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Adjudin, a potential male contraceptive, exerts its effects locally in the seminiferous epithelium of mammalian testes

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Abstract

Adjudin is a derivative of 1H-indazole-3-carboxylic acid that was shown to have potent anti-spermatogenic activity in rats, rabbits, and dogs. It exerts its effects most notably locally in the apical compartment of the seminiferous epithelium, behind the blood–testis barrier, by disrupting adhesion of germ cells, most notably spermatids to the Sertoli cells, thereby inducing release of immature spermatids from the epithelium that leads to infertility. After adjudin is metabolized, the remaining spermatogonial stem cells and spermatogonia repopulate the seminiferous epithelium gradually via spermatogonial self-renewal and differentiation, to be followed by meiosis and spermiogenesis, and thus fertility rebounds. Recent studies in rats have demonstrated unequivocally that the primary and initial cellular target of adjudin in the testis is the apical ectoplasmic specialization, a testis-specific anchoring junction type restricted to the interface between Sertoli cells and elongating spermatids (from step 8 to 19 spermatids). In this review, we highlight some of the recent advances and obstacles regarding the possible use of adjudin as a male contraceptive.

Introduction

Population growth in the next four decades, if unchecked, will become one of the major global issues because of limited resources on our planet. In the year 2005, human population in the world has reached about 6.5 billion (Cleland *et al*. 2006), and it is estimated that in the year 2050, this number will reach ~9 billion (Cleland *et al*. 2006). This poses a growing pressure to the limited resources, such as land, water, food, and fossil fuels. Moreover, over population has also threatened the environment via increasing industrial activities. For example, increase in the land use for food production and industrial activities has caused a loss in natural habitats and biodiversity (Cleland *et al*. 2006), and an increase in the level of toxicants in the environment has threatened the health of the public (Hunt *et al*. 2009, Siu *et al*. 2009, Cheng *et al*. 2011). In order to solve these problems, one of the best solutions is education through family planning and contraception. Indeed, besides preservation of

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Declaration of interest

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recourses and environment, population control can aid the economic development of underdeveloped and developing countries that have high birth rate by releasing women from domestic activities, contributing to financial stability and security in a family (Eastwood & Lipton 1999, Chen & Ravallion 2004). Therefore, there is a strong urge to develop more contraceptives to meet the needs of population control. Today, the burden of contraception rests mostly on women. Although recent surveys have indicated that men in different nations are willing to share the responsibility for contraception (Martin *et al*. 2000), choices for men are rather limited. Currently available contraception for men includes condom and vasectomy. Although condom has some advantages such as its convenience and cost effectiveness, its efficacy is relatively low and rather unreliable as there is still a 2% pregnancy rate (Trussell 2004). For vasectomy, this procedure is irreversible and an additional surgical procedure is needed to restore fertility. Also, some unwanted immunological consequences and pathological conditions are associated with vasectomy, at least in some men (Davis & Pollack 1994). Limited options for male contraception are due to a number of reasons. One of them is the difficulty in suppressing the production of a large number of sperm, which is up to 100 million sperm produced per day by each man from puberty throughout his entire lifespan (Sharpe 1994), without any adverse effects. It is noted that a 90% suppression of sperm production can still lead to unwanted pregnancy (Robaire 2003). One of the most investigated approaches to block sperm production is hormonal contraception, which disrupts the hypo-thalamic–pituitary–testicular axis, most notably with the use of testosterone by injection, implants, or gels, to suppress GNRH production, thereby reducing intratesticular androgen level to maintain spermatogenesis (Amory *et al*. 2006, Aitken *et al*. 2008, Page *et al*. 2008). Although promising clinical results were obtained from several recently conducted clinical trials, such as a 1.1 contraceptive failure rate per 100 persons (Gu *et al*. 2008), the development of hormonal contraception still has to overcome certain obstacles. These included the differences in efficacy of spermatogenesis suppression by testosterone from race to race (WHO Task Force on Methods for the Regulation of Male Fertility 1990, Handelsman *et al*. 1995) and the possible long-term adverse effects resulting from disruption of hypothalamic–pituitary–testicular axis, such as bone loss, prostate enlargement, and elevated blood pressure in men (Holmang *et al*. 1993, Ducharme 2010, Moulana *et al*. 2011). Therefore, there is a need for non-hormonal contraception. Immunocontraception may likely be another viable option (McLaughlin & Aitken 2011).Immunocontraceptive vaccines lead to infertility by raising antibodies against sperm-specific antigens or specific proteins related to sperm production (McLaughlin & Aitken 2011). A recent study on using a testis/ epididymis-specific protein called epididymis protease inhibitor (eppin) as the target antigen was able to induce infertility in seven out of nine monkeys (O'Rand *et al*. 2004). Despite the success in demonstrating the potential of eppin for the development of immunocontraceptive vaccine by the above-mentioned study, there are still concerns for immunocontraception to be developed as an ideal contraceptive. For example, the two monkeys that did not produce high-titer antibodies against eppin is a concern regarding its efficacy because there are differences in immunological response as a result of genetic variations among individuals in particular in men.

