

Drugs and nonsyndromic orofacial cleft: an update

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Abstract

Nonsyndromic orofacial cleft (OFC) derives from an embryopathy with failure of the nasal processes and/or fusion of the palatal shelves. This severe birth defect is one of the most common malformations among live births.

Human cleft is composed of two separate entities: cleft of the lip with or without palate (CL±P) and cleft palate only (CPO). Both have a genetic origin, whereas environmental factors contribute to these congenital malformations.

In this review we analyze the role of drugs related to the onset of cleft. The data were obtained from (i) epidemiologic studies (ii) animal models and (iii) human genetic investigations. These studies have demonstrated a relation between certain drugs (steroids and anticonvulsants) taken during pregnancy and a higher risk of generating offspring with OFC whereas no clear relation has been demonstrated between aspirin and OFC.

Key words:

Cleft; Steroid, Anticonvulsant; Cortisone; Phenytoin

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Introduction

Nonsyndromic cleft or orofacial cleft (OFC) is due to an embryopathy, which causes failure of the fusion of the nasal processes and/or palatal shelves. This severe birth defect is one of the most common malformations among live births. Indeed, incidence is in the range of 1/700-1/1,000 among Caucasians^{1,2}.

Clefts of the human face can be classified anatomically as those including the secondary palate alone (the posterior and/or soft palate) or cleft palate only (CPO), and those that involve the primary palate, encompassing cleft of the lip with or without the palate (i.e. CL±P)³. This distinction is biologically important and supported by embryological grounds: indeed, the primary and secondary palates are formed independently. Furthermore, it is unusual to find a familial CPO if the index case has CL±P, and vice versa¹.

Since one-fifth of patients in different populations have a positive family history, genetic factors are thought to play an important role in the etiology of this congenital defect. Fogh-Andersen⁴ provided the first population-based evidence that OFC has a strong genetic component. Fraser¹ divided CPO and CL±P. The elevated concordance rate observed in monozygotic twins (36%) with respect to dizygotic twins (4.7%) provides, further evidence of genetic predisposition⁵⁻⁸.

Population-based studies have shown that non-genetic factors play an important role in clefting: teratogens like phenitoin and valproic acid are known to cause OFC.

Murine models were developed to investigate drug-induced embryopathy and, more recently, to obtain information regarding genes and biochemical pathways. Comprehension of the results is complicated by the fact that nonsyndromic cleft lip in mice, as well as in humans, is genetically complex, and is distinct from isolated cleft palate⁹.

In this review we focus on drugs related to the onset of cleft. The data here presented have been obtained from epidemiologic studies (ii) animal models and (iii) human genetic investigations.

Steroids

Corticosteroids are first-line drugs used for the treatment of a variety of conditions in women of childbearing age; in animal models the clefting role of corticosteroids is well documented. Some studies have examined the association between corticosteroid use by women during the periconceptional period (one month before to 3 months after conception) and delivery of newborns with selected congenital anomalies. Carmichael and Shaw¹⁰ found an increased risk for nonsyndromic CL±P and CPO. Park-Wyllie et al.¹¹ have also demonstrated that, although prednisone does not represent a major teratogenic risk in humans at therapeutic doses, it does increase by an order of 3.4-fold the risk of oral cleft, which is consistent with the existing

animal studies. In a case-control survey of children with nonsyndromic cleft lip or palate, Edwards et al.¹² have shown a significant increase in the prevalence of maternal use of topical corticosteroid preparations in the first trimester of pregnancy (odd ratio 13.154).

In a case-control analysis, Pradat et al.¹³ demonstrated the possible association between oral cleft in the newborn and maternal exposure to corticoids during pregnancy. The study includes data on 11,150 malformed infants with a cleft palate or cleft lip and a history of maternal first-trimester drug exposure. They observed a slight association between exposure to corticoids for systemic use and the occurrence of cleft lip with or without cleft palate (OR, 2.59; 95% CI, 1.18-5.67).

Another study conducted on 1142 Sweden infants with OFC and maternal exposure to drug in early pregnancy found association between glucocorticoid use and infant cleft. In particular this risk seemed to be strongest for median cleft palate¹⁴. Additional strength was added by the same author in a recent report where anti-asthmatic drugs were analysed. An increased risk OFC, specifically for median cleft palate, was detected for inhaled corticosteroids¹⁵.

