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Effective Delivery of Male Contraceptives Behind the Blood-Testis Barrier (BTB) – Lesson from Adjudin

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Abstract

The blood-testis barrier (BTB) is one of the tightest blood-tissue barriers in the mammalian body. It divides the seminiferous epithelium of the seminiferous tubule, the functional unit of the testis, where spermatogenesis takes place, into the basal and the adluminal (apical) compartments. Functionally, the BTB provides a unique microenvironment for meiosis I/II and post-meiotic spermatid development which take place exclusively in the apical compartment, away from the host immune system, and it contributes to the immune privilege status of testis. However, the BTB also poses major obstacles in developing male contraceptives (*e.g.,* adjudin) that exert their effects on germ cells in the apical compartment, such as by disrupting spermatid adhesion to the Sertoli cell, causing germ cell exfoliation from the testis. Besides the tight junction (TJ) between adjacent Sertoli cells at the BTB that restricts the entry of contraceptives from the microvessels in the interstitium to the adluminal compartment, drug transporters, such as P-glycoprotein and multidrug resistance-associated protein 1 (MRP1), are also present that actively pump drugs out of the testis, limiting drug bioavailability. Recent advances in drug formulations, such as drug particle micronization (<50 μm) and co-grinding of drug particles with ß-cyclodextrin have improved bioavailability of contraceptives *via* considerable increase in solubility. Herein, we discuss development in drug formulations using adjudin as an example. We also put emphasis on the possible use of nanotechnology to deliver adjudin to the apical compartment with multidrug magnetic mesoporous silica nanoparticles. These advances in technology will significantly enhance our ability to develop effective non-hormonal male contraceptives for men.

Keywords

Testis; male contraceptive; blood-testis barrier; adjudin; nanotechnology

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

INTRODUCTION

The testes produce an upward of ~300 million spermatozoa per day in a man since puberty at ~12 years of age *via* spermatogenesis without interruption to support reproduction [1, 2]. Spermatogenesis takes place in the seminiferous tubule which is the functional unit that produces spermatozoa *via* cycles of spermatogonial self-renewal, germ cell differentiation and meiosis I/II, spermiogenesis and spermiation [3, 4]. For over a century, the seminiferous tubules in mammalian testes have been known to be protected by a unique blood-tissue barrier known as the blood-testis barrier (BTB) [5–8]. As noted in the cross-section of a seminiferous tubule, the BTB that locates near the basement membrane also segregates the seminiferous epithelium into the basal and the adluminal (apical) compartment (Fig. 1). Thus, all the events of meiosis I/II and post-meiotic spermatid development take place behind the BTB in the adluminal compartment (Fig. 1). In contrast to other blood-tissue barriers, which are constituted almost exclusively by the tight junction (TJ) barrier of endothelial cells of microvessels, such as the blood-brain barrier (BBB) [9] and the blood-retinal barrier [10], the BTB is constituted not just by TJ between adjacent Sertoli cells, but also by coexisting basal ectoplasmic specialization (basal ES, a testis-specific actin-rich adherens junction (AJ)) and gap junction, as well as intermediate filament-based desmosome. While the BTB is one of the tightest blood-tissue barriers, it undergoes extensive remodeling during the seminiferous epithelial cycle in particular at stage VIII when preleptotene spermatocytes are being transported across the BTB to enter the adluminal compartment to prepare for meiosis I/II. Studies have shown that these coexisting junctions are necessary to maintain the BTB function to avoid any disruption even transiently during the epithelial cycle [11, 12] and to ensure normal junction remodeling events such as during the transport of preleptotene spermatocytes across the immunological barrier at stage VIII of the epithelial cycle [13]. As such, the BTB poses a major obstacle to allow non-hormonal male contraceptives to get access to the seminiferous epithelium in particular if these drugs exert their effects behind the immunological barrier, such as the adluminal compartment wherein germ cell meiosis and post-meiotic spermatid development take place.

During the last two decades, significant advances are made in developing effective hormonal male contraceptives [14–16]. The approach is to use injectable androgen (*e.g.,* testosterone undecanoate with or without a progestin such as levonorgestrel or etonogestrel), oral androgen (*e.g.,* dimethandrolone undecanoate) or androgen implants in men to block spermatogenesis by down-regulating intratesticular testosterone level [15, 17–21], which must be maintained at a level of ~100-fold over the blood level in the systemic circulation in both humans and rodents to sustain spermatogenesis [22–24]. In short, this approach disrupts the hypothalamic-pituitary-testicular axis *via* an elevated serum testosterone level through the injectable androgen or androgen implants, which shuts down the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, thereby suppressing the secretion of luteinizing hormone (LH) from the pituitary gland, leading to a reduced androgen production by Leydig cells in the interstitium to disrupt spermatogenesis [15, 25]. However, since the hypothalamic-pituitary-testicular axis is disrupted, other androgen-dependent biological functions (*e.g.,* blood pressure) and organs (*e.g.,* prostate) as well as