Although the biology of the hypothalamic–pituitary– testicular axis and the intriguing interactions of multiple hormones (e.g. GNRH, FSH, LH, testosterone, and estradiol-17β)

that are crucial to spermatogenesis are known (Sharpe 1994, Winters & Moore 2007, Carreau *et al*. 2010), the cell and molecular biology of spermatogenesis, in particular the mechanisms that regulate the local events in the seminiferous epithelium, remains poorly understood. For instance, the cell cycle progression and regulation underlying meiosis remain largely unknown (Lie *et al*. 2009a, Wolgemuth & Roberts 2010). The biology and regulation of spermiogenesis, spermiation, and blood–testis barrier (BTB) pertinent to spermatogenesis such as cross talks between different sets of proteins (e.g. protein kinases, phosphatases) and ultrastructure (Cheng & Mruk 2002, 2010b, Kierszenbaum *et al*. 2007, Mruk *et al*. 2008, O'Donnell *et al*. 2011) are also poorly understood. Nonetheless, recent studies in the field have identified several potential compounds that exert their effects in the seminiferous epithelium, causing exfoliation of germ cells in the testis. One of these compounds is adjudin (Cheng *et al*. 2005, Mruk *et al*. 2008). We also briefly discuss gamendazole and CDB-4022 (Koduri *et al*. 2008, Tash *et al*. 2008a), which are also actively investigated as alternatives for non-hormonal contraception. Some recent studies have shown that these compounds exert their effects on the apical ectoplasmic specialization (ES; Cheng & Mruk 2002, Mruk & Cheng 2004a, 2004b, Koduri *et al*. 2008, Tash *et al*. 2008b) in the seminiferous epithelium. Since apical ES is a testis-specific cell–cell actin-based adhesion ultrastructure (Vogl & Soucy 1985, Vogl *et al*. 2000, Cheng & Mruk 2010b), and if these compounds indeed limit their effects to this ultrastructure, it is expected that these compounds would display high efficacy and low toxicity. In this review, we discuss recent development in the field regarding adjudin, gamendazole, and CDB-4022 and their potentials of becoming male contraceptives.

Adjudin

Background

Adjudin, 1-(2,4-dichlorobenzyl)-1H-indazole-3-carbo-hydrazide, formerly called AF-2364 (Fig. 1 and Table 1), is an analog of lonidamine (Cheng *et al*. 2001; Fig. 1). Lonidamine was initially explored as an anti-cancer drug without anti-mitotic activity, unlike most other anti-cancer drugs (Silvestrini *et al*. 1984). Interestingly, lonidamine was later found to be an effective anti-spermatogenic agent (Coulston *et al*. 1975, Corsi & Palazzo 1976, Lobl 1979, Lobl *et al*. 1979) that could induce vacuolation of endoplasmic reticulum of Sertoli cells and exfoliation of germ cells from the seminiferous epithelium (Buthala & Lobl 1979, Lobl 1979, Lobl *et al*. 1979, De Martino *et al*. 1981) in mammalian testes. However, it has a number of side effects including muscular pain, testicular pain, vomiting, and elevation of liver enzymes as a sign of liver damage (Silvestrini *et al*. 1984). Besides, its margin between its efficacy dose and toxicity is very narrow (Heywood *et al*. 1981, Silvestrini *et al*. 1984, Robustelli della Guna & Pedrazzoli 1991). Thus, there are some efforts among investigators to develop derivatives of lonidamine for the purpose of maintaining its anti-spermatogenic effect and at the same time minimizing its toxicity, and adjudin and gamendazole are the results of such efforts.

Efficacy and toxicity of adjudin

Adjudin (Fig. 1 and Table 1) was identified almost a decade ago in our laboratory as a potential male contraceptive (Cheng *et al*. 2001) following the screening of more than two

