 mg tapering (8-22)	S	1 yr	stippling/hypoplasia distal phalanges and pelvic bones	not reported
Kaplan[41], 1985	warfarin (8-12)	N	7 yrs	progressive thoracolumbar scoliosis/contracture right hip (needs walker)/bilateral equinovarus	seizures/blindness right eye/low IQ at 7 years (verbal 69, perf. 84)

Reference	Exposure drug (weeks)	‡	Follow-up	Face and Skeleton at follow-up	Central Nervous System at follow-up
Pawlow[42], 1985	phenprocoumon (0-12)	C	2 yrs	bilateral clubfoot	macrocephaly/normal development
Ruthnum[45], 1987	warfarin (0-6)	S	1 yr	weight P3/height P50/micrognatia/microglossia	normal psychomotor development
Tamburrini[46], 1987	warfarin (whole)	S	18 mos	some remaining stippling/abnormal ossification calcanei	not reported
Hosenfeld[50], 1989	phen 2.5-15mg (6-13)	S	32 yrs	midfacial hypoplasia/impaired nasal breathing/disproportional short stature	conductive hearing loss/normal intellect
De Vries[54], 1993	acenocoumarol (0-5 and 12-32)	C	11 yrs	disproportional short stature	psychomotor development normal/intelligence normal (IQ=98)/ hearing and vision normal
Howe[59], 1997	warfarin 6-11mg (0-38)	S	20 mos	maxillary hypoplasia/cervical kyphosis	Horner syndrome/syringomyelia/quadriplegia
Takano[60], 1998	warfarin (1sttrim)	S	17 yrs	abnormal ossification cervical vertebral bodies/cervical kyphosis/atlanto-axial instability	sudden loss of strength (2 yrs)/compression spinal cord with persistent hyperreflexia (17 yrs) /no motor weakness (17 yrs)
Van Driel[63]	acenocoumarol (8-12)	S	13 yrs	midfacial hypoplasia/normal growth	normal psychomotor development/normal intellect (IQ=101)

‡ type of congenital anomaly: S=skeletal anomalies, N=nervous system anomalies, C=combination of both

the child developed marked cervical kyphosis with narrowing of the spinal canal resulting in compression of the spinal cord. At 17 years of age, his neurological exam showed persistent hyperreflexia in both limbs without motor weakness. The spinal deformities in other children were reported after a shorter period of follow-up. They included butterfly thoracic vertebrae (at 15 months of age), cervical kyphosis with Horner syndrome, syringomelia and quadriplegia (at 20 months), atlanto-axial instability with hemiparesis (2 years), kyphoscoliosis (5 years), and progressive thoracolumbar scoliosis (7 years). The latter child had no facial or rontgenological anomalies at birth. He also developed a contracture of his right hip and needed a walker to move around. In addition, the ten-year-old child who died from pneumonia was born with midfacial hypoplasia. His post-mortem exam revealed progressive platyspondylisis, subluxation of C1-2, and posterior wedging of the thoracic vertebrae. The skeletal anomalies reported at follow-up in the other children included persistence of a disproportional or short stature during growth (n=4) and hypoplasia or abnormal ossification of bony structures (n=4). In three children only presenting skeletal anomalies at birth, hearing loss was diagnosed at follow-up. Of the eight cases with central nervous system anomalies at birth, normal psychomotor development was reported in three children. These children displayed hydrocephaly, macrocephaly, or hypotonia at birth. Four other children showed abnormal development at follow-up. Of these children, two were born with bilateral optic atrophy: a delay in motor development was described in one case at 16 months of age, while mental retardation with blindness was reported in the other child at five years of age. In addition, a child with microcephaly at birth displayed mental retardation, spasticity and blindness at follow-up, whereas a low verbal and performal intelligence quotient was found at seven years of age in a child presenting with a Dandy Walker cyst, hydrocephalus, and absent septum pellucidum at birth. The child who died at ten years of age had an occasional seizure and slow development in the first months of life. His long-term development was not described, but postmortem examination revealed a severe disorganization and atrophy of his cerebrum.

Prevalence of coumarin-related congenital anomalies

In order to estimate the prevalence of the congenital anomalies associated with exposure to coumarins during pregnancy, we reviewed 17 studies (Table 3) describing a total of 979 pregnancies[64-80]. In 327 of these pregnant women warfarin was prescribed, whereas 449 women used acenocoumarol during pregnancy and in 203 pregnancies the coumarin derivative was not specified. All the women were on long-term therapy with coumarin derivatives at the time of conception; indications were either a prosthetic heart valve or a bioprosthesis in combination with atrial fibrillation. Most women (n = 600) were treated with

Table 3. Foetal outcome in cohort studies describing maternal use of coumarins during pregnancy.

