

The H-Tx rat has a heritable methylation error affecting all tissues including the germline which underlies the congenital hydrocephalus of its offspring and is corrected by folate supplementation

Naila Naz¹, Ghazaleh Moshkdanian², Salma Miyan¹, Alice Cochrane^{1,+} Alicia Requena Jimenez¹, Jaleel Miyan¹

1 Faculty of Biology, Medicine and Health, Division of Neuroscience and Experimental Psychology, The University of Manchester, 3.540 Stopford Building, Oxford Road, Manchester, M13 9PT, UK

2 Department of Anatomy, The Medical University of Kashan, Kashan, Iran

j.miyam@manchester.ac.uk

Background

We have previously described a folate imbalance in the cerebrospinal fluid of affected hydrocephalic H-Tx rat fetuses and neonates. We further showed that this responds to maternal folate supplementation to reduce the incidence of hydrocephalus and generally improve brain development. We have been investigating two issues, firstly how to get the incidence to zero and secondly what is the origin of the methylation folate imbalance.

Materials and Methods

10 male female adult H-Tx rats were given daily subcutaneous injections of a combination folate supplement for 1 month before mating. After mating the males were sacrificed for analysis and the females continued on supplements throughout gestation. Heads of neonates and tissues from the dams were frozen for sectioning. Untreated male and female H-Tx as well as Sprague-Dawley control rats were also taken for analysis. Tissues including brain, liver, testis and ovaries were fresh frozen in isopentane cooled with dry ice, cut into 25µm thick sections and stained for immunofluorescence using antibodies for 5-methyl cytosine, 5-hydroxymethyl cytosine, folate binding proteins and folate.

Results

We found a general reduction in methylation in all tissues in the HTx rats including the brain and with a greatly reduced methylation in the testes compared to normal Sprague-Dawley rats. Folate supplemented rats showed improved/normal methylation in tissues including the brain and testes. Staining showed an apparent block to folate entry into the tubules of the testes compared to control rats. Moreover there was a block of entry into the nuclei of cells, including in the brain, of the folate enzyme 10-formyl tetrahydrofolate dehydrogenase (FDH) associated with the methylation error.

Conclusion

Our data indicate a germline methylation error may be causative in the general folate issue leading to hydrocephalus in the H-Tx rat. A failure of FDH to enter the nucleus may be responsible for the failure of the methylation process. Our combination folate formulation fixes the methylation error in all tissues and prevents hydrocephalus completely when given to both males and females.

Funded by a Nuffield Research Placement