muscle mass in men can be affected, leading to possible side-effects and concerns among men [26–29]. Furthermore, it takes several weeks for a male pill, including androgen-based implants or injectable androgen, to become effective, which inevitably associates with low patient compliance [30]. Additionally, it has been reported that some men are less susceptible to injectable testosterone known as non-responders, such as among Chinese men, leading to variations in androgen-mediated suppression of spermatogenesis [31]. Therefore, there is a need to develop non-hormonal contraceptives which preferably exert their effects locally in the testis without perturbing the hypothalamic-pituitary-testicular axis and serum androgen level. At present, several candidate compounds are being vigorously investigated by investigators, among them adjudin (1-(2,4-dichlorobenzyl)-1H-indazole-3-carbohydrazide) [32, 33] and gamendazole [34, 35] appear to be two promising lead compounds. Both drugs are derivatives of lonidamine [1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid], and are known to induce reversible germ cell exfoliation from the seminiferous epithelium by disrupting the testis-specific atypical adherens junction (AJ) known as apical ectoplasmic specialization (apical ES) between germ cells (step 8–19 spermatids in the rat testis) and Sertoli cells [36]. Studies have shown that adjudin possesses several added health benefits, such as anti-cancer [37], anti-neuroinflammatory and anti-neurodegenerative activity [38, 39] as well as anti-ototoxicity [40], suggesting its use as a male contraceptive has other added health benefits. While adjudin had passed the standard acute toxicity, mutagenesis, and chromosomal aberration tests conducted by licensed toxicologists, it failed the subchronic toxicity test in which rats were treated with adjudin by oral gavage at 50 mg/kg b.w. for 29 consecutive days since liver inflammation and skeletal muscle atrophy were detected in 3 out of 10 male rats even though the females did not display signs of similar liver and skeletal muscle damage [41]. These findings thus illustrate the margin between the safety and efficacy of adjudin must be considerably widened before it can be developed as a male contraceptive. This is not entirely unexpected since adjudin displays a relatively poor bioavailability largely because of its poor water solubility. According to a study using [^{3}H]-adjudin, fewer than 1% of adjudin could reach the testis following its administration by oral gavage in male rats [32]. Furthermore, the bioavailability of adjudin is compromised by the BTB which hinders drugs including male contraceptives such as adjudin from being transported from the interstitial space to the adluminal compartment of the seminiferous epithelium. This by and large is due to the presence of robustly expressed drug transporters by Sertoli cells at the BTB, including ATP-binding cassette (ABC)-transporters such as P-glycoprotein and multidrug resistance-associated protein 1 (MRP1), which are found on the plasma membranes of Sertoli cells, germ cells, peritubular myoid cells as well as the endothelial cells that constitute the microvessels in the interstitium [5, 8, 42]. Furthermore, ABC transporter breast cancer resistance protein (BCRP) is also robustly expressed by peritubular myoid cells in the tunica propria, and by endothelial cells of the microvessels in the interstitium [43], but not by Sertoli cells at the BTB or germ cells in the seminiferous epithelium. These efflux drug pumps are working in concert to actively pump exogenous drugs out of the testis if they somehow penetrated the BTB *via* specific drug transporters such as influx drug pumps. Also, these efflux drug pumps prevent drugs from entering the seminiferous epithelium, thereby limiting drug bioavailability in the testis. Thus, it has become imperative to develop better formulations based on current advances (*e.g.,* slow release formulations) to increase bioavailability of non-hormonal male contraceptives so as

to widen the margin between drug efficacy and toxicity. Herein, we briefly review some of the development (including the use of nanotechnology) in drug formulations that target tissue barriers, which will be applicable to develop new formulations of adjudin for its delivery to the testis.

OVERCOMING TISSUE/CELL BARRIERS FOR DRUG DELIVERY

Before a drug can exert its effects in a target organ such as the testis, it has to overcome one of the three anatomical primary barriers: the epidermal barrier of the skin to enter the systemic circulation, the gut barrier of the gastro-intestinal tract, or the epithelial lining of the respiratory tract for its initial uptake. If a drug can penetrate one of these barriers, an internal or secondary barrier is usually present to serve as a second line of defense to protect vital organs which include the blood-brain barrier (BBB) that guards the brain, the blood-ocular/retinal barrier that protects the eye, the maternal-fetal barrier in the placenta that guards the fetus, the blood-epididymal barrier that protects the epididymis, and the BTB that sequesters the seminiferous epithelium of the seminiferous tubules, among others. In short, the presence of these internal barriers further hinders a drug's bioavailability and its pharmacological actions, thus limiting its clinical uses. To improve drug bioavailability, two approaches are being actively pursued. The first approach attempts to penetrate tissue/cell barriers *via* the paracellular pathway, and the second focuses on increasing the transcellular drug uptake. Table 1 summarizes recent advances in both approaches to deliver drugs behind tissue/cell barriers.