Outcome	coumarins throughout pregnancy (n=600)	coumarins only in 1st trim (n=92)[1]	coumarins replaced by heparin in 6th- 12th week (n=118)	coumarins replaced by heparin after 6th week until end 1st trimester (n=84)	coumarins start after 1st trim (n=85)[2]
Liveborn, no complications	356 (preterm: 61/ ND: 1)	60	91 (preterm: 8)	55 (preterm: 3)	78 (preterm: 19 /ND: 1)
Liveborn, complications	38	1	3	5	2
embryopathy	22	-	0	2	-
CNS anomaly	-	1	1	1	1
combination	1	-	0	0	-
bleeding	11(7CNS + ND)	-	1 (ND)	0	-
other	4 (1 ND)	-	1	2	1
Spontaneous abortion	161	9	19	23	3
Elective abortion	13	13	-	-	-
Still birth	27 (1 WE; 2 CNS+WE)	6	-	1	1
Other	1 ectopic pregnancy and 3 mola	3 maternal death	4 maternal + fetal death and 1 abruptio/fetal death	-	1 maternal death

ND=neonatal death; WE=warfarin embryopathy; CNS=central nervous system

[1] withdrawal coumarin derivatives when gestation established

[2] some received heparin during 1st trimester

coumarins throughout pregnancy. In 118 pregnancies, coumarins were replaced by heparin from the 6th to the 12th gestational week in order to avoid the development of warfarin embryopathy.

Out of the 979 pregnancies, 689 children were born alive. In accordance with the proposed teratogenic window from the 6th to the 9th gestational week, no skeletal anomalies characteristic of warfarin embryopathy were reported in the offspring of the women in whom heparin was substituted for coumarins between the 6th and the 12th gestational week. In addition, no warfarin embryopathy was found in the children of women who used coumarins from the second trimester onwards. Twenty-two children with skeletal anomalies were found out of 394 children born alive to mothers using coumarin derivatives throughout pregnancy. This results in a prevalence rate of 6% for warfarin embryopathy. Two children with symptoms of warfarin embryopathy were found in the group of women in whom heparin substitution started after the 6th gestational week.

A total of 15 children were described with an anomaly of the central nervous system at birth, including hydrocephalus, anencephaly, mental retardation, myelomeningocele, and bifid spine. Out of 689 liveborn children, the estimated prevalence rate of central nervous system anomalies is 2%. All 15 children were at least exposed during the period of organogenesis, except a child with mental retardation who was exposed from the second trimester onwards. In eight children the central nervous system anomaly consisted of an intracranial haemorrhage, which was in three cases related to parturition under coumarin treatment.

Of the 979 pregnancies, 215 (22%) spontaneous abortions were reported, 26 elective abortions were carried out, and 35 (4%) stillbirths were described. The spontaneous abortions occurred most often during the first trimester. Vitale et al[80] showed a close dependency between warfarin dosage (> 5mg) and foetal death. In addition, prematurity was reported in 13% (n=91) of the 689 liveborn children.

Follow-up of cohorts of coumarin-exposed children

In addition to the follow-up of children born with coumarin-related malformations, we looked for studies describing long-term outcome of children who were born apparently normal after in utero exposure to coumarins. We found three studies describing the follow-up assessment of a cohort of children prenatally exposed to coumarin derivatives. Wong et al[81] examined 29 children who were exposed prenatally to warfarin; 25 children were at least exposed in the first trimester, while four children were only exposed during the second or third trimester of pregnancy. The age of the children at examination ranged from 0.5 to 11.3 years. In the cohort, two children were described with severe nasal hypoplasia at follow-up. In addition, two children were found to have a low intelligence quotient (between 70 and 90)

at 10.3 and 11.3 years of age. The authors described macrocephaly in five and speech problems in three children. Except for two children with speech problems who were only exposed during the second or third trimester, all children with anomalies at follow-up were exposed throughout pregnancy. In addition, a child of 0.5 years old was reported with gnatopalatoschisis, bilateral microphthalmia, cataract of the right eye, intraventricular haemorrhage, and hydrocephalus.

Another follow-up study[82] assessed 22 warfarin-exposed children at the average age of four years (range 1.9 to 5.6 years). Two children in this group were exposed throughout pregnancy, whereas 20 children were exposed during the second or third trimester. In comparison to 17 matched non-exposed children, no differences in physical and mental development were found.

