PCBs and Hemorrhagic Disease of the Newborn

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Introduction

Polychlorinated -dibenzodioxins (PCDDs), -dibenzofurans (PCDFs), and -biphenyls (PCBs) are prevalent in human breast milk samples of industrial countries.[1] PCBs in human milk samples are present at much higher levels compared to PCDDs/PCDFs. The highest level of PCDDs / PCDFs is 27 pg TEQs/g of fat,[2] and that of PCBs is 4 μg/g [3] of fat found generally in human milk samples from industrial countries. *Ortho*-substituted non-planar PCBs are more widespread in milk than co-planar PCBs primarily due to their greater prevalence in the environment. Around 63% of the total PCBs in human breast milk comprises *ortho*-substituted non-planar PCB-22,-52, -138, -153 and -180.[4] The corresponding the total level of *ortho*-substituted non-planar PCB-22,-52, -138, -153 and -180 has been estimated to be 2.5 μg/g of milk fat.[5]

PCBs have been found to be associated with vitamin K deficiency bleeding, otherwise known as Hemorrhagic Disease of the Newborn (HDN), encountered in some newborn infants nursed by mothers with high levels of these compounds in breast milk.[6,7]

Hemorrhagic Disease of the Newborn

Hemorrhagic Disease of the Newborn (HDN) was first described by Townsend[8] in 1894. In this report on HDN, the bleeding was observed typically from the gastrointestinal tract and was distinguished from inherited hemophilia because of its self-limiting nature. Of the first 50 neonates that were identified with this condition, 31 died and all had bleeding within the first two weeks. After the discovery of vitamin K and its

effect on coagulation in the 1930's by Dam[9], many neonates were shielded against this form of life threatening hemorrhaging with the administration of vitamin K. Vitamin K deficiency, which was usually the reason for HDN, seemed to be under control. The reasons for this was an increased practice of bottle feeding than breast feeding the infant, and decreased use of phenobarbital by pregnant mothers, which was found to deplete vitamin K. In the 1970's, breast feeding increased in the industrial countries and there were those that believed that vitamin K should be given only to those at risk[10] of hemorrhage. This list developed to include those infants that were to be entirely breast fed, those that had traumatic deliveries, those whose mothers had been administered vitamin K antagonizing drugs, including anti-convulsants (i.e. phenobarbital), and anti-tuberculous drugs, and those that were born preterm or with low birth weights.[11] The problem of HDN was declining once again; however, an emergence of a late form of the disease in the 1980's raised many questions.[12] In particular, it was seen that there was a relationship between the increasing number of cases of the late type of HDN with the increased rate of breast feeding of infants by mothers with higher levels of PCBs in breast milk.[6,7]

Vitamin K is essential for the activation of vital proteins, some of which are the coagulation factors II (prothrombin), VII, IX, and X.[13] Vitamin K affects the glutamic acid residues found on the vitamin K dependent proteins. The particular method of action is the post-translational carboxylation of these residues into gamma-carboxyglutamic acid, which changes them into active calcium binding sites.[14] Vitamin K acts as a strong base to split the hydrogen from a section of the glutamic acid, CH_2, which allows a carboxlyase enzyme to react with the glutamic acid to form gamma-carboxyglutamic acid.[15] With the gamma-carboxyglutamic acid, the proteins are able to bind to calcium atoms that are on the surface of blood platelets and then blood-clotting enzymes stick all of these cells together, thus aiding in blood clotting. Without the ability to bind calcium, the proteins are functionally defective. Many detailed reports describing the details of the importance of vitamin K exist.[16]

There have been three forms of HDN described, the early, classic, and late form. The early form can result in life threatening hemorrhaging within the first 24 hours of delivery. Generally, this early form is identified with mothers who have affected the metabolism of vitamin K by taking drugs, such as anti-convulsants (i.e. phenobarbital), oral coagulants, antibiotics, and tuberculostatics. It has been hypothesized that some drugs are able to induce microsomal enzymes in the liver which would increase the rate of degradation of vitamin K, thus producing a state of deficiency.[12,17] The classic form involves infants that are normal at time of delivery, but within 2-7 days develop bleeding. The causes of this form are delayed onset of feeding, insufficient intake of milk, and minimal vitamin K content in breast milk. The late form HDN occurs between the second week and the six month.[6]