The Paracellular Approach

The paracellular barrier is an important mechanism used by internal tissue/cell barriers to limit drug bioavailability. Thus, modifications of paracellular barrier permeability by pharmaceutical formulation are widely used in the field. Agents such as siRNA, medium chained fatty acids, antibodies and peptides have shown promising regulatory effects by specifically targeting epithelial junctions [44–48]. Multiple peptides have been reported to perturb TJ-permeability function by targeting the extracellular domains of crucial TJ-integral membrane proteins. For instance, a 22-amino acid peptide corresponding to a stretch of sequence of the second extracellular loop of rat occludin is known to induce reversible disruption of the Sertoli cell TJ-barrier *in vitro* and/or *in vivo* [48, 49]. Also, a synthetic peptide designated C1C2 which derived from rat claudin-1 is capable of modulating TJ function in rat perineurium to facilitate the entry of antinociceptive drug into the peripheral nervous system [50]. Recently, a peptide likely produced endogenously during spermiation at late stage VIII of the epithelial cycle *via* the action of MMP-2 on laminin chains at the apical ES [51, 52] has been shown to disrupt the Sertoli cell BTB function transiently with high potency [46, 53]. In short, this peptide coordinates the events of spermiation to release mature sperm and BTB remodeling to facilitate preleptotene spermatocyte transport across the immunological barrier, both of which take place at stage VIII of the epithelial cycle but at the opposite ends of the epithelium [54]. Moreover, this biologically active peptide designated F5-peptide derived from the laminin-γ3 chain also induces germ cell exfoliation effectively when administered to the testis intratesticularly [46], illustrating its potential as a reversible male contraceptive. This latter observation also suggests that besides exerting its

effects at the Sertoli cell BTB, the F5-peptide can potentiate apical ES disruption to facilitate sperm release at spermiation. Collectively, these findings suggest an attractive multidrug approach of using F5-peptide and adjudin in which F5-peptide perturbs the paracellular barrier at the BTB to facilitate the entry of adjudin into the adluminal compartment to induce germ cell loss. This thus widens the gap between the efficacy and toxicity of adjudin.

On the other hand, it may be a viable approach by manipulating the Sertoli cell TJ-barrier permeability through a down-regulation on the expression of TJ integral membrane or adaptor proteins (*e.g.,* ZO-1, oc-cludin and claudins) to improve drug bioavailability behind the BTB *via* the use of specific siRNA or shRNA. Studies in other tissue barriers have shown that an enhanced uptake of neuropeptide thyrotropin-releasing hormone behind the BBB in the mouse brain is observed within 48 hr following the administration of claudin-5 siRNA [55]. Furthermore, shRNA specific to claudin-5 can facilitate the entry of low-molecular weight drugs sunitinib malate (532 Da) and 17-AAG (585 Da) through blood-retinal barrier [56]. Nonetheless, the delivery of siRNA duplexes or shRNA is also hindered by the biological barriers, in part due to the negatively charged backbone of the nucleic acid, compounded with the intrinsic instability against endonu-cleases and rapid clearance [57, 58]. Thus, much work is needed to develop a better delivery system for successful application of siRNA or shRNA-based therapy. Recent attempts that protect siRNA with TEA-core PAMAM dendrimer [59], polyethylenimine/poly (lactide-co-glycolide) matrix [60], and chitosan derivatives [61, 62] have sparked some excitements for better siRNA delivery. Since studies have shown that the Sertoli cell TJ-permeability function can be transiently perturbed by using RNAi by targeting proteins that maintain the actin microfilament bundles at the basal ES/BTB such as palladin [63], ezrin [64], and Eps8 [65], a multidrug approach by combining adjudin and one of these siRNA duplexes should be considered in future investigations.

The Transcellular Approach

This approach aims at circumventing biological barriers by enhancing cellular uptake of drug molecules. There are several approaches to improve drug bioavailability *via* the transcellular pathway:

