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Review

Teratogenicity and underlying mechanisms of homocysteine in animal models: A review

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ABSTRACT

Background: Hyperhomocysteinemia in humans is a risk factor for adverse pregnancy outcome, especially congenital malformations. This review summarizes the studies directed on the teratogenicity of homocysteine carried out in animal studies, and elaborates on the underlying mechanisms.
Methods: Literature was searched in Pubmed (NCBI) through January 2010 and selected manually. Keywords comprised homocysteine, congenital abnormalities and animals.
Results: Increased frequencies of a wide range of congenital malformations are reported especially in the chicken embryo after exposure to homocysteine (Hcy) in various dosages and forms. Reduced embryonic growth and abnormalities of the vascularization of the yolk sac are described in mouse studies. A study in rats revealed a reduced development of blastocysts. The congenital malformations observed in the chicken embryo model share the mutual involvement of Hcy sensitive neural crest cells. Derangements in the behavior of these cells by interactions between Hcy and pathways involved in vascularization, growth, metabolism, signaling, and DNA synthesis and methylation may explain the wide range of effects on embryonic organs, the yolk sac and placental tissues.
Conclusions: The associations between human hyperhomocysteinemia and congenital malformations are substantiated by chicken and rodent studies. Moreover, derangements of several pathways induced by Hcy are demonstrated with adverse effects on both reproduction and long term health. Because of the high prevalence of hyperhomocysteinemia in both the reproductive and general population, research on underlying epigenetic mechanisms is warranted.

Contents

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1. Introduction

Congenital malformations are the most important cause of perinatal morbidity and mortality and affect over 8 million children worldwide each year [1]. More than 80% of congenital malformations have a complex etiology, in which interactions between subtle structural genetic, i.e., single nucleotide polymorphisms (SNP) and environmental exposures such as periconception malnutrition and unhealthy lifestyles are implicated.

Three decades ago the pediatrician Professor Smithells was the first to suggest that a maternal folate shortage plays a part in the development of neural tube defects (NTD). His group showed that a low maternal folate status in the first trimester of pregnancy, which coincides with the period of neural tube closure, i.e., 21–28 days after conception, was associated with an increased risk of NTD offspring [2]. In a nonrandomised trial they also showed that periconception multivitamin use, including 360 μg of folic acid per day, reduced the recurrence risk of NTD by 86% [3]. These findings stimulated both human and animal studies on associations between folate and other complex congenital malformations and on the clarification of underlying mechanisms, e.g. the homocysteine (Hcy) pathway (Fig. 1) [4–6]. Moreover, worldwide this has resulted in the recommendation of a daily intake of 400 μg of folic acid in the periconception period by governments and international organizations, such as the World Health Organization and the Food and Drug Administration from 1991 onwards. Additionally, this has led to the stimulation of folic acid use through local and national campaigns, and to the launch of food fortification programs in several countries. These programs have already shown to significantly reduce birth prevalence rates of NTD [7,8].

The most recent meta-analysis of systematically reviewed human intervention studies revealed that, dependent on study design, i.e., case control (cc) or cohort studies and randomised controlled trials (c-rct), folic acid-containing multivitamins protect against NTD (cc 33% and c-rct 48%), cardiovascular defects (cc 22% and rct 39%), limb defects (cc 52% and rct 43%), orofacial clefts (cc 24%–37%), urinary tract anomalies (cc 52%) and congenital hydrocephalus (cc 63%) [9].

In the early nineties Steegers-Theunissen et al. were the first to suggest that plasma Hcy is a more sensitive marker of the folate status than serum or plasma folate. This hypothesis was based on case–control studies in mothers with and without NTD offspring showing that a fasting Hcy concentration above approximately 14 μmol/L was associated with a 2–3-fold enhanced risk of NTD offspring [10]. This finding has been substantiated by studies of others [11,12]. The question remained, however, whether Hcy should be considered a sensitive biomarker of a compromised folate, vitamin B12, and vitamin B6 status, or a direct teratogen [5].

Experimental animal studies were performed to explore the direct effects of Hcy after exogenous Hcy application and showed varying effects [13–18]. The effects were different between species and models used and appeared to be dependent on the period of Hcy exposure and the chemical structure of Hcy used. The focus of this review is to describe the congenital malformations reported in chicken, mouse and rat studies after exogenous Hcy application in animal models without a known genetic contribution (mutation, knock out) and to elaborate on the suggested associations and underlying mechanisms.

2. Materials and methods

Literature was searched in PUBMED (NCBI) through January 2010 that contained the keywords "homocysteine"; "congenital abnormalities" and "animals". This was followed by the manual screening of the abstracts. In this review we only included original articles in which the teratogenicity of exogenous Hcy treatment in the chicken; mouse and rat were studied. Studies on genetic animal models of hyperhomocysteinemia were not in the scope of this review. This resulted in 22 articles; of which 16 studies were described in the chick; 5 in the mouse and 2 in the rat. One study contained both mouse and rat experiments [14]; and another study contained both chick and mouse experiments [19]. The articles were sorted per species and summarized in Tables 1 and 2 .

