Published in final edited form as:

Recent Pat Endocr Metab Immune Drug Discov. 2015 ; 9(2): 63–73.

Adjudin - A Male Contraceptive with Other Biological Activities

Yan-Ho Cheng^{1,2,*}, Weiliang Xia³, Elissa W.P. Wong¹, Qian R. Xie³, Jiaxiang Shao³, Tengyuan Liu³, Yizhou Quan³, Tingting Zhang³, Xiao Yang³, Keyi Geng³, Bruno Silvestrini⁴, and Chuen-Yan Cheng^{1,*}

¹The Mary M. Wohlford Laboratory for Male Contraceptive Research, Center for Biomedical Research, Population Council, 1230 York Ave, New York, New York 10065, USA

²Department of Medicine, Westchester Medical Center, 100 Woods Rd, Valhalla, New York 10595

³State Key Laboratory of Oncogenes and Related Genes, Renji-Med X Stem Cell Research Center, Ren Ji Hospital; School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, P.R. China

⁴S.B.M. Srl Pharmaceuticals, Rome, Italy

Abstract

Background—Adjudin has been explored as a male contraceptive for the last 15 years since its initial synthesis in the late 1990s. More than 50 papers have been published and listed in PubMed in which its mechanism that induces exfoliation of germ cells from the seminiferous epithelium, such as its effects on actin microfilaments at the apical ES (ectoplasmic specialization, a testis-specific actin-rich anchoring junction) has been delineated.

Objective—Recent studies have demonstrated that, besides its activity to induce germ cell exfoliation from the seminiferous epithelium to cause reversible infertility in male rodents, adjudin possesses other biological activities, which include anti-cancer, anti-inflammation in the brain, and anti-ototoxicity induced by gentamicin in rodents. Results of these findings likely spark the interest of investigators to explore other medical use of this and other indazole-based compounds, possibly mediated by the signaling pathway(s) in the mitochondria of mammalian cells following treatment with adjudin. In this review, we carefully evaluate these recent findings.

Methods—Papers published and listed at www.pubmed.org and patents pertinent to adjudin and its related compounds were searched. Findings were reviewed and critically evaluated, and summarized herein.

Results—Adjudin is a novel compound that possesses anti-spermatogenic activity. Furthermore, it possesses anti-cancer, anti-inflammation, anti-neurodegeneration, and anti-ototoxicity activities based on studies using different *in vitro* and *in vivo* models. Conclusion:

*Address correspondence to these authors at The Mary M. Wohlford Laboratory for Male Contraceptive Research, Center for Biomedical Research, Population Council, 1230 York Ave, New York, New York 10065, United States of America; Tel: 212 327 8738; Fax: 212 327 8833; Y-Cheng@popcbr.rockefeller.edu; ccheng@mail.rockefeller.edu Fellow, Oncology/Hematology, Department of Medicine, Westchester Medical Center, Valhalla, New York 10595, America; ChengY@WCMC.com.

CONFLICT OF INTEREST

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

Studies on adjudin should be expanded to better understand its biological activities so that it can become a useful drug for treatment of other ailments besides serving as a male contraceptive.

Keywords

Adjudin; anti-cancer activity; anti-ototoxicity; ectoplasmic specialization; germ cell loss; male contraceptive; Sertoli cell; testis