Drug Micronization—Micronization technology is the most widely used approach that substantially decreases the size of drug particles so that pharmaceutical agents can readily penetrate through cell membrane in a target organ. Adjudin *per se* is practically insoluble in water, and only ~1 mg/ml in ethanol but ~133 mg/ml in DMSO (a cytotoxic agent), which thus partially accounts for its poor bioavailability. Previous effort has been made to micronize adjudin by pulverization, which shrunk the particle size of adjudin to ~50 μm, thus significantly lowered the effective dose to ~16 mg/kg b.w. along with improved drug solubility [66]. However, conventional micronization methods such as ultrafine milling, spray-drying and liquid anti-solvent crystallization often result in variable distribution of drug particle size, which thus hinders their use for effective and consistent absorption. Consequently, other alternatives have been investigated during the past decade, which include supercritical fluid (SCF) techniques [67, 68]. By manipulating temperature and pressure, it is now possible to produce much smaller drug particles with significantly

narrowed size distribution. In addition, with appropriate solvents/co-solvents and procedures, the entire process can take place at ambient temperature, which is particularly ideal for the production of heat-sensitive drugs [68]. For instance, using SCF-CO_2 method, the production of more stable and uniform liposomes can be achieved that serve as better carriers for drugs [69]. Earlier SCF technologies such as the Rapid Expansion of Supercritical Solutions (RESS), Gas Anti Solvent (GAS) and Supercritical Anti Solvent (SAS) were used to produce particles of ~0.7–5.0 μm in size [70]. Recent SCF processes are capable of obtaining pharmaceutical particles at a nanometer range, such as <300 nm, which thus significantly increases dissolution rate and oral bioavailability of different drugs [70, 71]. Thus, work is in progress in our laboratory to encapsulate the practically water insoluble adjudin in liposomes or to prepare uniform fine particles of adjudin with the use of SCF technology to improve its bioavailability and efficacy.

Protein Transduction Domain for Delivery of Macromolecules—The transcellular entry of proteins *via* the plasma membrane was first reported in 1988 when the HIV TAT (HIV trans-activator of transcription) protein was shown to be capable of entering mammalian cells to activate HIV transcription [72, 73]. Conjugation of peptide fragments from HIV TAT or full length functional proteins was found to be an effective approach to deliver large molecules to mammalian cells [74]. Furthermore, only the amino acid residues spanning 48–60 of the TAT protein is necessary to induce effective cellular internalization [75]. For instance, administration of a 120 kDa TAT(47–57 amino acid residues)-β-galactosidase fusion protein to mice by i.p. was found to be effectively delivered to lung, heart muscle, and spleen *in vivo* [76]. Thus, following the discovery of this stretch of sequence of TAT from 47–57, a 11-amino acid peptide, known as Protein/Peptide Transduction Domain (PTD), multiple PTDs have since been identified and designated Cell Penetrating Peptides (CPPs) [77], such as low molecular weight protamine (LMWP) which is an effective CPP [78]. Most PTDs are basic peptides composed of multiple Arg residues between 9 and 20 amino acid residues, and can effectively transport proteins, peptides, siRNA, and siRNA nanoparticles across cell membranes [79]. It is now generally accepted that protein transduction occurs by first binding of the PTD with or without a cargo protein/molecule to the plasma membrane, to be followed by endocytic vesicle-mediated internalization, and the release of the cargo to the cell cytosol [79], analogous to the endocytic vesicle-mediated trafficking events that take place at the Sertoli cell BTB [80, 81]. In short, the use of PTD-conjugated adjudin or other potential male contraceptive is a novel and alternative approach of delivering adjudin behind the BTB for male contraception. For instance, a short PTD peptide can be conjugated to adjudin *via* the use of a heterobifunctional cross-linker (*e.g.,* SFB, succinimidyl 4-formyl-benzoate) which generate a benzaldehyde at the N-terminus of the PTD peptide, which then reacts spontaneously with adjudin at its hydrazide group in physiological buffer through a stable hydrazone linkage as recently reported by conjugating adjudin to keyhole limpet hemocyanin (KLH, an adjuvant), for producing an anti-adjudin antibody [82]. Such a conjugate is likely to have better penetrability across the testis, which should be explored in future studies.

Receptor-Mediated Transcellular Drug Transport—Integral membrane receptors or peptide transporters (*e.g.,* OATPs) are potential targets for drug delivery and also drug

selectivity. In short, conjugation of a drug to a synthetic peptide or recombinant protein that bears structural resemblance to the physiologically occurring substrates or ligands can be a novel approach to deliver drugs across plasma membrane [83]. In the mammalian testis, follicle-stimulating hormone (FSH) receptors are restricted to Sertoli cells [84, 85]. Thus, FSH is a promising carrier to deliver male contraceptives such as adjudin selectively to testis. In a proof-of-concept study, a FSH mutant was prepared through site-directed mutagenesis by deleting two glycosylation sites in the α subunit and one glycosylation site in the β subunit [41]. These modifications rendered the FSH mutant a loss of hormonal activity without compromising its receptor-binding capability. The FSH mutant was then conjugated to adjudin through a hydrazone bond [41]. This adjudin-FSH mutant conjugate was highly effective in inducing reversible infertility in adult rats with a significantly lower dose [41]. However, this approach is prohibitively expensive in particular if this adjudin-FSH conjugate contraceptive drug is to be used in developing countries. Also, it requires parenteral administration, posing an acceptability obstacle, unless this mutant-adjudin conjugate can overcome the proteolysis of FSH polypeptide in the GI tract when the conjugate is orally administered, such as using the approach developed for oral delivery of insulin [86], including the use of nanoparticles containing absorption enhancers (*e.g.,* surfactants, zonula occludens toxins) and proteolytic enzyme inhibitors (*e.g.,* bacitracin, aprotinin, soybean trypsin inhibitor, polymer-inhibitor conjugates) [86–88]. A recent study had also used this approach to conjugate a permanent male contraceptive melphalan (also a cytotoxic and gonado-toxic nitrogen mustard alkylating agent that kills murine testicular cells) to FSH-ß peptide to be used to target melphalan to the testis for chemical sterilization in wild-life animals and also pets such as cats and dogs [89].