Olthof et al[83] examined 21 coumarin-exposed and 17 control children between eight and ten years of age; three children were at least exposed to coumarins during the first trimester of pregnancy, while the other 18 children were exposed during the second or third trimester. No significant differences were found between the two groups. However, in the exposed cohort three children were found to have low intelligence (IQ < 80) at follow-up; one was exposed during the third trimester only and two children were exposed during the second and third trimester of pregnancy. In addition, one child with severe neurological abnormalities consisting of cerebral palsy with psychomotor retardation, epilepsy, and hypoplasia of both optic nerves was found. This child was exposed to coumarins during the second and third trimester of pregnancy; pediatric examination directly postpartum revealed no abnormalities.

Pathogenetic mechanisms

In order to understand the teratogenic interference in foetal development, insight into the pathogenesis of coumarin-related anomalies is essential. New insights are based upon the effect of coumarin derivatives on Vitamin K metabolism in the cell. Coumarins inhibit intracellular Vitamin K recycling (Fig. 1). During cellular processes, the active metabolite Vitamin K hydroquinone (KH2) is oxidized into Vitamin K 2,3 epoxide (K0). The microsomal enzymes Vitamin K epoxide reductase and Vitamin K reductase reduce the inert Vitamin K0 back into the active metabolite KH2. Coumarin derivatives inhibit the reductase enzymes, so that the supply of KH2 is exhausted[84].

Vitamin K is known to be an important cofactor in enzymatic processes in the cell, e.g. γ-carboxylation of glutamic acid (glu) residues into γ-carboxyglutamate residues (gla) in a variety of proteins[6] (Vitamin K-dependent proteins). The majority of known vitamin K-dependent proteins are involved in blood coagulation. Administration of coumarin
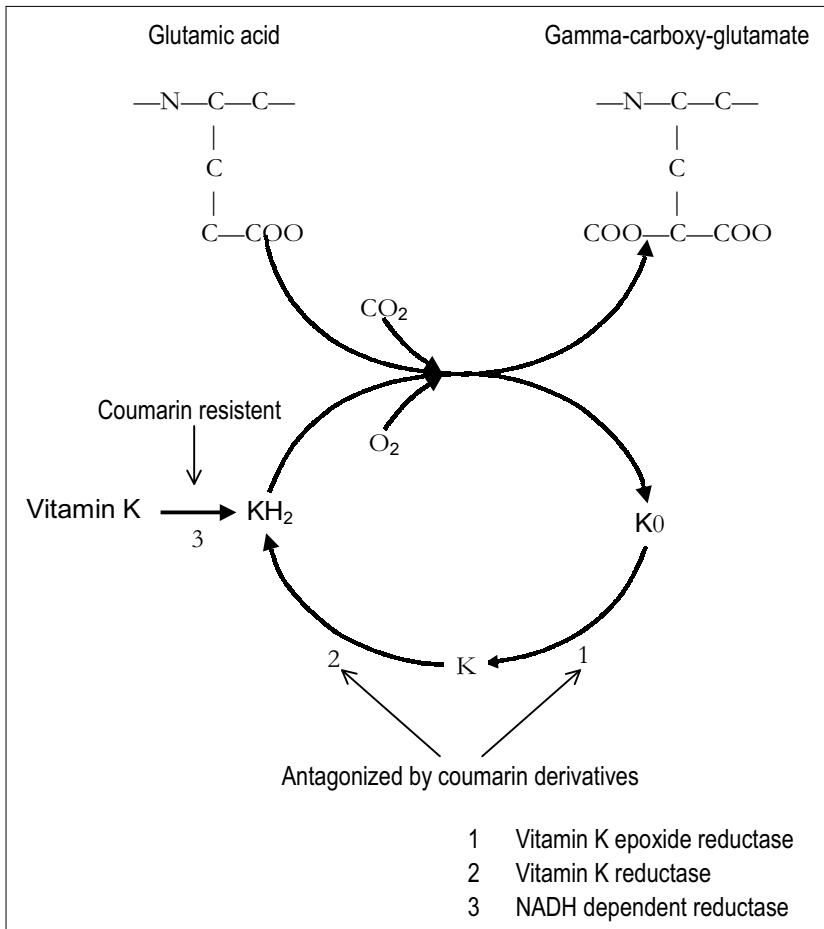
Figure 1. The effect of coumarin derivatives on the Vitamin K recycling in the cell

derivatives inhibits the essential posttranslational modification so that the coagulation proteins remain non-functional. Cartilage, bone, and the developing nervous system also contain Vitamin K-dependent proteins.