INTRODUCTION

Adjudin, 1-(2, 4-dichlorobenzyl)-1*H*-indazole-3-carbohydrazide, formerly called AF-2364 Fig. (1), is an analog of lonidamine. Lonidamine and related compounds such as tolnidamine [1] belong to a class of indazole-based compounds synthesized in the 1970s as part of the effort of developing innovative anti-cancer drugs, which were later found to have potent antispermatogenic activity [2, 3]. Unlike other anti-cancer drugs which are mostly anti-mitotic by targeting actively dividing tumor cells, lonidamine and its related compounds are not anti-mitotic, non-cytotoxic, and have no effects on cellular nucleic acids or protein synthesis [4–6]. However, lonidamine and its related compounds (e.g., tolnidamine) are mitochondrial hexokinase inhibitors (also known as glycolytic inhibitors), by impairing the energy metabolism of neoplastic cells [7–9], impairing mitochondrial function leading to mitochondria-mediated apoptosis in tumor cells [6, 10–13]. Specifically, lonidamine binds to adenine nucleotide translocator (ANT) in mitochondria, triggering mitochondrial membrane permeabilization (MMP) that leads to cancer cell apoptosis [6, 11–13]. Lonidamine also blocks lactate transport by inhibiting lactate efflux in cancer cells, and such lactate accumulation leads to intracellular acidification, thereby reducing cell proliferation, migration and invasion, and promoting apoptotic cell death in tumor cells [14–17]. It is noted that lonidamine also induces Sertoli cell injury in the testis, thereby inducing germ cell exfoliation, when administered by oral gavage in rodents [18]. In an effort to capture the anti-spermatogenic effects of lonidamine based on its indazole ring, we had synthesized a panel of analogs and derivatives of lonidamine and other indazole-based compounds to identify potential male contraceptives without the toxicity of lonidamine [19]. Adjudin was selected from more than two-dozen lonidamine analogs and derivatives that were synthesized in the 1990s. The screening was based on an *in vivo* assay developed in our laboratory [19] to select an indazole-based compound that could disrupt spermatid adhesion in the mammalian testis, which could serve as a potential non-hormonal and reversible male contraceptive. Since other germ cell types, in particular undifferentiated spermatogonia are not perturbed following treatment of adult male rats with adjudin [20], the adjudin-induced infertility is transient, this is because undifferentiated spermatogonia can repopulate the germ cell-depleted seminiferous epithelium in tubules via spermatogenesis, making adjudin a reversible male contraceptive. This assay was developed based on the observation that the level of testin, a signaling/structural molecule at the Sertoli-spermatid interface which is highly expressed in the testis [21], was transiently up-regulated, usually within 4–6 hr after treatment, when the adhesion of spermatids in the seminiferous epithelium was disrupted [19]. It was noted that if the testin level remained elevated well past -6 hr, the chemical entity usually associated with acute toxicity [19, 22–24]. Adjudin was selected based on this assay because it induced a drastic but transient up-regulation on the expression of testin in

the testis following treatment, usually peaked within a day vs. control rats and returned to the basal level rapidly based on Northern blot and/or immunoblot analysis [19, 24], consistent with subsequent gene profiling analysis [25]. Herein, we focus our discussion on adjuvin, however, we also provide a brief account on lonidamine and other structurally related compound since findings from these other chemical entities are helpful to better understand adjuvin. Also, we do not intend to provide detailed molecular mechanistic insights on the other biological activities of adjuvin but a brief summary of these recent findings since the intention of this brief review is to provide the latest update regarding some ongoing studies in the field. Furthermore, additional studies are needed to better explore the mechanistic insights pertinent to other biological activities of adjuvin besides its anti-fertility effects in male rodents.