dozen derivatives of lonidamine based on a novel assay involving testin, an apical ES signaling protein, to identify an analog that is highly specific to perturb apical ES function with minimal cytotoxicity (Cheng *et al*. 2001). Results showed that two doses of 50 mg/kg b.w. of adjudin (one dose per week) could induce 100% infertility by ~5 weeks following treatment in adult rats and their fertility rebounded by ~11 weeks to 100% (Cheng *et al*. 2001, 2005). Adjudin was effective to induce complete reversible infertility in adult rats (Cheng *et al*. 2001), rabbits (Hu *et al*. 2009), and dogs (Zhou *et al*. 2008) without fatality, and had no apparent side effects in treated male animals based on serum microchemistry to monitor the liver and kidney function. The lack of general toxicity of adjudin was also reflected by no reduction of body weight after treatment in these animals. Moreover, histological analyses had shown that cell adhesion in other organs, such as kidney, liver, brain, heart, epididymis, prostate, and seminal vesicles, was not affected in rat treated with adjudin (Grima *et al*. 2001). Furthermore, toxicity studies conducted by licensed toxicologists confirmed that adjudin has no genotoxicity and is not toxic in acute dose even at 2000 mg/kg b.w. in rats and mice (Mruk *et al*. 2006). More remarkably, the serum testosterone, FSH, and LH levels of the adjudin-treated animals had no detectable change compared with the control animals (Cheng *et al*. 2001, Grima *et al*. 2001). Thus, the hypothalamic–pituitary–testicular axis is not disrupted. Although adjudin appears to be safe as a male contraceptive based on a series of tests performed by licensed toxicologists including acute toxicity, genotoxicity, and mutagenesis (see Supplementary Data in Mruk *et al*. (2006)), the margin between its efficacy and toxicity was too narrow. Based on a 29-day subchronic toxicity study in ten male and ten female rats, it was shown that three out of the ten male rats, but none in the female rats, displayed signs of liver inflammation and skeletal muscle atrophy (Mruk *et al*. 2006). Therefore, we had developed a conjugate to deliver adjudin specifically to the testis using an FSH mutant to significantly reduce the efficacy dosing and thereby lowering the systemic toxicity (Mruk *et al*. 2006), by making use of the fact that FSH receptor is limited to the Sertoli cell in the mammalian body (Sprengel *et al*. 1990, Griswold *et al*. 1995). Although effective dose was lowered to 50 μg/kg b.w., this approach was proven to be prohibitively expensive for a male contraceptive because of the production costs (Mruk & Cheng 2008). We have made some advances in preparing different formulations of adjudin for its oral use in laboratory studies, and our goal is to reduce the effective dosing of adjudin to ~5–7.5 mg kg/b.w. from the current 50 mg/kg b.w. (for a review, see Wong *et al*. (2008b)), with some success based on ongoing studies in our laboratory.

Cellular targets of adjudin in the testis

The primary ultrastructural target of adjudin appears to be the apical ES. This conclusion is based on the following observations from recent published reports. First, it is known that apical ES is a unique actin-based adhesive ultrastructure in the testis. Apical ES is restricted to the interface between Sertoli cells and step 8–19 spermatids in the rat testis, which is typified by the presence of bundles of actin filament bundles sandwiched in between the cisternae of endoplasmic reticulum and the apposing plasma membranes of the Sertoli cell and the elongating spermatid. This ultra-structural feature, however, is visible only on the Sertoli cell side in the seminiferous epithelium by electron microscopy (Cheng & Mruk 2009, 2010b; Fig. 2). Once it appears, it is the only anchoring device that anchors

developing spermatids to the Sertoli cell during spermiogenesis, replacing desmosomes and gap junctions (Russell 1993, Cheng & Mruk 2010b). Studies by using micropipette pressure transducing system (MPTS) to quantify the physical force that is required to pull i) spermatocytes, ii) pre-step 8 spermatids, and iii) step 8–19 spermatids away from the Sertoli cell epithelium have demonstrated that apical ES is the strongest adhesive ultrastructure versus desmosomes and gap junctions (Wolski *et al*. 2005). In this context, it is noted that apical ES is present only in step 8–19 spermatids, but not in spermatocytes and pre-step 8 spermatids because desmosome and gap junction are restricted to the interface between Sertoli cells and spermatocytes or early developing spermatids (pre-step 8 spermatids; Cheng & Mruk 2010b, Lie *et al*. 2011). Yet, adjudin is more effective to disrupt the adhesive force that maintains the apical ES versus desmosomes and gap junctions found between spermatocytes/round spermatids and Sertoli cells as assessed by MPTS (Wolski *et al*. 2006), implying that apical ES is one of the primary targets of adjudin in the seminiferous epithelium. These findings are important and they are also consistent with an earlier *in vivo* study to assess the kinetics of germ cell loss from the epithelium in adjudintreated rats. It was reported that the halftime for the loss of elongating spermatids (i.e. the time it took for step 8–19 spermatids to be found in the tubule lumen in 50% of the seminiferous tubules examined based on morphometric/histological analysis) was only 6.5 h; in contrast, the halftime of round spermatid and spermatocyte depletion was 3 and 6 days respectively (Chen *et al*. 2003). Thus, this earlier *in vivo* study has shown that adjudin is significantly more effective in inducing the loss of elongating spermatids (i.e. step 8–19 spermatids) from the epithelium than the round spermatids and spermatocytes (Chen *et al*. 2003), consistent with the subsequent *in vitro* studies to quantify the ‘force’ required to disrupt adhesion between Sertoli cells versus spermatocytes, round spermatids (pre-step 8 spermatids), and step 8–19 spermatids (Wolski *et al*. 2006).