In bone and cartilage, bone glu-protein (BGP) and matrix glu-protein (MGP) have been identified[6]. These proteins are thought to play an important role as mineralization inhibitors in the developing skeleton. In rats, the levels of MGP and BGP decreased after warfarin treatment[85]. The exposed rats showed excessive mineralization in growth plates and the nasal septum together with maxillonasal hypoplasia[8,85]. Feteih et al[86] observed a disruption in the columnar arrangement of the hypertrophic chondrocytes in cartilage of foetal rats after warfarin exposure. It was not clear whether the abnormalities were due to overmineralization or whether they were the result of a more fundamental disturbance of the chondrocytes[86].

The congenital anomalies seen after exposure to coumarins in utero have long been associated with different types of chondrodysplasia punctata. The similarities in phenotype suggest that the genetic and drug-induced defects underlying these syndromes may be due to the same metabolic pathway. In rhizomelic chondrodysplasia punctata (RCDP), a defect in peroxisomal biogenesis has been established. Recently, different genotypes have been described pointing towards involvement of ether phospholipid biosynthesis in early skeletal development[87,88]. Warfarin embryopathy also shares phenotypical features with X-linked recessive chondrodysplasia punctata (CDPX). The gene for CDPX was assigned on the Xp22.3 region, the region coding for some members of the sulphatase family (i.e. arylsulphatase C,D,E, and F)[89]. Mutation analysis indicated arylsulphatase E as the gene responsible for CDPX. In vitro, a significant decrease of arylsulphatase E activity was observed with high concentrations of warfarin[90].

In the nervous system, Vitamin K has been shown to stimulate the activity of at least two microsomal enzymes in the sphingolipid pathway[9,91]. The requirement of phosphorus in these experiments suggests that a phosphorylation step is involved[92]. End products of this pathway are cerebrosides, sulfatides, gangliosides, and sphingomyelin. Sphingolipids are important structural components of myelin, but they also serve as second messengers in intracellular signal transduction pathways[93,94]. Sundaram showed that warfarin administration resulted in a significant reduction of sulfatides in mice brains through inhibition of the activity of galactocerebroside sulfotransferase (GST)[10].

Recently, a new Vitamin K-dependent protein (glu-protein) was discovered, Gas6[95]. This growth factor is a ligand for a subfamily of the receptor tyrosine kinases (RTK's). RTK's are receptors with an extracellular domain, which bind the ligand and an intracellular tyrosine kinase which is important for signal transduction[96]. RTK's were first described for their role in cell growth and proliferation, but are also known to function in cell migration, axonal pathfinding, cell survival and neural cell-type determination. Gas6 and its receptor, Tyro 3, were found to be widely distributed throughout the nervous system[7]. In the brain of developing chick embryos, tyrosine phosphorylation was enhanced by Vitamin K, whereas warfarin was shown to inhibit this phosphorylation cascade[97]. Since the development of the nervous system of the human embryo is dependent upon a highly co-ordinated repertoire of cell division, differentiation and migration, inhibition of regulatory growth factors like the Vitamin K-dependent Gas6 might cause disorganization of the central nervous system during development. Besides the nervous system, Gas6 has a wide tissue distribution, e.g. lung, intestine, heart, testis[94]. Therefore, it is conceivable that Gas6 inhibition might have a negative influence on the embryogenesis of other tracts.

Concluding remarks

Case reports describe a combination of facial and skeletal anomalies, which are relatively rare in the normal population, after in utero exposure to coumarins. Most cases of this so-called warfarin embryopathy were exposed during a teratogenic window from the 6th to the 9th gestational week. The embryopathy was not found in studies in which pregnant women used coumarin derivatives from the second trimester onwards. In addition, major structural malformations, including the central nervous system, were described after coumarin exposure in utero. These anomalies with a low incidence in the normal population could have been found by coincidence after coumarin exposure in utero. However, the combination with the typical facial and skeletal anomalies in 71% of these cases suggest a relation with the prenatal exposure to coumarin derivatives. Most of the major structural anomalies were found after exposure during the first trimester of pregnancy (organogenesis). There is little information about the long-term implications of in utero exposure to coumarins when prescription of these drugs during the first trimester of gestation is avoided. Only a few small cohort studies examined children who were born apparently normal after in utero exposure during the second or third trimester of pregnancy. These studies showed contradictory results including a normal as well as an impaired development of these children. In addition, the concepts of teratogenicity of coumarins are mainly based on animal models including rats and mice, which are born less mature than the human foetus. Therefore, the results of these studies are difficult to extrapolate to the human situation.

We conclude that in order to appropriately treat pregnant women who need anticoagulation, a large cohort study assessing growth and development of children exposed to coumarin derivatives during the second and third trimester of pregnancy is warranted.