ADJUDIN IS A POTENTIAL MALE CONTRACEPTIVE

As noted above, adjuvin has been explored to serve as a male contraceptive since its synthesis in the early 1980s [19, 26, 27]. Studies have shown that adjuvin exerts its effects by perturbing the apical ectoplasmic specialization (apical ES) function at the Sertoli-spermatid interface, a testis-specific actin-rich anchoring junction [22, 28], altering the function of adhesion protein complexes at the site, causing exfoliation of germ cells, initially elongating/elongated spermatids, to be followed by round spermatids and spermatocytes, but not spermatogonia [20, 26, 28]. During the epithelial cycle, the integrity of apical ES is notably supported by an array of actin microfilament bundles that lie perpendicular to the Sertoli cell plasma membrane, which are sandwiched in-between the Sertoli cell plasma membrane and the cisternae of endoplasmic reticulum in the Sertoli cell [29, 30]. In this context, it is of interest to note that a similar structural feature, namely the array of actin filament bundles, is not found in the apposing step 8–19 spermatid at the apical ES. Due to this array of bundles of actin microfilaments at the ES, study has shown that apical ES confers stronger adhesion vs. desmosome [31], the intermediate filament-based anchoring junction in the skin known to confer one of the strongest adhesion junctions [32, 33]. However, in order for spermatids to be transported across the adluminal compartment of the seminiferous epithelium during the epithelial cycle so that fully developed spermatids (i.e., spermatozoa) can be lined up near the tubule lumen to prepare for their eventual release at spermiation, these actin microfilament bundles must be rapidly re-organized via conversion between a “bundled” and “unbundled/branched” configuration to support spermatid transport until at stage VIII when spermiation takes place [30, 34]. Studies have shown this can be achieved via spatiotemporal expression of actin barbed end capping and bundling protein Eps8, and other actin bundling proteins such as palladin and ezrin vs. branched actin polymerization proteins such as the Arp2/3 (actin related protein 2/3) complex at the apical ES during the epithelial cycle [30, 35–37]. As such, actin bundles can be rapidly converted between a bundled and branched/unbundled configuration to confer plasticity to the apical ES to facilitate spermatid transport. Recent reports have shown that adjuvin exerts its effects by altering the spatiotemporal expression of actin binding proteins, such as Eps8 and Arp3 [38, 39], at the apical ES, thereby actin microfilament bundles fail to be re-organized from “bundled” to “unbundled/branched” configuration and vice versa in response to stages of the epithelial cycle to support spermatid transport and adhesion. This thus causes exfoliation of

spermatids, inducing infertility in male rats [28, 40] Fig. (2). Changes in the spatiotemporal expression of Arp3, Eps8 and other actin binding/regulatory proteins can possibly be regulated by p-FAK-Tyr³⁹⁷ and p-FAK-Tyr⁴⁰⁷, since studies have shown that these signaling molecules regulate actin microfilament organization at the ES based on studies *in vitro* and *in vivo*, and these proteins also display restrictive spatiotemporal expression at the apical ES during the epithelial cycle [41, 42]. It is noted that adjudin received a US6001865 patent granted on December, 1999. However, there are many advances in the field regarding the contraceptive use of other analogs of lonidamine and indazole-based derivatives such as H2-gamendazole as well as other medical use such as for treatment of multiple diseases including cancer, chronic and inflammatory disorders and others (see Table 1). In this section, we have briefly reviewed the latest updates on the likely mechanism of action of adjudin in the testis to perturb spermatogenesis, leading to transient male infertility. In the sections that follow, we also review other biological activities of adjudin illustrating the added health benefits of adjudin. Perhaps, these other biological activities of adjudin should be better explored in future studies. We first provide a brief update on the anti-cancer characteristics of lonidamine in the next section since adjudin is a close cousin of lonidamine.

LONIDAMINE, A DERIVATIVE OF INDAZOLE-CARBOXYLIC ACID AND AN ANALOG OF ADJUDIN, IS AN ANTI-CANCER DRUG

Lonidamine, 1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carboxylic acid shown in Fig. (1), is an anti-cancer drug using for different types of cancer including brain tumors, breast cancer, ovarian and lung cancer, including late stage cancer [6, 15, 43, 44]. Studies have shown that other similar analogs and/or derivatives of lonidamine also possess anti-cancer activities as noted in Table 1. Unlike many anticancer drugs, lonidamine, however, is not anti-mitotic [6, 8, 45, 46]. It was also shown to possess potent antispermatogenic activity by causing Sertoli cell injury due to the presence of the indazole ring in its chemical structure [4] as noted in Fig. (1). Lonidamine is an inhibitor of mitochondrial hexokinase 2, the predominant isoform of hexokinase found in cancer cells that is capable of phosphorylating hexoses by acting as a glycolytic inhibitor [7–9]. Thus, lonidamine effectively blocks the formation of glucose-6-phosphate from glucose - the initial step of glycolysis in mitochondria. This thus disrupts energy metabolism in cancer cells since mitochondrial hexokinase 2, especially in cells in advanced neoplasms, support cell growth by enhancing aerobic glycolysis [47], which is currently considered to be one of the major mechanisms of action of lonidamine. In this context, it is of interest to note that in normal mammalian cells, hexokinase I is predominantly used for glucose metabolism, and hexokinase 2 is restricted to rapidly dividing cells with condensed mitochondria such as those found in cancer cells in particular after they are sensitized by radiation [6, 8, 45, 48, 49]. Lonidamine also disrupts lactate transport, causing lactate accumulation in tumor cells, this thus leads to intracellular acidification which, in turn, induces tumor cell death [6, 14, 49]. Interestingly, lonidamine does not affect respiration, mitochondrial electron transport and glycolysis in Sertoli cells [50], perhaps due to the absence of condensed mitochondria in Sertoli cells. Lonidamine does disrupt the Sertoli cell cytoskeleton, affecting Sertoli cell actin microfilaments,