NANOPARTICLES (NPS) FOR DELIVERY OF NON-HORMONAL MALE CONTRACEPTIVES

Nanoparticles, with particle sizes ranging between 1 and 1000 nm, have been intensively investigated for the delivery of numerous therapeutic agents mostly as carriers for anti-cancer drugs for chemotherapy. Conventional drug molecules are either conjugated onto the surface of NPs or encapsulated into the core, if NPs carrying multiple drugs are delivered to a target organ to improve the efficacy. These ‘nanodrugs’ are designed to transport drugs that have low aqueous solubility or are susceptible to enzymatic cleavage since drugs are placed inside the core of the iron-based nanoparticles until they are uptaken by cells in a target organ, such as with the aid of a magnetic field [90, 91]. Furthermore, small NPs (*i.e.,* <100 nm) are having molecular sizes that facilitate their passage across internal biological barriers [92, 93]. NPs are usually made of nanomaterials such as lipids, metallic materials, polymers (*e.g.,* chitosan), dentrimers, nanocrystals (*e.g.,* semi-conductor quantum dots), carbon nanotubes, mesoporous materials and iron oxide-based magnetic nanomaterials. Among them, mesoporous materials are emerging as leading carriers for drugs including male contraceptive adjudin. For instance, mesoporous silica nanoparticles (MSN) are nanoparticles of ~40–70 nm in diameter with orderly arranged pores called mesopores (or tunable pore sizes) of ~3.8–6.1 nm, high surface areas (700–1100 m^2/gm) and large pore volumes (0.44–1.54 cm^3/gm) [94–96]. MSN are emerging vehicles for drug delivery [97–99], including small molecular drugs [*e.g.,* anticancer drugs paclitaxel (water *insoluble*) and

doxorubicin (water *soluble*), anti-hypertension drug telmisartan (poorly water soluble)] [100–102], proteins [97, 103–105] and siRNAs [106–109]. MSN are often loaded with multiple drugs [105, 106, 108, 110] so that drugs with different modes of action can be targeted to a specific organ/tissue to exert their effects to correct a pathological condition. More important, the use of MSN has now entered preclinical development stage for cancer treatment [111–113]. Furthermore, magnetic-based MSN (MMSN), in which MSN is constructed with a magnetic Fe_3O_4 core surrounded by hexagonally arranged mesopores (Fig. 2), is shown to have better cellular uptake to deliver multidrugs simultaneously to target tissues/organs *via* the use of strong magnet fielded established in the target organ [110, 114–116]. Studies have also shown that MMSN or MSN enter cells *via* either endocytic vesicle-mediated pathway [116, 117] or GTPase (*e.g.,* Rac1, Cdc42)-mediated macropinocytosis [118]. Administration of amorphous nanosilica particles to mouse testes (*via* i.v.) was shown to penetrate the BTB, leading to accumulation of NPs in the Sertoli and germ (*e.g.,* spermatocytes) cells without causing testicular injury [119]. Also, mesopores protect bioactive drugs from undesired enzymatic degradation before reaching the target cells/tissues due to the inaccessibility of the inner surface to enzymes in the systemic circulation and/or tissues (*e.g.,* intestine) [120]. Thus, it is possible that F5-peptide and adjudin can be loaded into hexagonally arranged mesopores of MMSN for their administration orally without the use of a needle, and they can be delivered to the testis specifically *via* the use of a magnetic field, such as by placing neodymium (NdFeB) permanent magnets in men's shorts to generate a strong magnetic field. Upon released into the Sertoli cell cyto-sol, F5-peptide disrupts the BTB to facilitate entry of adjudin to the adluminal compartment to induce germ cell loss from the seminiferous epithelium (Fig. 2). To further optimize the targeting of this multi-drug MMSN to the testis, an anti-FSH receptor IgG or recombinant FSH can be incorporated onto the surface of the adjudin/F5-peptide containing MMSN, so that the nanoparticles can home-in to the Sertoli cell for specific delivery besides under the influence of an external magnetic field (*e.g.,* NdFeB magnets) that is placed near the testis. This approach thus avoids parenteral administration, improving acceptability.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