References

1. DiSaia PJ. Pregnancy and delivery of a patient with a Starr-Edwards mitral valve prosthesis. Obstet Gynecol 1966;28(4):469-72.
2. Wright HP. Venous thrombosis during pregnancy treated with dicoumarin. J Obstet Gyneacol Brit Emp 1951;109:364-8.
3. Hirsch J, Cade JF, Gallus AS. Fetal effects of coumadin administered during pregnancy. Blood 1970;36(623):627.
4. Carlson B. Developmental disorders: causes, mechanisms, and patterns. In: Copland B, Caldwell L, editors. Human embryology and developmental biology. 2nd ed.St. Louis, Missouri: Mosby, 1999; 128-45.
5. Becker MH, Genieser NB, Finegold M, Miranda D, Spackman T. Chondrodysplasia Punctata. Is maternal warfarin therapy a factor? Am J Dis Child 1975;129:356-9.
6. Howe AM, Webster WS. Vitamin K: its essential role in craniofacial development. A review of

the literature regarding vitamin K and craniofacial development. Aust Dent J 1994;39(2):88-92.

7. Prieto AL, Weber JL, Tracy S, Heeb MJ, Lai C. Gas6, a ligand for the receptor protein-tyrosine kinase Tyro-3, is widely expressed in the central nervous system. Brain Res 1999;816:646-61.

8. Howe AM, Webster WS. The warfarin embryopathy: a rat model showing maxillonasal hypoplasia and other skeletal disturbances. Teratology 1992;46:379-90.

9. Sundaram KS, Lev M. Warfarin administration reduces synthesis of sulfatides and other sphingolipids in mouse brain. J Lipid Res 1988;29:1475-9.

10. Sundaram KS, Fan JH, Engelke JA, Foley AL, Suttie JW, Lev M. Vitamin K status influences brain sulfatide metabolism in young mice and rats. J Nutr 1996;126:2746-51.

11. Quenneville G, Barton B, McDevitt E, Wright IS. The use of anticoagulants for thrombophlebitis during pregnancy. Am J Obstet Gynecol 1959;77(5):1135-49.

12. Kerber IJ, Warr OS, Richardson C. Pregnancy in a patient with a prosthetic mitral valve. JAMA 1968;203(3):157-9.

13. Ikonen E, Merikallio E, Osterlund K, Seppala M. Mitral valve prosthesis, warfarin anticoagulation, and pregnancy. Lancet 1970;1252.

14. Tejani N. Anticoagulant therapy with cardiac valve prosthesis during pregnancy. Obstet Gynecol 1973;42(5):785-93.

15. Fourie DT, Hay IT. Warfarin as a possible teratogen. S Afr Med J 1975;49:2081-3.

16. Pettifor JM, Benson R. Congenital malformations associated with the administration of oral anticoagulants during pregnancy. J Pediatr 1975;86(3):459-62.

17. Shaul WL, Emery H, Hall JG. Chondrodysplasia punctata and maternal warfarin use during pregnancy. Am J Dis Child 1975;129:360-2.

18. Warkany J, Bofinger M. Le role de la coumadine dans les malformations congenitales. Med Hyg 1975;33:1454-7.

19. Barr M, Jr., Burdi AR. Warfarin-associated embryopathy in a 17-week-old abortus. Teratology 1976;14:129-34.

20. Carson M, Reid M. Warfarin and fetal abnormality. Lancet 1976;1127.

21. Holzgreve W, Carey JC, Hall BD. Warfarin-induced fetal abnormalities. Lancet 1976;914-5.

22. Pauli RM, Madden JD, Kranzler KJ, Culpepper W, Port R. Warfarin therapy initiated during pregnancy and phenotypic chondrodysplasia punctata. J Pediatr 1976;88(3):506-8.

23. Richman EM, Lahman JE. Fetal anomalies associated with warfarin therapy initiated shortly prior to conception. J Pediatr 1976;88(3):509-10.

24. Sherman S, Hall BD. Warfarin and fetal abnormality. Lancet 1976;692.

25. Abbott A, Sibert JR, Weaver JB. Chondrodysplasia punctata and maternal warfarin treatment. BMJ 1977;1(6077):1639-40.

26. Cox DR, Martin L, Hall BD. Asplenia syndrome after fetal exposure to warfarin. Lancet 1977;1134.

27. Raivio KO, Ikonen E, Saarikoski S. Fetal risks due to warfarin therapy during pregnancy. Acta Paediatr Scand 1977;66:735-9.

28. Vanlaeys R, Deroubaix P, Deroubaix G, Lelong M. Les antivitamines K sont-elles teratogenes? Nouv Press Med 1977;6:756.