intermediate filaments, and microtubules, and a disruption of these cytoskeletons thus leads to germ cell exfoliation as a result of Sertoli cell injury [18].

By contrast, all germ cells, unlike Sertoli cells, in particular post-meiotic spermatids, except spermatogonial stem cells, spermatogonia, and preleptotene spermatocytes, contain condensed mitochondria [51]. Thus the structural features of mitochondria in post-meiotic spermatids are similar to tumor cells. Unlike somatic or neoplastic cells, germ cells, such as spermatocytes and spermatids, do not utilize glucose and/or hexose; instead, they rely on lactate generated by Sertoli cells to serve as their energy source [52, 53]. Interestingly, in order to accommodate the physiological needs of germ cells, Sertoli cells also metabolize glucose preferably to lactate instead of pyruvate to enter the Krebs cycle to maximize the production of ATPs [54, 55]. In short, Sertoli cells are similar to cancer cells as noted in their energy management strategy by producing lactate from glucose [56]. Germ cells obtain lactate from Sertoli cells in the testis via their integral membrane lactate transporter MCT2 (monocarboxylate transporter 2). Once lactate enters a germ cell, it will be converted to pyruvate by a germ cell-specific lactate dehydrogenase (LDH) called LDHX, also known as LDHC4. Pyruvate then enters the Krebs cycle to produce ATPs to sustain germ cell development [53]. The effects of lonidamine on germ cells, in particular late spermatocytes and post-meiotic spermatids, are most obvious within the condensed mitochondrion, since lonidamine was shown to induce mitochondrial membrane swelling and also mitochondrial degeneration [57]. A significant reduction in oxygen consumption and a remarkable decline in ATP production by germ cells, but not by liver cells, are also evident after lonidamine exposure [57]. Other changes include the vacuolization of round spermatid nuclei and the formation to giant multinucleated round spermatids following lonidamine treatment have also been reported [57].

As noted above, Sertoli cells prefer to metabolize glucose to pyruvate, and pyruvate to lactate, instead of having pyruvate to enter the Krebs cycle to produce ATPs more effectively, almost analogous to tumor cells [53, 58]. In fact, the production of lactate via anaerobic glycolysis in Sertoli cells is significantly higher than that of normal differentiated or tumor cells such as Ehrlich ascites tumor cells [50]. In the testis, Sertoli cell-produced lactate is pumped out via the monocarboxylate transporter 1 (MCT1) and uptaken by meiotic and post-meiotic germ cells via the MCT2 [53]. Tumor cells generate energy by metabolizing glucose via anaerobic glycolysis even when oxygen is present in the environment [59, 60], which essentially yields two pyruvate molecules and two adenosine triphosphates (ATP) from a single glucose molecule; pyruvate is then metabolized to lactate. This phenomenon, the hallmark of tumor cell metabolism, is known as the Warburg effect [61, 62], and it explains how tumor cells maintain their active mitotic activity when there is a shortage of oxygen (i.e., hypoxia) because they are usually located away from the microvasculature during initial tumorigenesis [63]. Although Sertoli cells have access to oxygen from the microvasculature in the testis, they also produce pyruvate and lactate via anaerobic glycolysis, but supply lactate to developing germ cells [53], instead of putting pyruvate through the Krebs cycle that requires oxygen consumption. As each Sertoli cell requires to nurture ~30–50 developing germ cells at different stages of development [64], germ cell nourishment is no small task. Besides serving as the substrate for the production of ATP through mitochondrial respiration via the Krebs cycle, studies have shown that lactate

also regulates the reactive oxygen species (ROS) level in germ cells [65], sperm motility, protein tyrosine phosphorylation during capacitation, and fertilization [66].