As briefly discussed above, there are major advances in the field that will assist the optimization of delivering adjudin to the testis to serve as a non-hormonal male contraceptive. Both paracellular and transcellular approaches exhibit marked advantages in reducing side-effects and enhancing bioavailability of adjudin. It is obvious that much research is needed to gain a full understanding of the mechanisms under which adjudin perturbs germ cell adhesion (in particular spermatids) and BTB integrity following long exposure, and to better understand factors that limit the bioavailability of adjudin. It is noted that by modulating the BTB integrity, even transiently, this may expose meiotic germ cells and developing haploid spermatids to an unfavorable environment such as the systemic circulation, causing unwanted immunological responses. As discussed above, BTB restructuring near the basal compartment and apical ES reorganization during spermiation in the adluminal compartment are tightly coordinated events through an autocrine-based axis in the testis [54]. The reversible and transient disruption of BTB through paracellular approach

using siRNA duplexes or biological peptides, although highly effective, may cause unwanted effects such as by perturbing meiosis I/II or escalating germ cell exfoliation that leads to a prolonged recovery phase. Therefore, much challenge remains. Perhaps the nanoparticle-based multidrug approach is one of the most promising leads for future studies. Another challenge for non-hormonal male contraceptive development is the fact that the testis is equipped with an array of drug transporters, both efflux and influx drug pumps, that actively involved in determining the level of adjudin available to the testis following its administration by oral gavage [5, 8, 42, 121]. Furthermore, besides Sertoli cells that constitute the BTB, peritubular myoid cells in the tunica propria also express drug efflux transporters, such as BCRP (breast cancer resistance protein) [43, 122–124]. Moreover, germ cells, in particular spermatogonia, spermatocytes and spermatids including elongating/elongated spermatids, also express multiple drug transporters [5, 8, 42]. These findings illustrate that the bioavailability of adjudin in the testis is determined, at least in part, by an interaction of adjudin to these drug transporters [125]. In fact, studies have shown that the knockdown of P-glycoprotein, an efflux drug transporter, enhances the influx transport of adjudin across the Sertoli cell BTB [126], and studies by utilizing molecular modeling have identified putative docking pocket of adjudin with BCRP [127, 128] and P-glycoprotein [129]. Thus, an inhibitor or a set of inhibitors against drug transporters can also be included in the MMSN-based nanoparticles to optimize the entry of adjudin to the testis specifically (Fig. 2). Recently, SLC15A1, a peptide transporter which predominantly locates at the peritubular myoid cells in rat testes has been found to play a role in mediating the transport of F5 peptide into the seminiferous epithelium following administration of F5 peptide to the testis *via* intratesticular injection in rodents [130]. This finding indicates that transporters are at play to mediate influx and efflux of macromolecules in the testis and SLC15A1 may be a promising target to facilitate the transport of the F5-adjudin multidrug into the seminiferous epithelium. While much research is needed in the years to come, recent advances in the field have shed new lights in developing novel delivery strategies to the testis, making drugs such as adjudin to be more effective non-hormonal male contraceptives by exerting their effects behind the BTB in the adluminal compartment.

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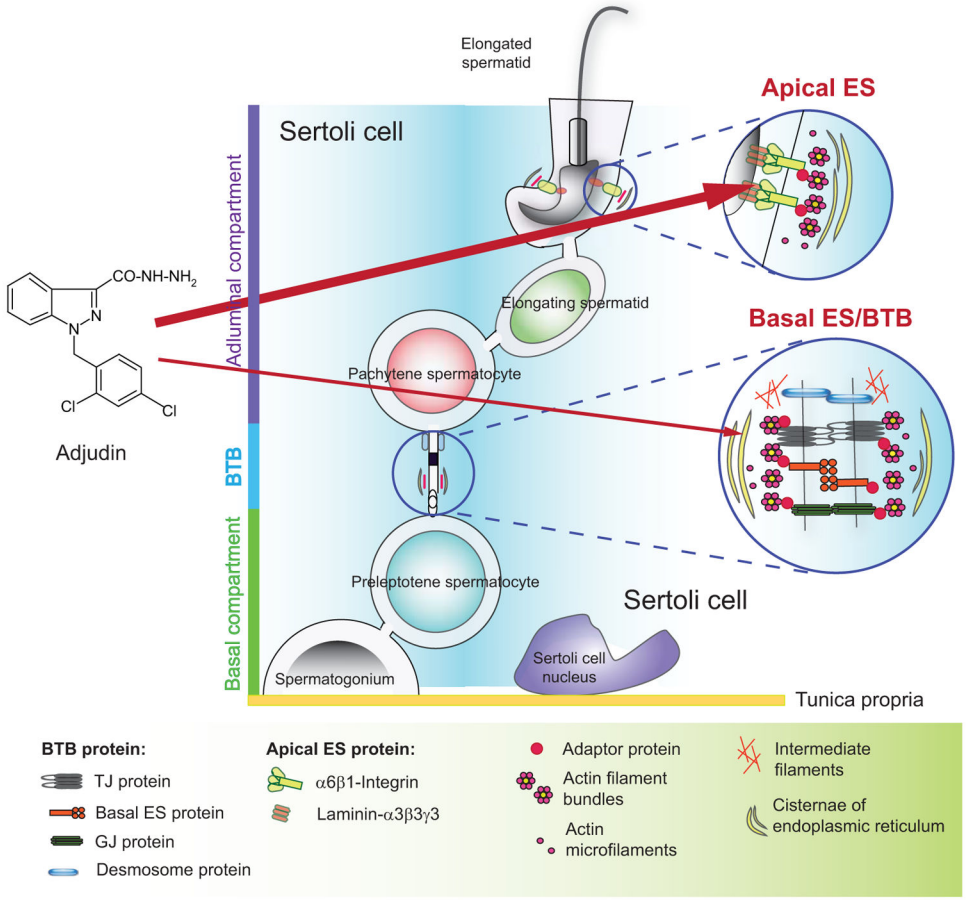