29. Robinson MJ, Pash J, Grimwade J, Campbel J. Fetal warfarin syndrome. Med J Aust 1978;1(3):157.

30. Smith MF, Cameron MD. Warfarin as teratogen. Lancet 1979;1(8118):727.

31. Baillie M, Allen D, Elkington AR. The congenital warfarin syndrome: a case report. Br J Ophthalmol 1980;64:633-5.

32. Curtin T, Mulhern B. Foetal warfarin syndrome. Irish Medical Journal 1980;73(10):393-4.

33. Hall JG, Pauli RM, Wilson KM. Maternal and fetal sequelae of anticoagulation during pregnancy.

Am J Med 1980;68:122-40.

34. Stevenson RE, Burton OM, Ferlauto GJ, Taylor A. Hazards of Oral Anticoagulants during Pregnancy. JAMA 1980;243(15):1549-51.

35. Whitfield MF. Chondrodysplasia punctata after warfarin in early pregnancy: case report and summary of the literature. Arch Dis Child 1980;55:139-42.

36. Harrod JE, Sherrod PS. Warfarin embryopathy in siblings. Obstet Gynecol 1981;57(5):673-6.

37. Weenink GH, Van Dijk-Wierda CA, Meyboom RHB, Koppe JG, Staalman CR, Treffers PE. Teratogeen effect van coumarine-derivaten. NTvG 1981;125(18):702-6.

38. Kaplan LC, Anderson GG, Ring BA. Congenital hydrocephalus and Dandy-Walker malformation associated with warfarin use during pregnancy. Birth Def Orig Art Ser 1982;18:79-83.

39. Lamontagne JM, Leclerc JE, Carrier C, Bureau M. Warfarin embryopathy - a case report. J Otolaryngol 1983;13(2):127-9.

40. Struwe FE, Reinwein H, Stier R. Coumarin embryopathie. Radiologe 1984;24:68-71.

41. Kaplan LC. Congenital Dandy Walker malformation associated with first trimester warfarin: a case report and literature review. Teratology 1985;32:333-7.

42. Pawlow I, Pawlow V. Kumarin-embryopathie. Z.Klin.Med. 1985;40:885-8.

43. Lapiedra OJ, Bernal JM, Ninot S, Gonzalez I, Pastor E, Miralles PJ. Open heart surgery for thrombosis of a prosthetic mitral valve during pregnancy. Fetal hydrocephalus. J Cardiovasc Surg 1986;27:217-20.

44. Zakzouk MS. The congenital warfarin syndrome. J Laryngol Otol 1986;100:215-9.

45. Ruthnum P, Tolmie JL. Atypical malformations in an infant exposed to Warfarin during the first trimester of pregnancy. Teratology 1987;36:299-301.

46. Tamburrini O, Bartolomeo-De Iuri A, Di Guglielmo GL. Chondrodysplasia punctata after warfarin: case report with 18-month follow-up. Pediatr Radiol 1987;17:323-4.

47. Zipprich KW, Canzler E, Hundsdörfer S. Zur teratogenen wirkung von kumarinen. Zent.bl.Gynäkol. 1987;109:364-8.

48. Balde MD, Breitbach GP, Wettstein A, Hoffmann W, Bastert G. Fallotsche Tetralogie nach Cumarineinnahme in der Fruhschwangerschaft-eine Embryopathie? Geburtsh u Frauenheilk 1988;48:182-3.

49. Hall BD. Warfarin embryopathy and urinary tract anomalies: possible new association. Am J Med Genet 1989;34:292-3.

50. Hosenfeld D, Wiedeman HR. Chondrodysplasia punctata in an adult recognized as vitamin K antagonist embryopathy. Clin Genet 1989;35:376-81.

51. Kreyberg-Normann E, Stray-Pedersen B. Warfarin-induced fetal diaphragmatic hernia. Case report. Br J Obstet Gynaecol 1989;96:729-30.

52. Freude S, Pabinger-Fasching I, Kozel-Lachmann D, Braun F, Pollak A. Warfarin-embryopathie bei mütterlicher coumarin-therapie wegen protein-S-mangels. Pädiatrie und Pädologie 1991;26:239-41.

53. Mason JDT, Jardine A, Gibbin KP. Foetal warfarin syndrome-a complex airway problem. Case report and review of the literature. J Laryngol Otol 1992;106:1098-9.

54. De Vries TW, Van der Veer E, Heymans HSA. Warfarin embryopathy: patient, possibility, pathogenesis and prognosis. Br J Obstet Gynaecol 1993;100:869-71.

55. Gärtner B, Seifert CB, Michalk DV, Roth B. Phenprocoumon therapy during pregnancy: case report and comparison of the teratogenic risk of different coumarin derivatives. Z.Geburtsh.u.Perinat. 1993;197:262-5.