Lonidamine is most effective as an antineoplastic drug when tumor cells are sensitized by radiation, which generates more condensed mitochondria, and in combination with other chemotherapeutic drugs, such as paclitaxel, which damages the plasma membrane and condensed mitochondrial membrane in tumor cells, causing mitochondrial degeneration and tumor cell death [6, 48, 67]. Adjudin has also been shown to possess anticancer activity in a mouse model as well as tumor cells cultured *in vitro*. For example, adjudin has been reported to reduce lung and prostate tumor cell growth *in vivo* after A549 (adenocarcinoma human alveolar basal epithelial cells) or PC3 (human prostate adenocarcinoma cells) cells were subcutaneously injected into adult nude mice [68]. Furthermore, adjudin has also been shown to possess anti-inflammatory activity in a brain model [69], indicating that it might alleviate symptoms associating with neurodegenerative diseases. However, these results must be interpreted with caution because outcomes from mouse models do not reliably predict outcomes in humans. Furthermore, most types of cancer and neurodegeneration have a strong environmental and/or behavioral underpinning, and they usually occur in aged individuals. Most mouse models that study cancer or neuro-degeneration do not mimic these features in particular humans. Below is a more detailed discussion on the other biological activities of adjudin.

It is also noted that when used at identical doses, adjudin is more potent than lonidamine and less toxic based on results of acute and subchronic toxicity tests conducted by licensed toxicologists [70], even though they share a common chemical structure of indazole. Comparison between adjudin and lonidamine perhaps are similar to salicyclic acid and aspirin. Salicyclic acid is a highly toxic active ingredient of willow bark leaves, but aspirin is acetylated salicyclic acid prepared in 1897 by Felix Hoffman in 1897 at Bayer AG, less toxic, and aspirin subsequently used to treat pain, fever, and inflammation, and remains one of the most widely used medicines with multiple biological activities [71–73]. Different lonidamine analogs currently under development by others (e.g., gamendazole) [74, 75] and our laboratory (e.g., adjudin) [22, 28] may prove to be viable non-hormonal male contraceptives with other added health benefits. In the following sections, we summarize relevant findings on adjudin in particular its other biological activities besides its anti-fertility activity as recently reviewed [27, 28].

BIOLOGICAL ACTIVITIES OF ADJUDIN IN ADDITION TO ITS ANTI-FERTILITY EFFECTS IN THE TESTIS

Anti-Cancer Activity

Since adjudin is an analog of the anticancer drug lonidamine, it is not surprising that it possesses anticancer activity. Studies *in vitro* using human cancer cell lines including SGC-7901 cells (human gastric adenocarcinoma cells), MDA-MB-231 cells (human breast adenocarcinoma cells), Smmc-7721 cells (human hepatoma cells), and MIA PaCa-2 cells (human pancreatic adenocarcinoma cells) have shown that adjudin is capable of inhibiting cell proliferation dose-dependently with an IC₅₀ of 58, 13.8, 72.3 and 52.7 μM, respectively,