Fig. (1).

A schematic drawing that illustrates the site of action of adjudin in the mammalian testis. Studies have shown that adjudin (left panel) exerts its effects primarily at the ectoplasmic specialization (ES) (right panel) in the seminiferous epithelium of mammalian testes [36, 131, 132]. Adjudin is more effective to perturb the apical ES function *versus* the basal ES function at the BTB. This is likely due to the presence of only a single array of actin filament bundles at the apical ES *versus* two arrays of actin filament bundles at the basal ES at the BTB [36, 133]. Furthermore, the BTB is constituted by actin-based tight junction (TJ) and GJ (gap junction) besides basal ES, as well as intermediate filament-based desmosome; whereas the apical ES does not have other parallel junctions at the Sertoli-spermatid interface. As such, apical ES is rapidly disrupted, usually within 6- to 9-hr following exposure of adult rats to adjudin by oral gavage [36, 134], but the basal ES/BTB is not disrupted until 2-wk following treatment to the rats unless a high acute dose of adjudin is used [133].

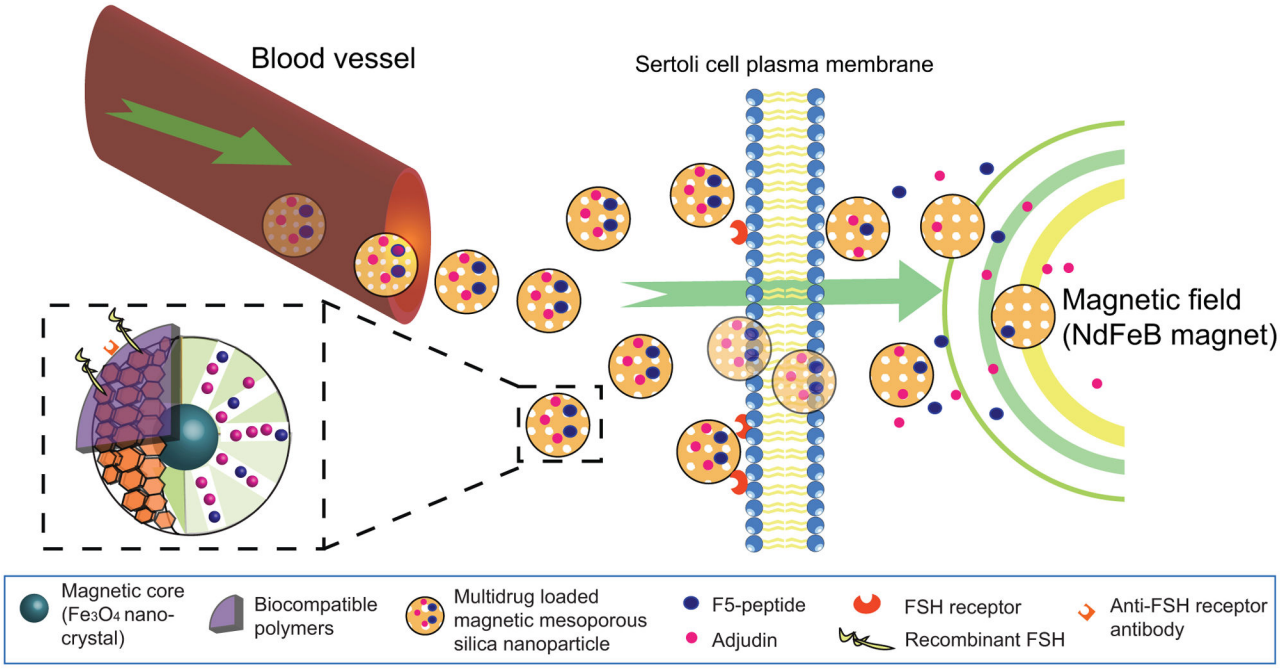

Fig. (2).