Of the three forms, there seems to be the least amount of information with the late

form of HDN. It is known for many of the cases of the early form of HDN in which pregnancies will be at risk and with this, there are safety guidelines to follow. With the classic form, it is known that through vitamin K administration at birth, prevention is possible. However, only recently has the late form emerged. The late form of HDN seems to be a serious cause of death in infants that are more than one month of age, yet the reason for this has not yet been identified.[18, 19, 20, 21, 22, 23] It has also been shown that there is an increase in incidence with breast-fed infants, and that the rate of incidence can be decreased with formula feedings given earlier.[15] This demonstrates an association of breast feeding involved with HDN, as breast feeding seems to be necessary for HDN.[23, 24] The reason suggested for the necessary of breast feeding is that the dietary requirements of vitamin K for newborns is not met by the concentrations of vitamin K in human milk.[25] The late form HDN is characterized by intracranial hemorrhage and seems to be a new disease exclusively found in infants breast-fed by mothers with high concentrations of PCBs in breast milk.[6, 7] Currently, the late form HDN is prevented by vitamin medications.

Mechanisms of Action for Depletion of Vitamin K by Ortho-substituted non-planar PCBs

Ortho-substituted non-planar PCBs depict the largest proportion of PCBs in human and wildlife tissues and have been identified with a series of neuroendocrine effects in animals,[26, 27, 28] and humans. [29, 30] The *ortho*-substituted non-coplanar PCBs , like phenobarbital, induce P450 CYP2B1/2 enzymes. Certain drugs are able to induce microsomal enzymes in the liver which would increase the rate of degradation of vitamin K, thus producing a state of deficiency.[12, 31] A relationship between PCB-153, but not TCDD, induction of either CYP2B1/2 or total P450 content and blood coagulation time dependent upon vitamin K, the vitamin K enzyme cycle, prothrombin, and/or factor VII has been demonstrated in female gnotobiotic rats.[32]

Male rats have been reported to be highly susceptible for vitamin K depletion when fed a diet containing irradiated ground beef.[33, 34] Similar vitamin K deficient hemorrhagic states have also been observed to be more prevalent in the male than the female rats maintained in gnotobiotic conditions.[35] Castration seems to profoundly increase survival time in male rats maintained on vitamin K deficient diet.[34] Administration of estrogen offers complete protection against hemorrhage in male rats maintained on a vitamin K deficient diet, whereas administration of androgen to the adult female rats results in increased susceptibility to vitamin K deficiency.[34] Prothrombin levels also increase in estrogen treated male animals, but androgen pretreatment of female rats decreases the prothrombin levels resulting in increased incidence of the hemorrhagic state.[36]

Vitamin K dependent coagulation factor VII depletion appeared by day 3 post administration of 4 μmole/ kg bw of PCB-153 in male rats where as in female rats it took

day 14 post administration of 0.75 μmole/ kg bw.[37] Although factor VII depletion was also found at higher dosed groups of males in this study, its depletion was absent in higher dosed female animals.

In human studies an increased prevalence of vitamin K deficiency hemorrhage has been reported in the male. In one study, 93 cases of the late form of vitamin K deficiency HDN was found to be comprised of 59 males and 34 females.[19] Between 1981/1985, there were 425 cases of late vitamin K deficiency bleeding were reported from Japan, which included 284 males and 141 females.[22] These human observations supported by animal studies suggest that vitamin K deficiency HDN is more prevalent in the male than females and protection in males can be offered in the males by administration of estrogen.

Neonatal diethylstilbestrol (DES) exposure of hamsters can induce premature uterine endometrial hyperplasia.[38] Similar uterotropic abnormalities in immature female rats have been used as an *in vivo* assay for determining the estrogenic activities of chemicals.[39, 40] PCB-153 has also been shown to increase uterotropic activity associated with induction of CYP2B1 activity, as measured by PROD, in prepubertal female rats.[41] Menadione, a synthetic form of vitamin K, has been reported to have uterotropic activities in prepubertal rabbits.[42] Similar uterotropic activity associated with cornification of the vaginal epithelium has also been observed in female rats and mice treated with vitamin K. [34]

Conclusions

These studies suggest that vitamin K deficiency is associated with endocrine disrupting activities of ortho-substituted PCBs . Vitamin K is estrogenic in nature as revealed by its uterotropic activities in females prepubertal rodents. Vitamin K deficiency is most prevalent in males and its depletion by exogenous endocrine modulating stress is protected by estrogens. Endocrine disrupting activities of *ortho*-substituted PCBs is accompanied with induction of CYP2B1 resulting in vitamin K deficiency in breast fed infants.

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