following treatment of 24hr [68]. Furthermore, the potency of adjuvin in blocking cancer cell proliferation is ~2–3 fold better than lonidamine when the IC₅₀ of adjuvin vs. lonidamine are compared in A549 cells (human lung adenocarcinoma cells) and PC3 (human prostate cancer cells) cells: 63.1 and 93 μ M for adjuvin vs. 200 and 120 μ M for lonidamine, respectively [68]. Adjuvin also induced cell apoptosis of A549 and PC3 cancer cells via the caspase 3-dependent apoptotic pathway, perturbing mitochondrial function, thereby reducing intracellular ATP levels in these cancer cells [68] noted in Fig. (2). Adjuvin apparently exerts its anti-cancer effects by triggering mitochondrial dysfunction via a loss of mitochondrial membrane potential, serving as a hexokinase 2 inhibitor by reducing cellular ATP level [68]. Adjuvin also induces cancer cell apoptosis via the caspase-3-dependent pathway [68]. More important, the anti-cancer activity of adjuvin noted *in vitro* has also been shown in studies *in vivo*. For instance, when adult nude mice were inoculated subcutaneously with A549 cells and PC3 cells to induce tumor up to a size of ~6-mm in diameter in 2-wk, an administration of adjuvin via i.p. at 100mg/kg b.w. (note: to obtain effective contraceptive dose in male rats, two weekly dose of 50mg/kg b.w. is required [22]) with ~6 doses in 2-wk, the tumor size was shrunk considerably, by as much as ~40–50% in ~10 days following adjuvin treatment [68]. These findings thus illustrate the added health benefits of adjuvin when used as a male contraceptive, in which adjuvin may be able to suppress cancer growth and/or cancer development in men taking adjuvin as a contraceptive. Nonetheless, the mechanism(s) by which adjuvin disrupts cancer metabolism and induces cancer apoptosis must be better delineated in future studies.

Anti-Neuroinflammatory Activity

Microglia cells are resident macrophages in the central nervous system (CNS) which play an important role in brain function including innate immunity, brain development, repair and also etiology of CNS disorders such as Alzheimer's disease [76–79]. Studies have also shown that activation of microglia that leads to chronic inflammation is closely related to neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, dementia and multiple sclerosis [80–83]. Indazole-based compounds, such as bindarit, and benzydamine, some structurally related compounds to lonidamine and adjuvin, have also been shown to possess anti-inflammatory activities, acting as non-steroidal anti-inflammatory drugs (NSAID) to relief some pathological conditions [84–91]. Using a murine BV2 microglia cell line that mimics primary microglia and is widely used to study microglial biology in Alzheimer's disease [92], adjuvin was shown to inhibit LPS (lipopolysaccharide)-induced microglia activation [69], the hallmark of microglia-mediated inflammation in the brain. For instance, adjuvin considerably inhibited LPS-mediated IL-6 release and also the expression of IL-6, IL-1 β , and TNF α in BV2 microglia cells [69]. More important, using a permanent middle cerebral artery occlusion (pMCAO) *in vivo* mouse model that closely mimics focal cerebral ischemia [93] by reducing the cerebral blood flow with ~10% of normal mouse to induce ischemia, adjuvin was found to significantly attenuate ischemia-induced microglia activation [69]. For instance, it was noted that after pMCAO, the expression of CD11b (also known as integrin α M chain), an indicator of microglia activation in various CNS diseases [80], was significantly up-regulated when frozen sections of pMCAO-induced ischemic cerebral cortex and striatum vs. controls (sham operated+vehicle control, and sham operated + adjuvin treated rats) were examined by immunofluorescence

analysis using an anti-CD11b antibody, and adjuvin treatment (50mg/kg b.w. by i.p.) 2hr before ischemia reduced CD11b expression [69]. Furthermore, this anti-inflammatory activity in microglia cells is mediated by inhibiting NF- κ B activation and ERK MAPK (but not p38 or JNK) phosphorylation [69]. Adjuvin also reduced ischemia-induced brain edema [69]. More important, adjuvin treated rats displayed behavioral improvement at 72hr after ischemia [69]. Subsequent studies have shown that adjuvin likely exerts its effects by preventing blood-brain barrier disruption after ischemia, and also by reducing the expression and the release of TNF α , IL-1 β and IL-6 in the brain based on the use of a tMCAO (transient MCAO) model [94]. Collectively, these findings illustrate the anti-neuroinflammatory activity of adjuvin in the CNS during ischemia-mediated brain damage, suggesting neuroprotective function of adjuvin as noted in Fig. (2).

