Asian Journal of Andrology (2015) 17, 939–941

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INVITED RESEARCH HIGHLIGHT

Sperm Biology

Zygotic chromosomal structural aberrations after paternal drug treatment

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Asian Journal of Andrology (2015) **17**, 939–941; doi: 10.4103/1008-682X.154307; published online: 22 May 2015

In recent years, the field of male-mediated reproductive toxicology has received growing attention. It is now well-established that many drugs, chemicals, and environmental factors can harm male germ cells by inducing DNA damage. Male germ cells have extensive repair mechanisms that allow detection and repair of damaged DNA during the early phases of spermatogenesis. However, during the later phase of spermiogenesis, when the haploid spermatids undergo chromatin condensation and become transcriptionally quiescent, their ability to repair damaged DNA is lost.[1,2] It is also thought that the highly compacted chromatin of the sperm can protect DNA against damage.[3] Therefore, it is expected that late spermatids will be most susceptible to DNA damaging agents. Unrepaired or misrepaired damage in the germ cells leads to the generation of spermatozoa with DNA damage that can be transmitted to the next generation. Fortunately, the maternal DNA repair machinery is capable of recognizing and repairing, at least to some degree, damaged paternal DNA after fertilization in the zygote. Therefore, the efficiency of the maternal repair machinery will greatly influence the risk of transmitting paternal DNA damage to offspring.[4]

Marchetti *et al.*[5] have recently published in *Scientific Reports* some novel studies in a manuscript entitled "Meiotic interstrand DNA damage escapes paternal repair and causes chromosomal aberrations in the zygote by maternal misrepair". The results from these studies highlight the fact that paternal exposure to a DNA damaging agent can induce effects that are severe enough that they cannot be corrected by DNA repair mechanisms during spermatogenesis or after fertilization by the maternal DNA damage sensing machinery.

In this article, Marchetti *et al.*[5] set out to define the differential sensitivity of male germ cells to melphalan (MLP), a bifunctional alkylating chemotherapeutic agent that causes interstrand cross-links (ICL), and to determine at which phase of spermatogenesis or fertilization DNA damage is misrepaired and converted into a chromosomal structural aberration (CSA). Using cytogenetic assays, they observed that MLP exposure to premeiotic (dividing spermatogonia), meiotic (diplotene spermatocytes) and postmeiotic (testicular and epididymal sperm) germ cells resulted in the formation of CSA in the first-cleavage stage zygote; however, no increase in CSA was observed after exposure of spermatogonial stem cells. The highest incidence of zygotic CSA was observed when meiotic germ cells were exposed. This increase in CSA closely correlated with negative pregnancy outcome, i.e., an increase in dominant lethality. However, the damage caused by MLP to male germ cells did not result in an increased rate of CSA observed in the meiotic (MI or MII spermatocytes) germ cells or epididymal sperm. These results suggest that the MLP induced damage persisted un-repaired through spermatogenesis and was subsequently misrepaired into CSA by the maternal DNA repair machinery in the zygote.

As the numbers of men of reproductive age who survive cancer and wish to father children increase, it is becoming particularly important to understand the effects of chemotherapy on male germ cells and reproductive outcome. Because MLP treatment induced chromosomal damage in the dividing spermatogonia, it could be expected that men receiving treatment could experience adverse reproductive outcomes for many months or years posttreatment, as has been reported after chemotherapy for testis cancer and lymphoma.[6] On the other hand, the fact that MLP did not induce CSA when spermatogonial stem cells were exposed suggests that there may be no long-term consequences on the quality of the germ cells after treatment with this particular drug. Although it is tempting to suggest that such an exposure may not have long lasting reproductive effects, it is unclear if MLP treatment has any more subtle effects on the spermatogonial stem cells that could be detected with more sensitive tests and that may be transmitted to the zygote. For instance, ethylnitrosourea, cyclophosphamide, and isopropyl methanesulfonate, also alkylating agents, as well as particulate air pollution, and cigarette smoke have been found to cause point mutations in spermatogonial stem cells.[7–11]

The damage induced by MLP and the inability of the germ cells and zygote to repair this damage have important implications for couples using assisted reproductive technologies such as *in-vitro* fertilization and intra-cytoplasmic sperm injection. Currently, routine semen analysis based on the World Health Organization (WHO) guidelines only evaluates parameters such as sperm concentration, motility, and morphology.[12] These tests do not take into consideration the genetic integrity of spermatozoa. Even the application of the cytogenetic assays used by Marchetti *et al.*[5] may not detect chromosomal abnormalities in the sperm if the damaged DNA is only converted to CSA after fertilization. It would be interesting to see if other tests that assess the genetic integrity of the sperm,[6] such as SCSA, Comet assay

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and tunnel staining, which measure DNA fragmentation, would correlate with the cytogenetic results presented. It is possible that the extent of damage to the sperm genome determines whether the maternal machinery will be able to properly repair the paternal DNA. Perhaps these tests will be able to predict the extent of misrepair by the maternal machinery of DNA damage into CSA in the zygote.