Cleft lip (CL) and cleft palate (CP) are induced in the mouse progeny when glucocorticoids were administered to pregnant mice. The incidence varies among inbred mouse strains and with the dosage and stage of gestation when the drug is given. The inbred A/J strain, which has spontaneous cleft lip, displays lateral median processes that are smaller than in other resistant strains; these diverge slowly to impede the fusion process. Diewert et al.¹⁶ found not only that cortisone affected the content of the extracellular matrix and the number of palatal shelf cells in A/J mice, but also shelf elevation was delayed; besides, only half of the cortisone-treated palates achieved complete horizontal positioning of the shelves in all regions of the palate. Melnick et al.¹⁷ studied the teratologic effects on lip morphogenesis following administration of triamcinolone hexacetonide at the eighth day of gestation. The frequency of cleft lip in treated A/J mice was found to be more than three times higher than spontaneous frequency in untreated controls. Affected A/J embryos showed a severe reduction in the size of the lateral nasal processes. Gasser et al.¹⁸ examined strains of mice for susceptibility to cortisone-induced cleft palate, and confirmed the role of genes linked to H-2 complex (homologous to human HLA system) on chromosome 17. Later, the same group¹⁹ refined the map of the chromosome region carrying the Cps-1 gene (cleft palate susceptibility-1). Juriloff et al.^{9,20} mapped a major CL±P-causing gene on mouse chromosome 11, in a region having linkage homology with human 17q21-24. By setting up an *in vitro* organ culture system with developing mouse palates, Shimizu et al.²¹ were able to demonstrate that exposure to hydrocortisone (HC) in a concentration-dependent manner inhibits the palatal fusion process by preventing apoptosis

in the epithelial cells at the tip of palatal shelves.

Jaskoll et al.²² analyzed the developmental expression of four glucocorticoid-responsive genes (TGF-beta 1, TGF-beta 2, TGF-beta 3, and EGF-R) in developing mouse palates in the presence or absence of exogenous glucocorticoids. These molecules delay the down-regulation of palatal TGF-beta 2 transcript, which is known to inhibit cell proliferation. The authors thus hypothesized that TGF-beta 2 is the mediator of the glucocorticoid effect. In the same year, Fawcett et al.²³ demonstrated that endogenous corticosterone did not contribute significantly to the incidence of cleft palate induced by the exogenous corticosteroid. These studies support the concept of a threshold in the dose-response relationship for corticosteroid-induced cleft palate in mice.

Anticonvulsants

Anticonvulsants (phenytoin/hydantoin, oxazolinediones and valproic acid) are associated with a clearly demonstrated increased risk of congenital defects²⁴. All three therapeutic classes induce cleft lip and/or cleft palate, as a part of severe embryopathies.

It is worth noting that a significant increase of benzodiazepine usage was detected in mothers of infants with CPO, while no such significant increase was found in mothers of CL±P infants²⁵. Safra and Oakley²⁶ instead reported association of CL±P with first-trimester exposure to diazepam. These evidences were confirmed in 1990 by Laegreid²⁷ for benzodiazepine in general. In a further study, Eros et al.²⁸ addressed the question of benzodiazepine teratogenicity as a whole, verifying undetectable teratogenic risk due to treatment with this kind of anticonvulsants. Although diazepam is a weak teratogen in susceptible mice at very high doses, its interference with the forming human face is probably very modest or inexistent. In a more recent study Wikner et al.²⁹ studied the effects of benzodiazepines during foetal life by means data derived from the Swedish Medical Birth Register and they do confirm the earlier proposed increased risk for OFCs.

It is well known that women with epilepsy have an increased risk of offspring with CL±P. This risk has been attributed mostly to the teratogenic effects of antiepileptic drugs, though other risk factors have been suggested, such as epilepsy itself, or some underlying genetic defects associated with epilepsy³⁰. However, it has been noted that a monotherapy of antiepileptic drugs reduces the risk of developing fetal anticonvulsant syndrome³¹.

In 1987 Tocco et al.³² demonstrated that diazepam produced a significant increase in cleft palate frequency in mice. Subsequently, it was confirmed that the fusion of palatal shelves was inhibited dose-dependently by treatment with diazepam³³. Moreover, knockout mice for the gamma aminobutyric acid (GABA)-producing enzyme glutamic acid decarboxylase (Gad1) gene or the beta3 subunit of the

GABAA receptor (Gabbr3) gene results in neurological alteration and clefting of the secondary palate³⁴⁻³⁵. The striking similarity in phenotype between the receptor and ligand mutations clearly demonstrated a role for GABA signaling in normal palate development. A well-devised experiment showed that neural expression of Gabrb3 in knockout mice can rescue the neurological phenotype, but not avoid cleft palate³⁶. This indicates that non-neuronal GABA signaling is implicated in palate development. Additional evidences of the pivotal role of GABA came from a microarray-based experiment³⁷.

In a recent study, our group³⁸ observed a significant relationship between the beta 3 subunit of the gamma-aminobutyric acid receptor (GABRB3) and CL±P, suggesting that the GABRB3 gene is involved in this congenital disease. Although GABR is the target of benzodiazepine, none of our patients presented neurologic diseases. In the same study, it was also demonstrated that the GAD1 gene, which encodes the GABA-producing enzyme, is not involved in CL±P pathogenesis.

Kanno et al.³⁹ studied the possible association between Glutamic acid decarboxylase 67 (GAD67) and development of nonsyndromic OFC in Japanese patients. They screened 99 parent-offspring trios using 5 SNPs at the GAD67 gene. The frequency distribution of the haplotype differed between OFC patients and controls. In transmission disequilibrium test (TDT) they found haplotypes preferentially transmitted to the patients with CL±P suggesting that GAD67 is involved in the pathogenesis of OFC in the Japanese population.