Otoprotective Activity in Cochlea Hair Cells

In mammals, hair cells in the cochlea of the ear convert sound signals into electrical impulses [95, 96]. However, degeneration of hair cells due to aging, acoustic trauma and exposure to aminoglycoside antibiotics (e.g., gentamicin is known to cause ototoxicity in ~5–10% of the patients when given intravenously or during peritoneal dialysis in humans) are generally irreversible [97, 98]. However, a recent study using rat cochlear explant cultures and cochlear primary cell cultures subjected to gentamicin-induced ototoxicity, adjuvin has shown to offer otoprotection by up-regulating mitochondrial Sirt3, a member of the Sirtuin family protein known to regulate reactive oxygen species (ROS) production [99]. In short, adjuvin protects hair cells from gentamicin-mediated apoptosis [99]. Treatment of hair cells with adjuvin also induces a surge in mitochondrial Sirt3 expression, concomitant with a significant reduction in ROS level [99], illustrating adjuvin exerts its otoprotection via the mitochondrial Sirt3-ROS pathway Fig. (2). These findings are consistent with an earlier report illustrating that Sirt3 mediates reduction of oxidative damage by reducing ROS production in the inner ear, and promoting hair cell survival, thereby preventing age-related hearing loss following caloric restriction using a Sirt3^{-/-} mouse model [100]. The otoprotective effects of adjuvin on cochlea hair cells are further confirmed by overexpressing Sirt3 using lentivirus (lenti-Sirt3) in cochlear explant cultures, illustrating that Sirt3 overexpression in cochleae indeed can rescue gentamicin-induced hair cell loss, analogous to adjuvin treatment [99]. Furthermore, using an *in vivo* gentamicin injury mouse model combined with hearing function tests including Auditory Brainstem Response (ABR) and Compound Action Potential (CAP) tests, adjuvin was shown to have significant otoprotective effects on gentamicin-induced hearing loss in mice [99]. Adjuvin was also shown to reduce hair cell loss from cochleae in gentamicin-treated mice in this mouse model, likely by blocking cell apoptosis induced by gentamicin [99]. In short, adjuvin is an otoprotective drug.

CURRENT & FUTURE DEVELOPMENTS

As briefly reviewed herein, adjuvin is a male contraceptive that exerts its effects at the testis-specific Sertoli-spermatid interface known as apical ES. Adjuvin alters the spatiotemporal expression of actin regulatory proteins such as actin barbed end capping and bundling protein Eps8, and branched actin inducing protein Arp3, thereby perturbing the proper

organization of actin microfilament bundles at the apical ES, leading to germ cell exfoliation from the seminiferous epithelium. In this context, it is of interest to note that adjuvin has been subjected to a battery of standard toxicity studies, including its acute toxicity tests performed in both mice and rats, bacterial mutation assay, mammalian erythrocyte micronucleus test and mammalian chromosome aberration test, performed by licensed toxicologists. It was noted that adjuvin had passed all the standard toxicity tests [70]. However, in a 29-day subchronic toxicity tests with $n = 10$ males and $n = 10$ females, the no-observable-adverse-effect level (NOAEL) for adjuvin when administered once daily by oral gavage for female rats was shown to be 50 mg/kg/day since adjuvin displayed no noticeable signs of damage in organs in female rats examined by a licensed pathologist. A NOAEL for males, however, could not be determined, since at 50 mg/kg/day for 29 days, one male rat displayed signs of chronic-active portal inflammation of minimal severity, and another male rat had midzonal vacuolization of minimal severity; also 3 out of the 10 male rats displayed skeletal muscle degeneration atrophy even though there were no adverse effects on other organs such as brain, kidney and heart (see Final Report of Subchronic Toxicity Report of Adjuvin in [70], and reviewed in [26–28]). These findings thus illustrate that in order to develop adjuvin as a male contraceptive for humans, the efficacy and toxicity dose must be significantly widened, such as by lowering the efficacy dose of adjuvin from 50mg/kg b.w., to <5mg/kg b.w. Work is now in progress to develop a better formulation [70], including the co-delivery of adjuvin with another drug that can induce transient disruption of the blood-testis barrier to improve its bioavailability. These recent advances have been recently reviewed (see [26–28]) and work is in progress in our laboratory. Recent studies, however, have shown that adjuvin has other added health benefits, such as anti-cancer activity that appears to be more potent than lonidamine with less toxicity as summarized in Fig. (2). Besides, adjuvin also has neuroprotective activity by inhibiting neuroinflammatory response. Since neuroinflammation is one of the hallmarks of neuro-degenerating diseases, such as Alzheimer's disease and Parkinson disease, long-term use of adjuvin may have added protection to such ailments as noted in Fig. (2). A more recent report illustrating the otoprotective function of adjuvin based on the use of a gentamicin-induced ototoxicity study model *in vitro* and *in vivo* further illustrates the medical benefits of adjuvin as summarized in Fig. (2). It is of interest to note that recent patents also report other medical benefits of lonidamine, its relative indazole-containing compounds including their protective roles in inflammation and neurodegeneration besides their anti-cancer activities as noted in Table 1 [100–115], consistent with the observations noted for adjuvin as discussed herein. Nonetheless, many open questions remain to be addressed. Does long-term low-dose use of adjuvin also offer otoprotective effects due to aging? Does the *in vivo* otot-protective effects of adjuvin based on the *in vivo* gentamicin model is mediated by mitochondrial Sirt3-ROS pathway analogous to the studies *in vitro*? Does Sirt3-ROS pathway is also involved in the anti-cancer activity or neuroprotective (i.e., anti-inflammatory activity in the CNS) activity of adjuvin? The answers to some of these questions will provide the incentive to better explore the medical use of adjuvin besides male contraception. With the rapid advances in the field at the technological level and the availability of different *in vitro* and *in vivo* models to study major human diseases, we will likely witness a smooth development in the next decade regarding the use of adjuvin as a non-hormonal male contraceptive and also for other pathological conditions, such as for cancer treatment, and treatments for neurodegeneration