2.1. Homocysteine pathway

The Hcy pathway is presented in Fig. 1. Folate as 5-methyltetrahydrofolate serves as a substrate in the remethylation of Hcy into methionine. A deficient folate intake induces a mild to moderate hyperhomocysteinemia. Elevated Hcy concentrations enhance the synthesis of Hcy thiolactone [20]. Thiolactone is a reactive metabolite to proteins which may explain some of the observed pathological features [21]. In most cases hyperhomocysteinemia and folate deficiency can be treated by folic acid use or by an increased intake of folate-rich foods [22]. Besides folate, numerous of other determinants are implicated in Hcy homeostasis varying from other vitamins, e.g. vitamin B12, vitamin B1 and vitamin B6, unhealthy lifestyles, e.g., high caffeine intake and smoking, medication use, renal and liver function, endocrine and physiological processes, to SNPs in folate related genes, e.g. MTHFR 677 C > T [23]. Although maternal hyperhomocysteinemia is significantly associated with NTD, orofacial clefts and congenital heart disease, current knowledge strongly suggests that Hcy is an epiphenomenon [12,24–26].

2.2. Animal studies

In Table 1 an overview is given of the studies on the embryotoxic effects of Hcy in the chicken embryo. Three studies reported embryonic death (0–85%) [13,27,28] and NTD was observed in five studies, with prevalence ranging from 11% to 100% [13,29–32]. Orofacial clefts were described in one study (5.8% prevalence) [30], cardiovascular disease in three studies (28–83%) [19,31,33], somite deformities were reported in one study (92%) [31], brain abnormalities in two studies (86%) [31,34] and ocular malformations in one study with a range of 9.5–100% [35].

The variations in frequencies of these malformations can partly be explained by differences in experimental set-up. Although similar chicken strains were used, variations in the chickens' diets, possibly influencing the yolk sac composition, and variations in developmental rates may have led to outcome differences [36]. The time of first treatment varied from HH1 to HH9 and the frequency of Hcy exposure varied from one to three times between these studies. There were also differences in study design, *in vitro* or *in ovo*, and the absence of tissue and intestinal barriers *in vitro* may result in a higher Hcy exposure than in the *in ovo* experiments. Additionally, the manner of Hcy application, i.e., injection into the neural tube, the circulatory system or the amniotic sac, or the *in ovo* administration of Hcy through droplets on the inner shell membrane, and the *in vitro* addition of Hcy to the medium, is also of influence. *In ovo* administration of Hcy leads to less precise timing and concentration of exposure.

Table 2 summarizes the studies on the embryotoxic effects of Hcy in the mouse and the rat. Ten different mouse strains were studied, and only one *in vitro* study in the CD-1 strain and one *in vivo* study in the C57 strain showed significantly more embryonic abnormalities after Hcy exposure [16,19]. No increase in NTD frequency was reported in both the *in vivo* [37] and the *in vitro* [18] rodent studies. Morphological features of derangements in early neural tissues were observed in two *in vitro* rodent studies [14,16]. Reduced embryonic growth and abnormalities of the yolk sac vascularization and diameter were reported in two *in vitro* mouse studies [16,18]. One non-invasive *in vivo* ultrasound study showed 66% valve regurgitation after Hcy exposure caused by cardiac valve defects [19]. Hcy also decreased placental weight, crown-rump length and body weight. One *in vitro* mouse study revealed a 4-fold reduction of embryos developing into blastocysts [14]. *In vitro* mice studies, Hcy showed to have a dose-dependent effect, and high Hcy concentrations added to the medium caused blisters, growth retardation and abnormalities in somite development [16]. The observed dose-dependent lethality, growth retardation and frequency of congenital malformations is supported by the toxic effects of very high Hcy concentrations and the beneficial effects of low Hcy exposure, resulting in a reduction of dysmorphological features in the rat *in vitro* compared to control embryos [15]. Van Aerts et al. state that this beneficial effect may be explained by the conversion of L-homocysteine (L-Hcy) to L-methionine, probably a limiting amino acid in embryonic development. Due to this conversion THF is also liberated and available for its essential functions, such as purine and thymidine synthesis.

The variability of the results in rodent studies are comparable to chicken studies, and can partly be explained by differences in the moment and duration of Hcy exposure, the finally achieved Hcy level, the maternal diet, the period between exposure and morphological outcome assessment, the method of outcome assessment and the rodent strain studied. The study by Han et al. emphasizes the critical importance of the application of Hcy at the right moment during development to induce a defect. The heart defects described in this study were only seen if exposure was very early at embryonic day 6.75. If treated earlier lethality of the embryos occurred, and if treated 24 h later no defects were seen [19]. Also the importance of the method of outcome assessment was shown by this study. With the use of non-invasive ultrasound examination changes in cardiac function were demonstrated after Hcy exposure in a mouse strain without a genetic predisposition for NTD, as no cardiac defects were reported in all other nonmutant mouse studies after morphological examination. Han et al. only analyzed malformations of the heart,