Secondly, adjudin was shown to induce the expression of several integral membrane proteins at the apical ES in the testis, such as β1-integrin (Siu *et al*. 2003) and N-cadherin (Lee *et al*. 2003), and to activate the downstream signaling pathways that eventually perturb cell adhesion at the apical ES in the testis. One of these pathways is the integrin/FAK (focal adhesion kinase)/ phosphatidylinositol 3-kinase (PI-3 kinase)/p130Cas/ MAP kinase signaling pathway (Siu *et al*. 2003; Fig. 2), which is known to regulate the apical ES by affecting the underlying actin-based cytoskeleton network (Lee & Cheng 2004b). Thus, the effects of adjudin on the β1-integrin-based adhesion protein complex at the apical ES may account for the preferential disruption of adhesion between elongating spermatids and Sertoli cells (Chen *et al*. 2003, Wolski *et al*. 2006; see above). Since β1-integrin is found in virtually all epithelia/ endothelia but at the cell–matrix interface, one will argue that if adjudin indeed exerts its effects on β1-integrin and its downstream signaling pathway at the apical ES, the adhesion function in multiple epithelia would have been affected systemically. However, in both acute and subchronic toxicity studies performed by licensed toxicologists and pathologists, it was shown that adjudin did not affect cell adhesion function in multiple tissues and organs (Grima *et al*. 2001) based on pathological and histological analysis (see Supplementary Data in Mruk *et al*. (2006)) except the seminiferous epithelium in the testis. Interestingly, it is noted that β1-integrin is restricted to focal adhesion complex (or focal contact) at the cell–matrix interface in multiple epithelia;

however, β1-integrin is an integrated component of the cell–cell actin-based anchoring junction apical ES. Apical ES is a hybrid anchoring junction type composed of the physicochemical features of adherens junction, tight junction, desmosome junction, and gap junction (Mruk & Cheng 2004a, Mruk *et al*. 2008, Wong *et al*. 2008a). Furthermore, the ultrastructural features of apical ES are unique, distinguishable from all other anchoring junction types found in mammalian tissues and organs (Mruk & Cheng 2004a, Yan *et al*. 2007, Vogl *et al*. 2008, Cheng & Mruk 2010b). As such, it is likely that the unique architectural/ molecular features of apical ES have rendered this anchoring junction type in the seminiferous epithelium to be the prime (or more susceptible) target of adjudin.

Thirdly, recent studies have shown that adjudin affects two actin regulatory proteins: i) epidermal growth factor receptor pathway substrate 8 (EPS8) and ii) actin-related protein 3 (ARP3), at the apical ES in the rat testes with some unexpected ramifications. These two proteins were shown to play a role in mediating the apical ES restructuring to facilitate the rapid morphological changes in developing elongating spermatids during spermiogenesis by their highly restricted temporal and spatial expression in the seminiferous epithelium at the ES during the seminiferous epithelial cycle (Lie *et al*. 2009b, 2010). It was noted that EPS8, an actin bundling protein (Higgs 2004, Hertzog *et al*. 2010), was normally down-regulated at the apical ES at stage VIII tubules to facilitate the ‘debundling’ of the actin filament network, thereby destabilizing apical ES to prepare step 19 spermatids to undergo spermiation. However, upon adjudin treatment, EPS8 was found to be down-regulated at the apical ES to facilitate actin filament ‘debundling’ in all tubules instead of restricted to stage VIII tubules (Lie *et al*. 2009b; Fig. 2). In short, EPS8 is used to maintain the apical ES integrity by stabilizing the F-actin filament organization via its ability of actin bundling in the epithelium during the normal seminiferous epithelial cycle of spermatogenesis. Thus, down-regulation of EPS8 by adjudin causes disorganization of F-actin, which leads to disruption of apical ES analogous to inducing ‘spermiation’ in immature elongating spermatids (Lie *et al*. 2009b). On the other hand, ARP3 (actin-related protein 3) is part of a functional complex containing ARP2, ARP3, ARPC1–5 (ARP2/3 complex subunit 1 to 5), and WASP (Wiskott-Aldrich syndrome protein) to regulate actin nucleation/branching (Goley & Welch 2006, Cheng & Mruk 2011, Campellone & Welch 2010). The expression of ARP3 was found to be abundant at the apical ES in stage VII tubules to promote actin branching, thereby ‘destabilizing’ the apical ES to prepare for spermiation that occurs in stage VIII (Lie *et al*. 2010; Fig. 2). However, treatment of rats with adjudin induced truncated localization of ARP3 (Lie *et al*. 2010), which is likely being used to disrupt the highly organized actin branching and bundling activity induced by ARP3 and EPS8 respectively, thereby leading premature ‘spermiation’ on all elongating spermatids (Fig. 2).

