Chapter 43
Can we protect germ cells against testicular insults?
Male reproductive toxicology

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Exposures to testicular insults

The germinal epithelium of the human testis is often subjected to chemical and physical insults as a result of medical, occupational, and environmental exposures. Necessary medical treatments with anticancer agents or immunosuppressive drugs, frequently cause reductions in sperm count (oligospermia); particularly treatment with radiation or alkylating drugs, can cause permanent reductions in sperm count and may even result in complete lack of sperm (azoospermia). Industrial solvents, such as bromopropane, compounds released from plastics used in food containers and medical tubing, such as phthalates and bisphenol A, and numerous fungicides and pesticides also damage spermatogenic function. Methods to prevent these damaging effects and to restore gonadal function after toxic treatments are of great importance to men who desire to have children.

Two exposure scenarios will be considered separately: (1) short-term medical treatments or accidental exposures and (2) prolonged occupational, environmental and medical exposures.

Short-term medical treatments or accidental exposures

Treatment for cancer usually involves exposures to radiation and chemotherapy during a period of several weeks to several months. Many of the anticancer agents preferentially kill proliferating cells, which in the testis are primarily the rapidly proliferating differentiating spermatogonia (Fig. 1). However, killing of differentiating spermatogonia will only result in transient damage to sperm production provided that the stem cells survive and can repopulate the tubules. Protection against these short-term effects is rarely warranted since the patients are advised against procreation at this

time because of possible genetic alterations in these developing germ cells, and the possibility that the tumor cells will also be protected.

Recovery from these short-term effects can occur as the more slowly proliferating stem cells (Fig. 1) that survive repopulate their numbers and differentiate through spermatogenesis within several months, though sometimes several years are required. But the damage might kill too many stem cells or damage the supporting cells (e.g. Sertoli, Fig. 1) or paracrine factors required for recovery. This results in prolonged azoo- or oligospermia, which are of greater consequence to the patient and protective measures would be desirable.

Accidental short-term exposure to radiation or chemicals that affect spermatogonial stem cells, such as dibromochloropropane, are also of concern. However, in these cases it is not possible to plan protective methods but only methods to stimulate recovery may be applicable.

Protection from short-term toxicant exposures

The only example of protection from testicular injury that has been reliably used in humans is shielding of the testes during radiation therapy. The testes can be well-shielded from the direct radiation beam, although scattered radiation still presents some concerns.

Since many anti-cancer agents are selective for killing proliferating cells, the concept of placing the stem spermatogonia in a non-cycling state has been proposed as a protective mechanism. It was erroneously proposed that suppression of male reproductive hormones, [luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone] would reduce the proliferation of the target cells and render them more resistant to the toxicant. But suppression of these hormones only blocks the completion of spermatogenesis and has no effect on the proliferation of the crucial target cells, the stem spermatogonia.

Despite this incorrect theoretical basis, hormonal suppression of rats prior to, during, and even after exposure to chemotherapy or radiation dramatically enhanced the subsequent recovery of spermatogenesis by a mechanism that is still unknown. This result was not due to protection of the stem spermatogonia from being killed but rather is attributable to the restoration of the ability of the somatic cells of the testis to support the differentiation of the surviving stem spermatogonia. This strong effect seems to be unique to rats, where the stem spermatogonia are blocked from differentiating

after cytotoxic treatments unless there is a period of hormonal suppression. Hormonal suppression immediately after irradiation can also modestly stimulate the rate of recovery of spermatogenesis in mice and possibly in non-human primates. Clinical trials of protection or restimulation of spermatogenesis by hormonal suppression have generally been unsuccessful.

