

Sex, death and the (nerve) cell

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1. ABSTRACT

Men and women not only look different, but they have different risks of multiple diseases like migraine, neurodegenerative disorders or numerous cancers. Even the nerve cells may die in different ways and exhibit different sensitivity to pro-apoptotic factors. Some of the differences can be explained by the action of sex hormones, but the experiments on four core genotype mouse model, in which XX and XY mice can be of either sex showed that not all differences are due to hormones. An example of a disease with no simple explanation of sex bias is Leber hereditary optic neuropathy, a mitochondrial disease with about 4:1 male to female ratio. The apoptotic death of retinal ganglion cells forming an optic disc is a proposed mechanism of the disease pathophysiology. The mechanisms causing different sensitivity of the nerve cells of male and female subjects may be responsible for the gender bias in LHON and merit further studies.

2. INTRODUCTION

Sex bias is quite a common feature in human disease. Differences in prevalence, course, and reaction to therapy between females and males are observed in numerous disorders. For example, migraine pains are about four times more frequent in females (1-2); hypothyroidism is about five times more frequent in females (3) while schizophrenia (4) is more common in males, although it becomes more frequent in older women whereas autism (5) is about four times more frequent in men. A significant group of diseases exhibit this feature, from autoimmune disorders like multiple sclerosis (MS, higher incidence in women) (6), type 1 diabetes mellitus (higher incidence in men) (7-8) or arthritis (higher incidence in women) (9). Sex bias is also observed in different types of neoplasms: thyroid cancer is more common in females (10) while pancreatic cancer in males (11). Neurological and neurodegenerative disorders are another group

demonstrating gender differences. For example males are more susceptible to Parkinson's disease (PD) (12) and amyotrophic lateral sclerosis (ALS) (13).

Formerly the differences between males and females were ignored in most studies. Indeed female subjects were completely excluded in many of them because they were more difficult to study than males because of their hormonal instability. Currently as the importance of sex differences has been noticed much of the research is concentrated on understanding their origins.

The first and most obvious culprits of this phenomenon are the hormonal differences between females and males. Their influence is discussed below. Another reason may be social conditions. Although lung cancer is more frequent in men this is not due to higher susceptibility, but only to higher frequency of cigarette smoking among males (14). Genetic factors, like X-linked mutations, are responsible for a significant part of sex differences in intellectual disability (15-16).

The protective role of estrogen is well known in atherosclerosis, as the disease and its common consequence, myocardial infarction, are less frequent in pre-menopausal women in comparison to age-matched men, but this effect disappears with age when the estrogen level drops after the menopause. The same observation is true for ischemic stroke (17).

A similar effect is observed for some neurological disorders with proposed inflammatory response involved in the mechanism of pathology like Alzheimer disease (AD), Parkinson's disease, amyotrophic lateral sclerosis or multiple sclerosis. Estrogen seems to play an anti-inflammatory role in all of them, but the exact mechanism is not yet known. The involvement in the expression of pro-inflammatory cytokines via a putative estrogen receptor has been proposed. Unfortunately estrogen neuroprotection is not straightforward, and is not the only explanation of the sex bias in neurological disorders (18-19).

As mentioned above, women are less likely to develop Parkinson's disease and frequently motor symptoms are delayed in them. At the same time they are more susceptible to PD-related complications. It seems that the bioavailability of levodopa (the drug commonly used in PD treatment) is higher in women. The role of estrogens in females with PD was studied and postmenopausal estrogen therapy (estrogen replacement therapy, ERT) was found to decrease the risk of PD in women (18-19).

In amyotrophic lateral sclerosis male predominance decreases with age, and the risk is higher for post-menopausal women. Although in different experiments neuroprotective features of estrogens for spinal motor neuron in SOD-1 male mice (SOD-1 mice are the murine model of ALS) were demonstrated, ERT not only was not beneficial for women but also women who used estrogens had an earlier onset of ALS in comparison to non-users (18-19).

Hormonal status is known to affect the course of the disease in multiple sclerosis. In pregnant women with the disease remission is frequently observed in the first trimester and the symptoms worsen after the delivery. Hormonal contraceptives lower the risk of MS (18-19).