Taken collectively, these recent findings have demonstrated unequivocally that one of the primary targets of adjudin is the apical ES, wherein adjudin exerts its effects to induce ‘premature spermiation’ in step 8–19 spermatids by perturbing the highly restricted temporal and spatial expression of ARP3 and EPS8 (see Fig. 2), leading to transient infertility because of the loss of functional mature sperm in semen.

Gamendazole and CDB-4022

In this context, it is of interest to note that besides adjudin, two other compounds, namely gamendazole and CDB-4022, appear to exert their effects, at least in part, in the seminiferous epithelium to disrupt germ cell adhesion to induce infertility in rodents. These findings are summarized in this review and briefly discussed in light of the data based on adjudin.

Gamendazole

Gamendazole (see Fig. 1 and Table 1) is another analog of lonidamine. Recent studies have shown that in rats, a single dose of gamendazole at 3 and 6 mg/kg b.w. was able to induce 67 and 100% infertility ~4-week post-treatment respectively. Fertility of the 3 mg/kg b.w. treated group rebounded to 100%, whereas only 57% was achieved in the 6 mg/kg b.w. treated group (Tash *et al*. 2008a). Besides being effective in inducing transient infertility, gamendazole appeared to have no side effects to the animals at the dose that was effective to block fertility. There was no loss in body weight of rats treated with gamendazole indicating that this lonidamine derivative had no general toxicity at that dosage. Although mortality was resulted in three out of five animals at the dosage 200 mg/kg b.w., no observable abnormality including inflammation, necrosis, hemorrhage, or tumor could be detected at dosage lower than 200 mg/kg b.w. (Tash *et al*. 2008a). Moreover, the circulating testosterone levels for gamendazole-treated animals had no significant difference from control animals, whereas the FSH levels of the treated animals had only a transient but insignificant increase (Tash *et al*. 2008a). This indicates that the hypothalamic–pituitary–testicular axis is not disrupted. The fatality that was reported for gamendazole, but not for adjudin that served as a comparative control in the same study (Tash *et al*. 2008a), is likely due to the presence of the trifluoro group in the indazole ring (see Fig. 1), which was observed even a single fluoro group was present (Cheng *et al*. 2001), and this issue of toxicity has recently been discussed (Cheng & Mruk 2010a).

As both gamendazole and adjudin are analogs of lonidamine (see Fig. 1), it is not surprising to see that both compounds induce similar phenotypic changes in the seminiferous epithelium of rat testes by depleting germ cells from the tubules (Cheng *et al*. 2001, 2005, Tash *et al*. 2008a, 2008b). Interestingly, gamendazole was shown to exert its action by selectively inhibiting part of the functions of eukaryotic elongation translation factor 1 (eEF1A1; Tash *et al*. 2008b). eEF1A1 was first identified for its role in protein synthesis as an elongating factor during transcription (Carvalho *et al*. 1984). It was later found to interact with actin cytoskeleton (Yang *et al*. 1990) and this function is conserved from yeast (Munshi *et al*. 2001) to human (Liu *et al*. 2002). Thus, partial inhibition of eEF1A1 might lead to disruption of the eEF1A1-mediated bundling of F-actin and therefore impairing the actin filament bundles necessary to maintain the integrity of the apical ES (Tash *et al*. 2008b). It was also proposed that gamendazole could induce a temporary reduction in functions of a heat-shock protein HSP90, leading to degradation of its client protein AKT1 (Tash *et al*. 2008b). As AKT1 (also known as PKB, protein kinase B) was shown to be involved in maintaining the adhesion between Sertoli cells and spermatids (Siu *et al*. 2005), a loss of AKT1 thus destabilizes germ cell adhesion, thereby inducing germ cell loss from the

epithelium (Tash *et al*. 2008b). It remains to be determined in future studies if the action of eEF1A1 downstream would involve changes in the restricted expression and/or activation of EPS8 and ARP3 (Lie *et al*. 2009b, 2010) that leads to an alteration of the actin cytoskeleton as discussed above.

