Testicular and epididymal toxicity: Pathogenesis and potential mechanisms of toxicity

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Introduction

The toxicologic literature abounds with examples of drugs and environmental chemicals that cause changes in spermatogenesis and/or epididymal sperm in laboratory animals. Unless proven otherwise, the assumption must be made that similar changes are likely to occur in humans exposed to these chemicals. For those working in the pharmaceutical industry, this can mean the costly termination of a drug development program and the loss of a potentially valuable therapeutic molecule unless studies can be conducted to investigate potential mechanisms of toxicity and/or realistically predict human risk. For those working in regulatory environmental toxicity, mechanistic studies are rarely conducted but demonstration of a mode of action (MOA) can provide valuable information to allow a better understanding of the real threat of environmental toxicants versus the perceived threat. For both disciplines, it is essential to understand the basic science underlying spermatogenesis and sperm maturation in order to address any mechanistic approach to investigating a toxicologic lesion.

The toxicologic pathologist is generally the person to first identify and sound the alarm regarding a male reproductive toxicant in a drug development or environmental chemical program. Organ weights and histopathology will be the main (often only) information that will be available from repeat dose studies. Given a good understanding of spermatogenesis and spermatogenic staging, the pathologist may be able to take a guess at the earliest cell type affected (Sertoli or germ cell), get a feel for the pathogenesis and recovery of the lesion over time, and they may even be able to assess whether endocrine disturbance is a major and primary event. Very soon after sounding the alarm, a lot of questions will be asked regarding the likely mechanism of toxicity, whether the spermatogenic disruption is “on or off target” for the therapeutic molecule, and is the toxicity, (which often affects one species and not the other) relevant to man? Such questions are extremely difficult to answer or even address when dealing with disturbances of spermatogenesis because of the complexity of the cellular interactions within the testis, the relative lack of knowledge of the physiology and molecular biology of spermatogenesis and the fact that we are often working with species (e.g. dog and monkey) for which there is remarkably little basic biologic

information. In fact, many of these questions are never addressed by pharma companies with a reproductive issue, partly because of time and money constraints, but also because the main objective of repeat dose regulatory studies is risk assessment. So if any follow up mechanistic work is conducted, it is limited and only aimed at whether an effect is relevant to man or justifying why a higher dose can safely be used in clinical trials. This approach contrasts with basic research on cell physiology, endocrinology and molecular biology of spermatogenesis where individual processes are studied in great depth and where occasionally, chemicals may be used to disrupt a process. The objective of this special issue is to try and bring regulatory toxicology and academic, investigative toxicology together to provide relevant information that can benefit both academic and toxicologic-driven disciplines. Specifically, the authors of the main chapters here have been charged with explaining the biology underlying their topic. When we see a lesion, what is going on in the cells to produce that kind of lesion? The intent is to provide a quick path to understanding, and access to relevant literature.

This Special Issue begins with a pictorial review of the broad types of changes that confront the pharmaceutical toxicologic pathologist on a daily basis. *Morphologic manifestations of testicular and epididymal toxicity* by Justin Vidal and Katherine Whitney provides an atlas and review of the typical features that lead the pathologist to draw conclusions regarding the main cell type injured, possible subcellular targets and the progression of a lesion from its subtle early features through to the non-specific end- stages of tubular degeneration and tubular atrophy. This provides the backdrop for the subsequent detailed reviews from academic contributors explaining what might be expected (the Signature Lesion) when specific aspects of reproductive physiology are disturbed and the ways potential regulatory pathways could be disrupted to explain that lesion. This includes a detailed review of the cytoskeleton and the various critical functions that it regulates in *Testicular histopathology associated with disruption of the Sertoli cell cytoskeleton* by Kam Johnson, which details the roles of actin microfilaments, the intermediate filaments, and microtubules. The cytoskeleton is integral to the structural support of the seminiferous epithelium and the functioning of the multiplicity of cell junctions between Sertoli and germ cells and