56. Ville Y, Jenkins E, Shearer MJ, Hemley H, Vasey DP, Layton M, Nicolaides KH. Fetal intraventricular haemorrhage and maternal warfarin. Lancet 1993;341:1211.

57. Barker DP, Konje JC, Richardson JA. Warfarin embryopathy with dextrocardia and situs

inversus. Acta Paediatr 1994;83:411.

58. Pati S, Helmbrecht GD. Congenital schizencephaly associated with in utero warfarin exposure. Reprod Toxicol 1994;8(2):115-20.

59. Howe AM, Lipson AH, De Silva M, Ouvrier R, Webster WS. Severe cervical dysplasia and nasal cartilage calcification following prenatal warfarin exposure. Am J Med Genet 1997;71:391-6.

60. Takano H, Smith WL, Sato Y, Kao SCS. Cervical spine abnormalities and instability with myelopathy in warfarin-related chondrodysplasia: 17-year followup. Pediatr Radiol 1998;28:497-9.

61. Wellesley D, Moore I, Heard M, Keeton B. Two cases of warfarin embryopathy: a re-emergence of this condition? Br J Obstet Gynaecol 1998;105:805-6.

62. Tongsong T, Wanapirak C, Piyamongkol W. Prenatal ultrasonographic findings consistent with fetal warfarin syndrome. J Ultrasound Med 1999;18:577-80.

63. Van Driel D, Wesseling J, De Vries TW, Sauer PJJ. Coumarin embryopathy at birth: follow-up of two cases. Submitted.

64. Chen WWC, Sing Chan C, Kei Lee P, Wang RYC, Wong VCW. Pregnancy in patients with prosthetic heart valves: an experience with 45 pregnancies. Q J Med 1982;203:358-65.

65. O'Neill H, Blake S, Sugrue D, MacDonald D. Problems in the management of patients with artificial valves during pregnancy. Br J Obstet Gynaecol 1982;89:940-3.

66. Larrea JL, Nunez L, Reque JA, Gil Aguado M, Matorras R, Minguez JA. Pregnancy and mechanical valve prosthesis: a high-risk situation for the mother and the fetus. Ann Thorac Surg 1983;36(4):459-63.

67. Salazar E, Zajarias A, Gutierrez N, Iturbe I. The problem of cardiac valve prostheses, anticoagulants and pregnancy. Circulation 1984;70(suppl I):169-177.

68. Matorras R, Reque JA, Usandizaga JA, Minguez JA, Larrea JL, Nunez L. Prosthetic heart valve and pregnancy. Gynecol Obstet Invest 1985;19:21-31.

69. Iturbe-Alessio I, Del Carmen Fonseca M, Mutchinik O, Angel Santos M, Zajarias A, Salazar E. Risks of anticoagulant therapy in pregnant women with artificial heart valves. N Engl J Med 1986;315:1390-3.

70. Lee PK, Wang R, Chow J, Cheung KL, Wong VCW, Chan TK. Combined use of warfarin and adjusted subcutaneous heparin during pregnancy in patients with an artificial heart valve. J Am Coll Cardiol 1986;8:221-4.

71. Vitali E, Donatelli F, Quaini E, Groppelli G, Pelligrini A. Pregnancy in patients with mechanical prosthetic heart valves. J Cardiovasc Surg 1986;27:221-7.

72. Sareli P, England MJ, Berk MR, Marcus RH, Epstein M, Driscoll J, Meyer T, McIntyre J, van Gelderen C. Maternal and fetal sequelae of anticoagulation during pregnancy in patients with mechanical heart valve prostheses. Am J Cardiol 1989;63:1462-5.

73. Cotrufo M, de Luca TSL, Calabro R, Mastrogiovanni G, Lama D. Coumarin anticoagulation during pregnancy in patients with mechanical valve prostheses. Eur J Cardio-thor Surg 1991;5:300-5.

74. Born D, Martinez EE, Almeida PAM, Santos DV, Carvalho ACC, Moron AF, Miyasaki CH, Moraes SD, Ambrose JA. Pregnancy in patients with prosthetic heart valves: the effects of anticoagulation on mother, fetus, and neonate. Am Heart J 1992;124:413-7.

75. Caruso A, De Carolis S, Ferrazzani S, Paradisi G, Pomini F, Pompei A. Pregnancy outcome in women with cardiac valve prosthesis. Eur J Obstet Gynecol 1993;54:7-11.

76. Sbarouni E, Oakley CM. Outcome of pregnancy in women with valve prostheses. Br Heart J 1994;71:196-201.

77. Lecuru F, Desnos M, Taurelle R. Anticoagulant therapy in pregnancy.Report of 54 cases. Acta Obstet Gynecol Scand 1996;75:217-21.