CDB-4022

CDB-4022, [4aRS,5SR,9bRS]-2-ethyl-2,3,4,4a,5,9b-hexahydro-8-iodo-7-methyl-5-[carbo-methoxyphenyl]-1H-indeno-[1,2-c]-pyridine-hydrochloride (Fig. 1 and Table 1), is an indenopyridine that was shown to have anti-spermatogenic effects in rodents and monkeys (Hild *et al*. 2001, 2007). A single dose of CDB-4022 at 2.5 mg/kg b.w. or seven daily doses of CDB-4022 at 12.5 mg/kg b.w. was able to induce 100% infertility in rats (Hild *et al*. 2001) or monkeys respectively (Hild *et al*. 2007). Interestingly, the irreversible fertility was different from species to species because CDB-4022 induced irreversible infertility in rats (Hild *et al*. 2001), but monkeys treated with CDB-4022 could have their fertility rebounded by 16-week post-treatment (Hild *et al*. 2007). Furthermore, no observable adverse effects such as loss in body weight were found in the treated animals, indicating that this compound had no general toxicity (Hild *et al*. 2001). One of the sites of action of CDB-4022 was the adherens junction between Sertoli and germ cells. A previous study showed that after CDB-4022 treatment, germ cell adhesion was perturbed due to a loss of nectin-3 and its partner afadin (Koduri *et al*. 2008) because the nectin-3/afadin adhesion complex is crucial to confer spermatid adhesion at the apical ES (Ozaki-Kuroda *et al*. 2002). In addition, an activation of ERK–MAPK signaling pathway was also detected (Koduri *et al*. 2008) during CDB-4022-induced germ cell loss from the epithelium, analogous to the adjudininduced germ cell loss from the testis (Lee & Cheng 2004a, Xia *et al*. 2005, Li *et al*. 2009).

Concluding remarks and future perspectives

As briefly discussed in this review, adjudin and two other potential male contraceptives under investigation, namely CDB-4022 and gamendazole, share some similar action in the testis by disrupting germ cell anchoring junctions in the seminiferous epithelium, perhaps mediated by similar molecular mechanism(s), to induce germ cell loss from the testis. However, results of acute and subchronic toxicity for gamendazole are not known. It appears that the next phase of study will likely focus on the preparation of better formulations in order to widen the margin between efficacy and safety so that these compounds, or their second-generation analogs, can move forward in the development pipeline. Although this is a tedious process and can sometimes be frustrating, new and some unexpected findings will be obtained. For instance, the use of adjudin-treated rats becomes a novel *in vivo* model to study the biology and regulation of different anchoring junctions, including the apical ES and the basal ES, as well the BTB.

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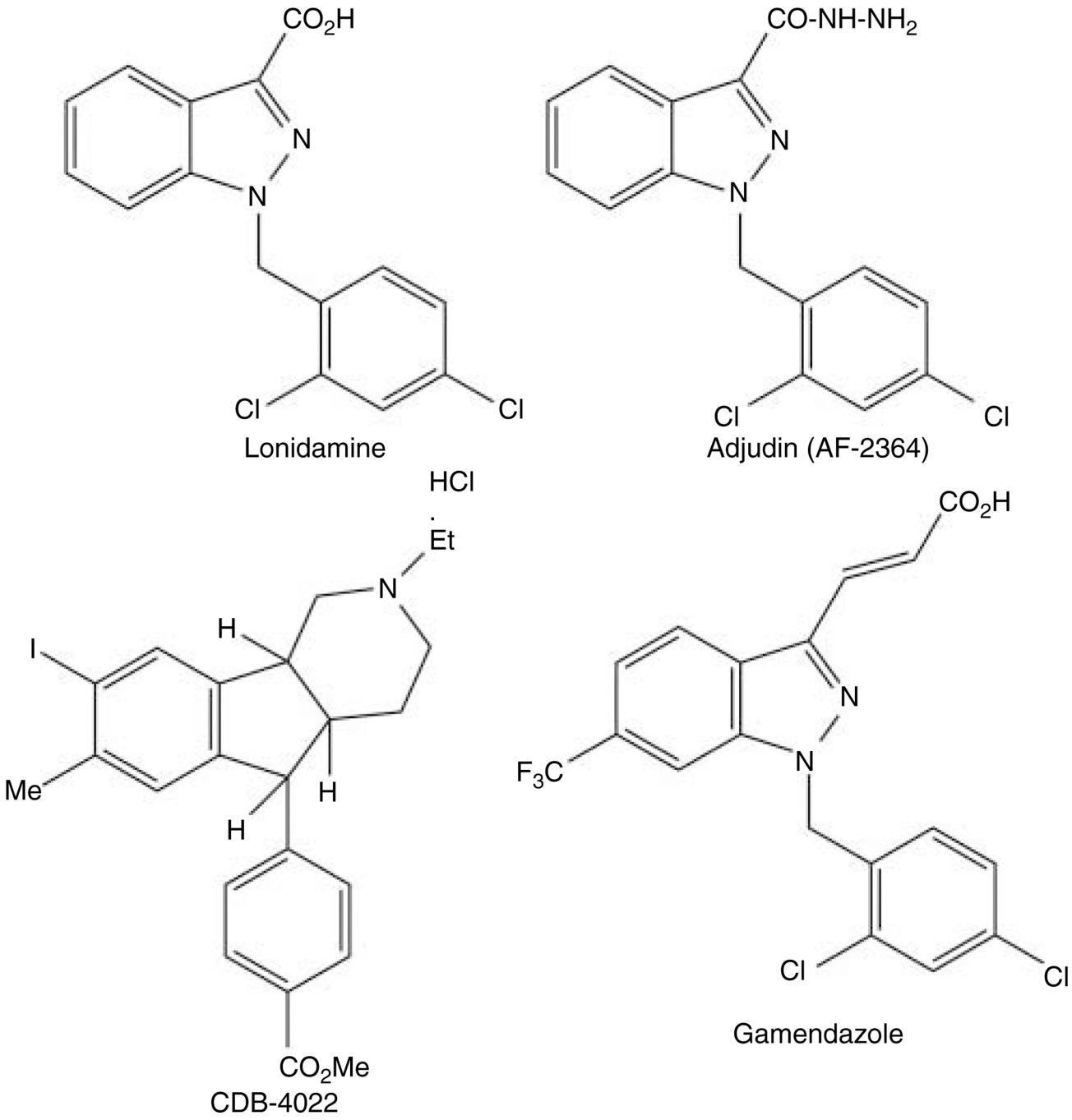