For Alzheimer disease the situation is more complicated. The prevalence of AD is higher in females and the pathologic lesions in female brains are generally more serious. The data on estrogen influence in AD are confusing, some of them show protective influence of ERT, but for women older than 65 no such effect is observed. It seems that estrogen can influence the disease but there definitely are the other factors responsible for sex bias in this disease (18-19).

3. SEX-DEPENDENT DEATH OF NEURONS

3.1. Leber hereditary optic neuropathy

Although, as mentioned above, in some cases there is at least a partial explanation of gender bias, there are many cases where it is still a mystery. One of them is Leber hereditary optic neuropathy (LHON), a maternally inherited mitochondrial disease caused by mutations in mitochondrial DNA (mtDNA) (20). In comparison to the other mitochondrial disorders – multi-systemic, mostly neuromuscular diseases, LHON is characterized by several extraordinary features: only the optic nerve is affected, the penetrance is quite low and men suffer from visual loss 3-4 times more frequently than women (21, 22). Two questions remain unanswered in LHON research: why only the optic nerve is affected and why men are more susceptible? To answer the first one it is important to point out the differences between the optic and other nerves and determine how the optic nerve dies in LHON. Some features of the structure of the optic nerve, formed from the axons of retinal ganglion cells, are unique. First, it is morphologically divided into the parts lying before and behind the lamina cribrosa. The first, containing the body of the retinal ganglion cells (RGC) is unmyelinated and contains more mitochondria than the myelinated second part. The specific structure, the exposure to light and high energy requirement of the pre-laminar part of RGC can make the nerve cells more sensitive to reactive oxygen species (ROS) and decline in energy production. How do they die? The present consensus is that in LHON optic nerve atrophy is caused by apoptosis as a consequence of higher ROS production due to mutations in mtDNA respiratory complex I genes (23). Tissue culture studies on cybrids (transmitochondrial hybrids formed from the fusion of rho0 cells lacking mtDNA and cytoplasts bearing mitochondrial mutations) with LHON mutations show higher sensitivity to Fas mediated apoptotic death, but there are no studies performed directly on the optic nerve (24). Such an experiment should not be expected soon because, although the model of murine RGC exists (25), it is impossible to mimic human LHON mutations in mice because there is no way to transform mitochondria with mtDNA carrying the appropriate mutations. At the same time there are no cultures of human RGC.

3.2. What, if not hormones?

The question of gender bias in LHON remains unanswered as well. Because of low penetrance it is obvious that mtDNA mutations are not sufficient to trigger the disease although they are necessary. There are other genetic and environmental factors co-acting in disease formation. The simplest idea of the X-linked factor responsible for sex differences has been tested for the last 20 years giving only a few susceptibility loci with no specific genes and no supporting data from other groups of researchers (26). Skewed X chromosome inactivation in females with fully expressed LHON has been excluded (27). Surprisingly, there are no studies on hormonal influence in LHON patients. Families where women are affected as frequently as men or even more often may be of great help in the future (28). Interestingly no sex bias was observed, in “the nuclear twin” of LHON, autosomal dominant optic atrophy, a disease in which the same cells, RGCs are affected, demonstrating many similar features (29). Hormonal influence was tested on cybrids with LHON mutations. 17 β -estradiol lowered ROS production, activated mitochondrial biogenesis and slightly improved energetic competence (30). These data have not been reproduced in other cell types and the level of female hormones has not been tested in LHON patients.

The differences which have been mentioned above can be caused by numerous factors. The obvious are sex chromosome differences and differences in structure and gene expression induced permanently by the presence of hormones and persisting in their absence (organizational differences), as well as those requiring their constant presence (activational differences) (31). It is difficult to distinguish between the two types of effects of sex hormones in cells after any kind of hormonal exposure. Therefore various experimental models have been developed to dissociate the effects of chromosomes and hormones.