Some studies show that neuroprotective peptides prevented fetal death and learning deficits caused by prenatal alcohol exposure⁴⁰. The gamma-aminobutyric acid A (GABA) receptor subunit GABAbeta3 plays a critical role for nervous system and palate development. Toso et al.⁴¹ demonstrated, with a study in vivo, that treatment with neuropeptides prevents the alcohol-induced decline in GABAbeta3 expression 10 days after alcohol exposure. Because palate formation continues through E18, neuropeptides may be beneficial for the prevention of cleft lip and palate.

As well as GABAergic signaling systems, other molecules like transforming growth factor-beta (TGF-beta) and retinoic acid (RA), are potentially involved in palatogenesis.

Baroni et al.⁴² studied the phenotypic differences between primary cultures of fibroblasts from subjects with familial nonsyndromic CL±P and age-matched normal fibroblasts. They found that CL±P fibroblasts exhibit an abnormal phenotype in vitro and respond differently to RA treatment. They suggest that altered crosstalk between RA, GABAergic, and TGF-beta signaling systems could be involved in human CL±P fibroblast phenotype. Moreover they demonstrated that GABA receptor (GABRB3) mRNA expression was up regulated in human CL±P fibroblasts.

By using an animal model, Hansen et al.^{43,44} analyzed the mechanism of phenytoin (PHT) teratogenicity. In a first report, they found an increased incidence of cleft palate and a fall in maternal plasma folate levels in mice treated with PHT during gestation⁴³. Since methylenetetrahydrofolate reductase (MTHFR) activity was decreased in the hepatic tissue of pregnant mice, but it was unaltered in embryos, they inferred that PHT affects maternal folate metabolism. In a second report the same authors found a relationship between the plasma levels of PHT and corticosterone⁴⁴. They suggest that the lengthy increase in plasma corticosterone during organogenesis may represent a factor in the increased incidence of cleft lip and palate observed after administration of PHT to A/J mice. By exposing explants of mouse palates to diphenylhydantoin (DPH), Shimizu and colleagues²¹ obtained results indicating that the teratogen causes cleft palate by inhibiting mesenchymal and epithelial cell proliferation.

Recent epidemiologic studies to evaluate the possible association between some drug treatments during pregnancy and OFC confirmed the inducing effect of phenytoin⁴⁵.

Experimental evidence suggests that the fetal adverse effects of PHT's are associated with potential embryonic bradycardia/arrhythmia and hypoxia-related damage during a restricted developmental period⁴⁶⁻⁴⁸. This hypoxia, through an undefined downstream mechanism, leads to the development of CL.

An hypoxia-related teratogenic mechanism by PHT is supported by indirect evidence from teratology studies^{46,49,50}. Longer periods of hypoxia result in embryonic death while shorter periods of severe hypoxia result in growth retardation and the same type of stage-specific malformations (distal digital reductions, orofacial clefts, and cardiovascular defects).

Nonsteroidal anti-inflammatory drugs

It has been controversial whether aspirin use during pregnancy increases the risk of congenital abnormalities. Saxen⁵¹ suggested that aspirin consumption during the first trimester of pregnancy was involved in the etiology of oral cleft. However, a review of a large body of published experimental animal and human epidemiological data provided no direct conclusive evidence of adverse effects in the pregnant woman and her developing fetus⁵².

A meta-analysis, based on 22 studies published between 1971 and 2002, has suggested an increased risk of neural tube defects (odds ratio [OR] = 2.2; 95 %CI: 0.93-5.17), gastroschisis (OR = 2.37; 95% CI: 1.44-3.88), and cleft lip and palate (OR = 2.87; 95% CI 2.04-4.02) after aspirin use in early pregnancy⁵³.

Nørgård et al.⁵⁴ examined the association between maternal aspirin use in the first weeks of gestation and the most frequent congenital abnormalities: neural tube defects,

gastroschisis, CL±P and CPO. By using a large case-control dataset from Hungary they revealed no increased risk of congenital abnormalities

Finally, Ericson and Källén⁵⁵ performed a study on congenital malformations in infants whose mothers used nonsteroidal anti-inflammatory drugs in early pregnancy. An increased risk was demonstrated for OFC and it correlated with the use of naproxen.

Concluding, it is well-established that nonsyndromic OFC is composed of two separate entities: CL±P and CPO. Both have a genetic origin, and environmental factors play a role in the onset of these malformations. Epidemiologic studies have demonstrated a relationship between certain drugs (steroids and anticonvulsants) during pregnancy and a higher risk of having a child with OFC. In all cases, the molecular mechanisms whereby these environmental factors produce their effects are still unknown. Further investigations will be necessary before a complete picture can be obtained of the main factors involved in lip and palate formation. These elements will enable to better understand this complex disease and to provide improved treatments.

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