78. Salazar E, Izaguirre R, Verdejo J, Mutchinik O. Failure of adjusted doses of subcutaneous heparin

to prevent thromboembolic phenomena in pregnant patients with mechanical cardiac valve prostheses. J Am Coll Cardiol 1996;27(7):1698-703.

79. Suri V, Sawhney H, Vasishta K, Renuka T, Grover A. Pregnancy following cardiac valve replacement surgery. Int J Gynecol Obs 1999;64:239-46.

80. Vitale N, De Feo M, De Santo LS, Pollice A, Tedesco N, Cotrufo M. Dose-dependent fetal complications of warfarin in pregnant women with mechanical heart valves. J Am Coll Cardiol 1999;33(6):1637-41.

81. Wong V, Cheng CH, Chan KC. Fetal and neonatal outcome of exposure to anticoagulants during pregnancy. Am J Med Genet 1993;45:17-21.

82. Chong MKB, Harvey D, De Swiet M. Follow-up study of children whose mothers were treated with warfarin during pregnancy. Br J Obstet Gynaecol 1984;91:1070-3.

83. Olthof E, De Vries TW, Touwen BCL, Smrkowsky M, Geven-Boere LM, Heymans HSA, Van der Veer E. Late neurological, cognitive and behavioural sequelae of prenatal exposure to coumarins: a pilot study. Early Hum Dev 1994;38:97-109.

84. De Boer-van den Berg MAG, Thijssen HHW, Vermeer C. The in vivo effects of acenocoumarol, phenprocoumon and warfarin on Vitamin K epoxide reductase and Vitamin K-dependent carboxylase in various tissues of the rat. Biochim Biophys Acta 1986;884:150-7.

85. Price PA, Williamson MK, Haba T, Dell RB, Jee WSS. Excessive mineralisation with growth plate closure in rats on chronic warfarin treatment. Proc Natl Acad Sci USA 1982;79:7734-8.

86. Feteih R, Tassinari MS, Lian JB. Effect of sodium warfarin on vitamin K-dependent proteins and skeletal development in the rat fetus. J Bone Min Res 1990;5:885-94.

87. Braverman N, Steel G, Obie C, Moser A, Moser H, Gould SJ, Valle D. Human PEX7 encodes the peroxisomal PTS2 receptor and is responsible for rhizomelic chondrodysplasia punctata. Nature Genet 1997;15:369-76.

88. Biermann J, Gootjes J, Humbel BM, Dansen TB, Wanders RJA, Van den Bosch H. Immunological analyses of alkyl-dihydroxyacetone-phosphate synthase in human peroxisomal disorders. Eur J Cell Biol 1999;78:339-48.

89. Daniele A, Parenti G, d'Addio M, Andria G, Ballabio A, Meroni G. Biochemical characterization of Arylsulfatase E and functional analysis of mutations found in patients with X-linked chondrodysplasia punctata. Am J Hum Genet 1998;62:562-72.

90. Franco B, Meroni G, Parenti G, Levilliers J, Bernard L, Gebbia M, Cox L, Maroteaux P, Sheffield L, Rappold G, et al. A cluster of sulfatase genes on Xp22.3: mutations in Chondrodysplasia Punctata (CDPX) and implications for warfarin embryopathy. Cell 1995;81:15-25.

91. Sundaram KS, Lev M. Regulation of sulfotransferase activity by Vitamin K in mouse brain. Arch Biochem Biophys 1990;277(1):109-13.

92. Sundaram KS, Lev M. Vitamin K and phosphate mediated enhancement of brain sulfotransferase activity. Biochem Biophys Res Com 1990;169(3):927-32.

93. Kinney HC, Karthigasan J, Boenshteyn NI, Flax JD, Kirschner DA. Myelination in the developing human brain: biochemical correlates. Neurochem Res 1994;19(8):983-96.

94. Tsaioun KI. Vitamin K-dependent proteins in the developing and aging nervous system. Nutr Rev 1999;57(8):231-40.

95. Manfioletti G, Brancolini C, Avanzi G. The protein encoded by a growth-arrest-specific gene (GAS6) is a new member of the Vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. Mol Cell Biol 1993;13:4976-85.

96. Varnum BC, Young E, Elliot G. Axl receptor tyrosine kinase stimulated by the Vitamin K-dependent protein encoded by growth arrest-specific- gene-6. Nature 1995;373:623-6.

97. Saxena SP, Fan T, Li M, Israels ED, Israels LG. A novel role for Vitamin K1 in a tyrosine phosphorylation cascade during chick embryogenesis. J Clin Invest 1997;99(4):602-7.