One of these models is the four core genotypes mouse model, in which XX and XY mice can be of either sex. This complement of four mouse karyotype/sex combination makes it possible to separate some of the chromosomal from some of the hormonal effects (32). Many differences were found to be mediated by the XX versus XY chromosome complement, and not by the presence of gonadal hormones. No data on apoptosis of nerve cells have been presented; however the chromosome complement, and not gonadal hormones, was found to affect behavior, gene expression and neural tube closure (33).

Another approach is to look at mouse embryonic cells in stages of development before any sex hormones are produced. Penalzoza *et al.* (34) have compared the effects of a number of stress-inducing compounds in cells taken from mice at 10.5 and 17.5 days post-conception. In the former case the cells had not been subjected to any hormonal influence, in the latter sex hormones were already produced. Both gene expression and reactions to stressors were found to be different even before exposure to sex hormones. These experiments were not performed on nerve

cells, but they indicate that there are differences between embryonic cells of male and female mice before any hormonal effects are exerted.

Innate differences in the apoptosis pathway were found in embryonic rat neurons (35). Neurons derived from female embryos were more sensitive to etoposide and staurosporine induced apoptosis and less sensitive to nitrosative stress and excitotoxicity. Du *et al.* (35) also found that 17 day old male rats, in contrast to female rats, were unable to maintain reduced glutathione levels in the cerebral cortex after asphyxial cardiac arrest. Nitrosative/oxidative stress caused cell death via poly(ADP-ribose) polymerase (PARP) in male neurons whereas female neurons died in a pathway involving cytochrome c release.

This fundamental difference the way nerve cells die was also observed in mice subjected to ischemia (36). Not only are quantitative differences in cell death observed, such as smaller infarct volumes in female than in male mice, but here again in females the major pathway of cell death after ischemic injury is through caspase activation, in males the trigger is poly(ADP-ribose) polymerase (PARP) activation and the nuclear translocation of apoptosis inducing factor (AIF) (37). Caspase inhibitors benefited females, but not males. On the other hand, both PARP and nitric oxide synthase inhibitors protected males, but not females (38). The PARP-mediated cell death is androgen dependent, as ischemia causes an increase in PARP expression which is lower if androgens are absent (39). Moreover, if the PARP-1 gene is knocked out in mice, the males are more resistant to focal cerebral ischemia (39).

The name parthanatos has been proposed for the PARP-mediated cell death pathway (40, 41) though the name is not used extensively in the literature, and many authors do not distinguish between parthanatos and apoptosis. Moreover, the differences in cell death well established in the rat and mouse brain ischemia model may not be universal, they do not appear to occur in a similar model in gerbils. (B. Zabłocka, personal communication).

Differences have also been observed in cells which are components of the nervous system, though they are not themselves nerve cells. In astrocytes, which are glia cells present in the brain, estrogen and progesterone were found to affect mitochondrial fission and fusion gene transcription (42) in a different way in male and female mice. In females estrogen and progesterone stimulated the transcription of both groups of genes, whereas in males both hormones stimulated apoptosis. These differences may contribute to the differences in how neurological diseases affect men and women; moreover, even though astrocytes are not present in nerves outside of the brain, similar effects could occur in other types of glia cells and be pertinent for apoptosis occurring in nerves, including the optic nerve.

4. SUMMARY AND PERSPECTIVE

It seems that gender bias is much more complicated than being just the result of pre- and postnatal

hormonal action. The sex differences in PARP-mediated neuronal death may be responsible for distinctive features in women and men in multiple neurological disorders including LHON. Unfortunately no experiments on the optic nerve with its specific morphology and localization in the body have ever been conducted and may only be possible if an appropriate animal model is established.

Greater understanding of the mechanisms of neuronal death will not only widen our basic knowledge of the sex bias phenomenon but may also be helpful in establishing the way to appropriately manage male and female patients taking into account that the molecular causes of their diseases may not be identical. This may be particularly important as PARP inhibitors are already used as a therapeutic agent.

5. ACKNOWLEDGMENTS

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Abbreviations: AIF, apoptosis inducing factor, LHON, Leber hereditary optic neuropathy, PARP, poly(ADP-ribose) polymerase, RGC, retinal ganglion cells

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