Figure 1.
Structural formulae of lonidamine, adjudin, gamendazole, and CDB-4022.

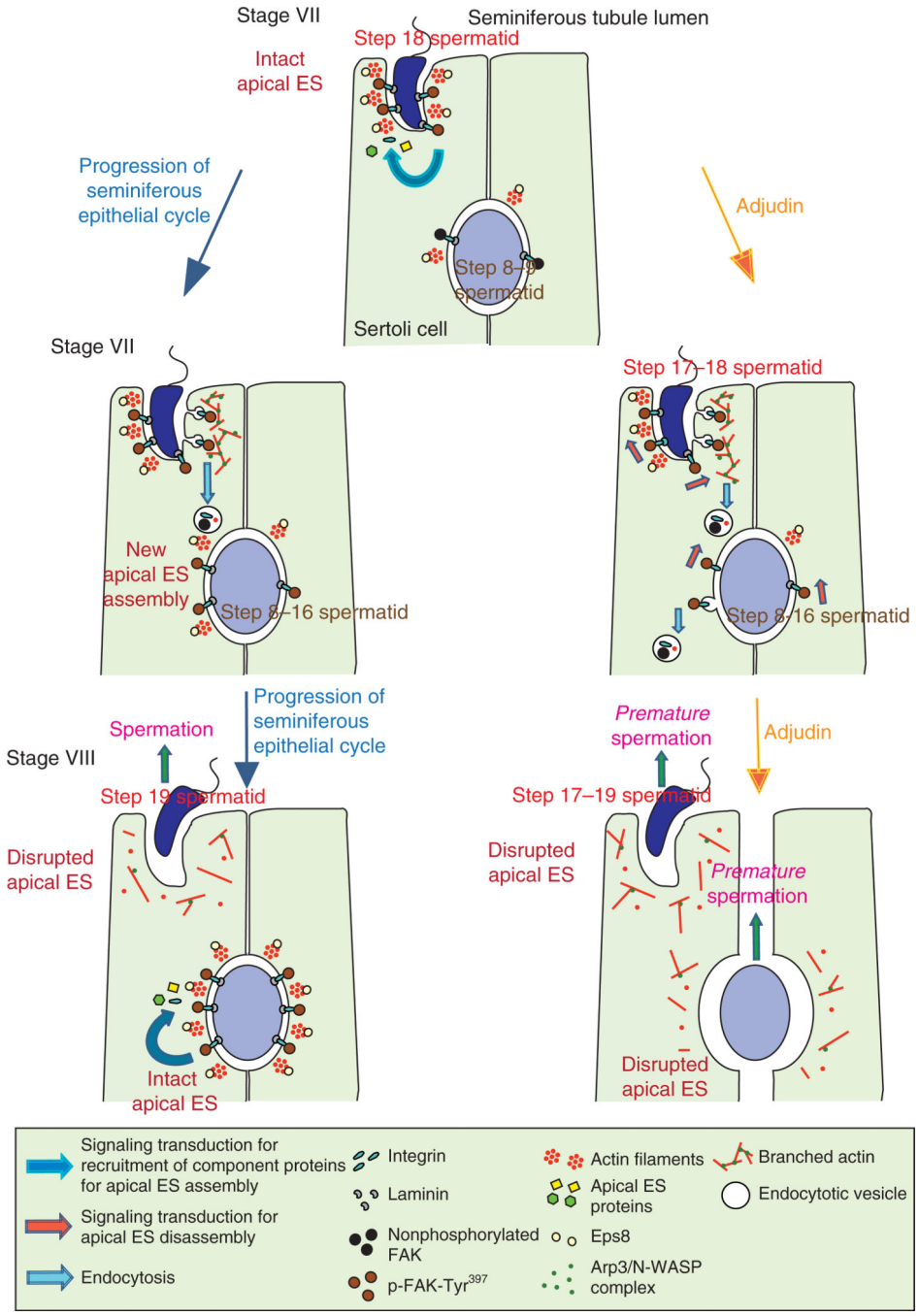

Figure 2.