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http://dx.doi.org/10.1080/21565562.2014.1005511

between adjacent Sertoli cells. Staying with the importance of cell junctions, *Toxicants target cell junctions in the testis- insights from the indazole-carboxylic acid model* by Yan Cheng reviews the various types of junctions and explains how they mediate transport of germ cells up through the epithelium, are responsible for the complex process of spermiogenesis and spermiation, form the blood testis barrier and mediate communication between the various cell types within the epithelium, and what happens when they are disrupted. The complex morphogenesis of spermatids from a conventional round cell to an elongated motile sperm (spermiogenesis) and the precisely timed release of those fully formed sperm into the lumen (spermiation) are obvious targets for chemical disruption. *Mechanisms of spermiogenesis and spermiation and how they are disturbed* by Liza O'Donnell describes the various ways these processes can be disrupted and the morphologic features that can be used to recognize where in the process the disturbance has occurred. Germ cell death (generally by apoptosis) is a ubiquitous feature of testicular toxicants and it can be initiated by toxicants that apparently target either germ cells or Sertoli cells. Stage-specificity of germ cell death sometimes can be used to differentiate these different pathways. *Implications of Sertoli cell induced germ cell apoptosis to testicular pathology* by Caitlin Murphy and John Richburg describes the pathways that regulate germ cell death and how they can be impacted by toxicants. Much of spermatogenesis is driven by testosterone, which in turn is regulated by the neuroendocrine pathways of the hypothalamic pituitary gonad axis. The morphologic consequences of endocrine disruption in rodents can be cell- and stage-specific, allowing the pathologist to recognize androgen insufficiency. However, there are significant species differences in the response to endocrine imbalance that need to be appreciated when evaluating dogs and monkeys. These are reviewed in *Endocrine control of spermatogenesis: Role of FSH and LH/testosterone* by Suresh Ramaswamy and Gerhard Weinbauer.

Although most male reproductive toxicants disrupt spermatogenesis, post-testicular functions in the epididymis can be the primary target for toxicity, or the epididymis can exhibit changes as a secondary consequence to testicular events. *Interpreting histopathology in the epididymis* by Wilma Kempinas and Gary Klinefelter reviews the ways in which the epididymis can be disrupted by toxicants and by androgen deprivation, while *Disruption of estrogen receptor signaling and similar pathways in the efferent ductules and initial segment of the epididymis* by Rex Hess discusses the important (and sometimes missed) consequences of disturbances

in fluid reabsorption by the efferent ductules on testis histopathology. Sperm, which are antigenically foreign, are protected from the host immune system by tight junctions, which comprise the blood testis and the blood epididymal barrier. Inflammation is the result if these barriers are compromised. *The blood epididymis barrier and inflammation* by Mary Gregory and Dan Cyr discusses the morphologic, physiologic and pathologic components associated with the blood epididymal barrier, the role of immune function on its regulation, and how disturbance of these factors can result in inflammatory lesions of the epididymis.

Access of toxicants to the germ cells is believed to rely on those toxicants crossing the blood testis barrier. This can depend on the physiochemical properties of the compound or occur by transporter-mediated access. *Organic and Inorganic transporters of the testis: a review* by David Klein and Nathan Cherrington provides an overview of how transporters function and the main transporters that may be involved in controlling the access of a toxicant to the adluminal compartment of the testis. Many toxicants have the ability to produce reactive oxygen species during their metabolism. *Redox reactions in mammalian spermatogenesis and the potential targets of reactive oxygen species under oxidative stress* by Junichi Fujii and Hirotaka Imai gives the reader an overview of how such molecules can interact with spermatogenic cells as well as the role they play in signal transduction within the testis and epididymis. Finally, *Kinases as targets for chemical modulators: structural aspects and their role in spermatogenesis* by Pranitha Jenardhanan and Premendu Mathur is a valuable insight into the roles that the different families of kinases play in spermatogenesis and what might be expected if a toxicant interferes with some of the associated pathways.

The attentive reader will notice that the amount of information available for each chapter can vary widely: for example, much more information is available on the hormonal regulation of the epithelium, and on the junctions, than on the specific role of kinases in the spermatogenic process. We would view the less-populated fields as opportunities for contributions, places where some scholarship and data would be prominent and most welcomed.

In any case, we hope that these chapters will help get the reader quickly to the relevant literature and will ease the task of answering the questions of mechanism of toxicity and relevance to humans, questions which are key to advancing the relatively-safe molecules, or eliminating those molecules with hopeless toxicities. We wish you the best of luck!