A schematic drawing illustrating the strategy that can be used to prepare multidrug MMSN (magnetic mesoporous silica nanoparticles) as non-hormonal male contraceptive. Adjudin together with F5-peptide can be loaded into the hexagonally arranged mesopores of MMSN with a magnetic Fe_3O_4 core. When administered orally, multidrug MMSN enter the blood vessel as demonstrated in published studies *via* macropinocytosis (see text for detail). The presence of a static magnetic field in the target organ, such as the testis, can be created by placing permanent neodymium (NdFeB) magnet near the testis such that MMSN enter the Sertoli cell preferentially. The targeted delivery of MMSN to the Sertoli cell in the testis can be further improved by coating either recombinant FSH or anti-FSH receptor antibody on the biocompatible polymers since FSH receptor is expressed only in Sertoli cells in the mammalian body. Thus, MMSN can reach Sertoli cell cytosol *via* endocytosis and/or macropinocytosis. As such, adjudin and/or F5-peptide can be released from the MMSN and exerts their effects at the ES in the testis to induce transient BTB disruption and germ cell exfoliation. In short, this multidrug MMSN can be further modified by including: (i) a fluorescence tag (*e.g.,* FITC or Cy3) to track its cellular uptake, (ii) an anti-FSH receptor IgG or FSH recombinant protein so that it can be better targeted to the Sertoli cell (even without the magnetic core), (iii) inclusion of an efflux drug transporter inhibitor and/or specific siRNA duplexes to inactivate P-glycoprotein at the BTB if necessary, and (iv) biocompatible polymers to improve its bioavailability and/or resistant to degradation. It is likely that the BTB will eventually be disrupted even when a lower dose of adjudin is used by adopting the multi-drug MMSN nanotechnology for its delivery because of the F5-peptide intrinsic BTB-disrupting activity, and there are concerns about the production of anti-sperm antibodies in men receiving adjudin/F5-peptide MMSN. However, since germ cells would be depleted from the seminiferous epithelium before the BTB is compromised

given that the apical ES is more susceptible to adjudin treatment than the basal ES/BTB [36, 131, 132], the production of anti-sperm antibodies is not likely to be an issue.

Table 1

Typical strategies used for delivery of selected therapeutic agents across tissue/cell barriers*.

	Strategies	Drugs	Targeted Tissue Barrier(s)	References
The Paracellular Ap- proach	Attachment of surfactant-like agents to the drug (*e.g.,* sodium caprate)	Berberine	Gut barrier	[135]
	Delivery of specific peptides to modulate tight junction proteins (*e.g.,* synthetic peptide corresponding to the C-terminal ex tracellular loop 1 of rat claudin-1 that transiently perturbs the TJ- permeability barrier)	DAMGO, tetrodotoxin	Perineurial barrier	[50]
	Delivery of specific siRNA or shRNA that targets junction proteins (*e.g.,* claudin-5 shRNA or siRNA)	Small molecules (<1 kDa) (*e.g.,* sunitinib malate)	Blood-brain barrier, inner-blood- retina barrier	[44, 56, 136]
The Transcellular Ap- proach	Design of lipophilic drugs (*e.g.* ester-linked/acetylated prodrugs or drug-in-liposome)	Thiorphan	Blood-brain barrier, blood-cerebrospinal fluid barrier	[137, 138]
	Design of prodrugs that target specific protein transporters or receptors on the cell surface (*e.g.* valacyclovir that targets trans porter PEPT1 & 2 or a vitamin B12 based insulin conjugate)	Acyclovir Insulin	Renal epithelial barrier, Gut barrier	[139] [140]
	Drug particle micronizton (*e.g.,* preparation of drugs using super-critical fluid technology)	Tetracycline	Gut barrier	[70]
	Nanoparticulate strategies (*e.g.,* combination of lipid-based or polymer-based nanoparticles)	Nitrendipine Rivastigmine	Blood-brain Barrier	[141] [142]

*This Table is not intended to be exhaustive. Only selective examples are shown herein to support discussion for delivery of male contraceptives (see text for detail). Thus, the use of different delivery routes such as nasal or transdermal delivery to circumvent biological barriers are not included. DAMGO, [D-Ala^2, N-MePhe^4, Gly^5-ol]-enkephalin; PEPT1 & 2, peptide transporter 1 and 2.