A more scientifically based method for manipulating stem spermatogonial cycling, would be the modulation of growth factors that are known to affect stem cell proliferation (Fig. 1), of which glial-derived neurotrophic factor (GDNF) and fibroblast growth factor (FGF) are prime examples. As yet there are no methods for reducing the proliferation of the stem cells without the loss of these cells. However, there are reports that modulation of some growth factors can stimulate the recovery of spermatogenesis in mice after exposure to busulfan, a stem cell cytotoxic chemotherapeutic drug (Fig. 1). These methods include treatment of mice with granulocyte colony-stimulating factor (G-CSF), a stimulant of hematopoietic stem cell proliferation, treatment with an inhibitor of transforming growth fact beta (TGFβ)-receptor 1, down-regulating the G-protein-coupled bile acid receptor (TGR5) signaling pathway, and injection of mesenchymal stem cells which secrete a variety of factors.

Radiation and alkylating agent chemotherapy often produce testicular damage by increasing oxidative stress by generating reactive oxygen species (ROS) and reactive alkyl groups that directly damage the DNA, lipids, and impair sperm function (Fig. 1). However, caution must be exercised about using antioxidants or radical scavengers to protect the testis against the toxic effects of ROS since protection of the tumor cells may also occur. One agent, amifostine, was originally identified because it selectively protected some normal tissues but not tumors against radiation. Although it does partially protect mouse spermatogonial stem cells against radiation, amifostine shows direct toxicity to stem spermatogonia, which in conventional fractionated anti-cancer regimens would outweigh the protective benefit. Similarly, there is some indication that it may reduce oxidative damage in the testis after chemotherapy, but there is only minimal evidence that it may enhance the survival of the spermatogonial stem cells. The benefit of this marginal survival effect is again outweighed by the fact that there appears to be increased genetic damage in the sperm produced. Most of the many other studies of antioxidant protection against radiation and chemotherapy have not addressed the effects

on stem spermatogonial cells, which is the significant target for the subsequent fertility of the cancer patient.

Anti-apoptotic compounds, such as sphingosine-1-phosphate, have been studied for protection against toxicants that kill germ cells by apoptosis (Fig. 1). This procedure partially protects differentiating spermatogonia from radiation but the question of whether stem spermatogonia were also protected was not investigated.

Reducing blood flow to testes by transient ligation or cryptorchidism has been investigated to protect spermatogenesis against damage by chemotherapy drugs with short half-lives. Indeed, reversible ligation does protect animal testes against Adriamycin and produced minimal direct damage, but its potential to cause ischemic damage remains a concern.

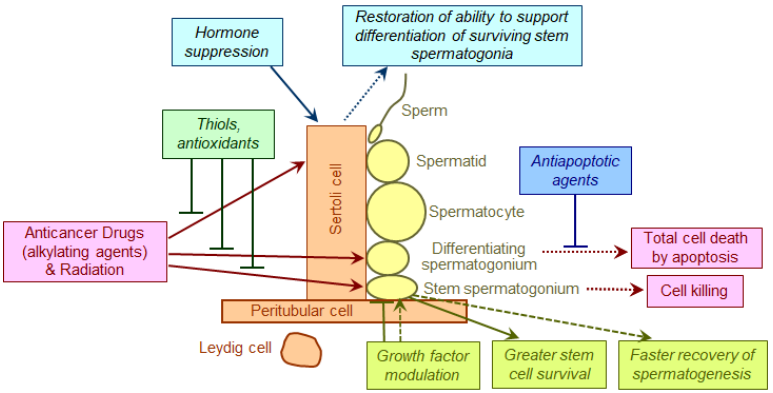

Figure 1. Diagrammatic representation of action of anti-cancer agents on specific cells of the testis and outcomes. Different targets for protection are shown: blocking the initial damage (thiols, antioxidants), restoring somatic cell function to enable surviving spermatogonia to reinitiate spermatogenesis (hormone suppression), and enabling the damaged cells to survive by blocking apoptosis (anti-apoptotic).