A schematic illustration of the current concepts of mechanism by which adjudin perturbs apical ES function in the seminiferous epithelium to induce germ cell loss from the seminiferous epithelium. During spermatogenesis, such as in the seminiferous epithelium of a stage VII tubule (left panel), the expression of EPS8 is high at the apical ES for the maintenance of F-actin organization via its actin bundling activity to stabilize that apical ES. The integrity of the apical ES in this stage VII tubule is also maintained via an elevated expression of p-FAK-Tyr^{397}, perhaps being used to maintain the proper phosphorylation

status of the constituent proteins at the apical ES (Siu *et al*. 2003), as well as to recruit additional adaptors (e.g. p130 Cas, vinculin) to the site to reinforce cell adhesion (Siu & Cheng 2008). As spermatogenesis proceeds (lower panel), ARP3, whose expression is also high at the apical ES in stage VII (Lie *et al*. 2010), causes 'debundling' of actin filaments by promoting actin branching. At the same time, endocytosis facilitates recycling of apical ES proteins for the assembly of 'new' apical ES that arises via spermiogenesis such as newly formed step 8 spermatids (Cheng & Mruk 2010b). At stage VIII of the seminiferous epithelial cycle when spermiation occurs, the apical ES is destabilized as the result of 'debundling' of actin filament due to the down-regulation of EPS8 with concomitant actin branching promoted by ARP3 (Lie *et al*. 2009b, 2010). On the other hand, new apical ES is being assembled for developing step 8–9 spermatids, such as via protein transcytosis and/or *de novo* synthesis of 'new' apical ES proteins. When rats are treated with adjudin (right panel), the above-described events, which only take place for step 18–19 spermatids during stage VII–VIII tubule, are 'triggered' to occur 'prematurely' at the apical ES in other stages of tubules with step 8–19 spermatids. Therefore, spermiation takes place 'prematurely' in developing spermatids in other staged tubules besides stage VIII. In addition, p-FAK-Tyr^{397} is highly expressed at the apical ES for the induction of the integrin/FAK/PI3-kinase/p130Cas/MAP kinase signaling pathway (Siu *et al*. 2003) to further destabilize apical ES to cause 'premature' spermiation in spermatids from steps 8 to 19.

Table 1

A summary of the efficacy, toxicity, and sites of action for adjudin versus gamendazole and CDB-4022.

	Compound		
	Adjudin	**Gamendazole**	**CDB-4022**
Min. effective dosage for 100% efficacy	Two doses of 50 mg/kg b.w. (one dose per week)	Single dose of 6 mg/kg b.w.	Single dose of 2 mg/kg b.w. for rats Seven daily doses of 12.5 mg/kg b.w. for monkeys
Reversibility at min. effective dosage	100%	57%	0% in rats 100% in monkeys
Level of FSH, LH, and testosterone after treatment	FSH (−), LH (−), and testosterone (−)	FSH (NS ↑), and testosterone (−)	FSH (↑), LH (−), and testosterone (−)
Lethal dosage	No toxic effects for single dose up to 2000 mg/kg b.w. and no fatality at 50 mg/kg b.w. per day for 29 days for 10 male and 10 female rats (subchronic toxicity test)	200 mg/kg b.w.	Not determined
Sites of action	Activation of integrin/FAK/ERK signaling pathway	Partial inhibition of eEF1A1 Partial inhibition of HSP90	Alteration of expression of apical ES proteins
	Activation of integrin/RhoB/cofilin signaling pathway		Activation of ERK–MAPK signaling pathway
	Down-regulation of EPS8 Causing truncated localization of ARP3		Decreasing the ratio of membrane to soluble form SCF Activation of pro-apoptotic factors
Effect(s)	Premature spermiation due to disruption of apical ES	Premature spermiation due to disruption of apical ES	Premature spermiation due to disruption of apical ES An increase in germ cell apoptosis
References	Cheng *et al*. (2001, 2005), Grima *et al*. (2001), Lie *et al*. (2009b, 2010), Lui *et al*. (2003), Siu *et al*. (2003) and Mruk *et al*. (2006)	Tash *et al*. (2008a, 2008b)	Hild *et al*. (2001, 2007) and Koduri *et al*. (2008)

↑, Stimulation; −, no change; min, minimal; NS, not significantly; SCF, stem cell factor.