Cytogenetic assays, as used by Marchetti *et al*.[5] in this mouse study and Tempest *et al*.[13] in human studies, have proven to be a very elegant and powerful tool in assessing genotoxicity. The association found between CSA in the zygotes, and dead implants[5] supports a previous study by this group reporting that the frequency of paternally transmitted CSA is predictive of abnormal embryonic development.[14] In addition, the frequency of observed reciprocal translocations in zygotes sired by MLP treated mice at the meiotic cell stage is in agreement with a previously published frequency observed at the same stage by the standard heritable translocation (HT) test.[15] Using the standard HT test, this same study also observed high frequencies of embryo death and HT at other germ cell stages, particularly when the early to mid-spermatids were treated with MLP. Conceivably, a high frequency of CSA would also be observed in the zygotes after treatment at the early to mid-spermatid stage.

The finding that maximum damage from alkylating agents arises when diplotene spermatocytes are targeted diverges from what has been previously understood regarding the sensitivity of different types of germ cells to genotoxic substances. It is believed that spermatids are most sensitive to DNA damaging agents because of a declining capacity to repair DNA as the chromatin is condensed, and transcription shuts down.[16] However, this notion has been challenged recently. There is evidence that late spermatids maintain an active DNA repair system throughout the chromatin remodeling steps.[17] Whether this repair system is capable of responding to DNA damage induced by a genotoxic agent such as MLP is unknown. Studies with other alkylating agents seem to suggest that the late spermatids are unable to repair drug-induced DNA damage. Paternal exposure to cyclophosphamide (CPA), another bifunctional alkylating chemotherapeutic and immunosuppressant agent that also causes ICLs, results in the highest incidence of CSA in the zygotes after exposure of mice at the late spermatid stages.[18] Other studies in rats have also shown maximal sensitivity to CPA at the postmeiotic stages.[19,20] The different outcomes observed after paternal treatment with MLP and CPA may be due to the pharmadynamic differences between the two drugs. MLP is active in its native form while CPA needs to be metabolized before it is active. Additionally, the two differ in their kinetics when it comes to ICL formation, with MLP forming more stable ICLs.[21] However, as mentioned previously, it is possible that MLP treatment targeting spermatids would also result in a high incidence of CSA in zygotes.

The conclusion that DNA damage occurring in the diplotene spermatocytes goes unrepaired through phases of germ cell development known to be DNA repair competent is surprising. However, ICL repair appears most efficient in S phase in cycling cells as the ICLs are first detected at stalled replication forks.[22] Therefore, as suggested by Marchetti *et al*.[5] late meiotic and postmeiotic germ cells may not have the ability to detect or repair this type of damage. Further studies to assess whether markers of DNA damage detection and repair are present in the germ cells after treatment are needed to determine if MLP induced DNA damage does indeed remain unrepaired in these cells and whether MLP treatment affects the DNA repair machinery. As the postmeiotic germ cells progress through spermiogenesis, the chromatin becomes highly compacted which may limit the ability of MLP to interact with DNA and form ICLs. Although data on targeting mid-spermiogenesis stages with MLP are not available, the increased level of zygotic CSA when testicular or epididymal sperm are targeted clearly demonstrates that these very advanced germ cells are still susceptible to damage, as was previously shown using CPA.[23] Determination of effects along the epididymis would be of great interest to define at what point in epididymal transit the sperm are most affected by MLP treatment.

Clearly there remains much to be understood about male-mediated reproductive toxicology. To date, a relatively small number of drugs, chemicals, and environmental factors have been classified as male reproductive toxicants. The exact mechanisms of action of these compounds on the male germ cells and the capacity of the germ cells to respond to insult are not clearly understood. The complex damage-sensing and repair mechanisms during spermatogenesis, as well as those found in zygotes that can correct paternally transmitted DNA damage, are only beginning to be understood. The additional layer of complexity associated with modification of the paternal epigenome by chemicals and environmental factors and how the zygote and early embryo respond to such marks should provide exciting new avenues for research and therapies in years to come.

COMPETING INTERESTS

The authors declare no competing interests.

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