Protection from Chronic Toxicant Exposures

The major emphasis in reports of attempts to protect spermatogenesis against damage from chronic exposures has been protection against oxidative stress. In addition to radiation and the alkylating agents used in cancer therapy, a variety of occupational and environmental toxicants produce testicular damage by increasing oxidative stress (Table 1). These toxicants generate ROS which are free radicals with unpaired valence electrons which are capable of oxidizing lipids, proteins, and nucleic acids. Damage occurs when these ROS overwhelm the cellular protection mechanisms, such as endogenous antioxidants like glutathione and enzymes like superoxide dismutase, catalase, and glutathione peroxidase. In addition to the process of spermatogenesis, the mature sperm function and sperm DNA integrity are also very sensitive to oxidative stress.

Table 1: Toxicants Producing Reactive Oxygen Species (ROS) and Protective Scavengers

Examples of other agents producing reproductive toxicity by oxidative stress			
Endosulfan	Hexanedione	Sodium fluoride	Acrylamide
Aflatoxin B1	Phthalates	Acetylene	Cadmium
Acetaminophen	Chlorpyrifos	Malathion	Diazinon
Silver nanoparticles	Deltamethrin	Gentamycin	Acetonitrile
Antioxidants and radical scavengers shown to protect spermatogenesis from toxicants			
Melatonin	Vitamin C	Vitamin E	Glutathione
N-acetyl-cysteine	Retinoic acid	Selenium	Doxycycline
Dietary polyphenols	Folate	Carnitines	Ubiquinone-10
Curcumin	Caffeic acid	5-acetyl sialic acid	Naringenin (flavone)
Lycium	α-lipoic acid	CeO_2 nanoparticles	Apigenin
Caryophyllene	Green tea extract	Lycopene	Royal jelly

A wide variety of antioxidants can be used to reduce tissue oxidative damage (Table 1). Most act by scavenging free radicals either directly or in concert with other scavengers. Among the antioxidants and radical scavengers are a variety of foods, fruits, vegetables, and spice products, and extracts from these products. There are numerous studies showing that these radical scavengers

can reduce damage produced by the oxidative agents to the testes of mice and rats. That is often measured by the degree of lipid peroxidation, using malondialdehyde as a marker, in the testis after exposure to ROS-producing agents; some of these studies also show that there is protection of spermatogenesis, sperm function, and/or fertility.

Despite the large literature showing protection of rodent spermatogenesis from ROS-generating toxicants, it is difficult to pinpoint a practical treatment applicable to cases of specific human exposures from environmental, occupational, or medical treatment exposures. There is debate about the benefit of consuming sufficiently high doses antioxidants to protect against oxidative damage exposure, due to concerns that consuming excessive doses of antioxidant supplements may be harmful.

There have also been numerous studies investigating the possibility that anti-oxidants can have beneficial effects in the treatment of unexplained male infertility, on the assumption that endogenous and/or exogenous ROS were contributing to the infertility. A number of these studies did show favorable effects on spermatogenesis, but others failed to do so or even showed a negative influence on the sperm parameters. This variation may be attributed to the heterogeneity of the studied population. One study with subjects who were heavy smokers of cigarettes, which produce ROS in the smoke, did show a benefit to sperm quality with daily vitamin C supplementation, suggesting protection against ROS-induced damage. However, the doses of antioxidants necessary to balance the redox system are not generally known and overconsumption of antioxidants may result in reductive stress that could cause detrimental effects on human health and well-being.

Conclusions

As yet there are no proven methods for protecting the human germinal epithelium when it is directly exposed to chemical insults nor proven therapeutic options to improve spermatogenesis after damage has occurred. However, protection may be achieved by removal of germ cells prior to exposure and storage for later use. Sperm banking (Chapter 24) done prior to medical exposures routinely results in successful pregnancies. In experimental animals, cryopreservation of spermatogonial stem cells or testicular tissue, and later reintroduction into the testicular tubules or grafting into a subcutaneous site, respectively, result in production of viable sperm

that yield live offspring (Chapter 8). Awareness of such novel research in experimental animals designed to either protect the germinal epithelium from toxic insults or restore its function after the insult could lead to their application to humans (Chapters 46, 47).

Suggested reading

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