and ototoxicity once the underlying molecular mechanisms and efficacy are better understood.

Acknowledgments

All authors were involved in the initial studies including design, performance, analysis and reporting findings of experiments that explored the anti-fertility, anti-cancer, anti-inflammation, anti-neurodegeneration, and anti-ototoxicity activities of adjudin as discussed herein. Authors listed herein have contributed substantially to the design and writing of this review, both intellectually and in the preparation of the manuscript. All authors have approved the publication of this review.

This work was supported in part by grants from the National Institutes of Health, NICHD U54 HD029990 Project 5 to C.Y.C., and R01 HD056034 to C.Y.C.; and grants from the Chinese Ministry of Science and Technology (2013CB945604), the National Natural Science Foundation of China (31270032) and SJTU Interdisciplinary Research Grant (YG2012ZD05), all to W.X.

References

1. Rossi V, Cheng CY, Gunsalus GL, Bardin CW, Spitz IM. The differential effects of the indazole-carboxylic acid derivative, tolnidamine, on Sertoli cell protein secretion. *J Androl.* 1989; 10:466–71. [PubMed: 2621153]
2. Corsi G, Palazzo G, Germani C, Scorza BP, Silvestrini B. 1-Halobenzyl-1H-indazole-3-carboxylic acids. A new class of antispermatogenic agents. *J Med Chem.* 1976; 19:778–83. [PubMed: 950645]
3. Mok KW, Mruk DD, Lie PPY, Lui WY, Cheng CY. Adjudin, a potential male contraceptive, exerts its effects locally in the seminiferous epithelium of mammalian testes. *Reproduction.* 2011; 141:571–80. [PubMed: 21307270]
4. Silvestrini B, Palazzo G, De Gregorio M. Lonidamine and related compounds. *Prog Med Chem.* 1984; 21:111–35.
5. Silvestrini B. Basic and applied research in the study of indazole carboxylic acids. *Chemotherapy.* 1981; 27(Suppl 2):9–20.
6. Di Cosmio S, Ferretti G, Papaldo P, Carlini P, Fabi A, Cognetti F, et al. Lonidamine: Efficacy and safety in clinical trials for the treatment of solid tumors. *Drugs Today (Barc).* 2003; 39:157–74. [PubMed: 12730701]
7. Guaragnella N, Giannattasio S, Moro L. Mitochondrial dysfunction in cancer chemoresistance. *Biochem Pharmacol.* 2014; 92:62–72. [PubMed: 25107705]
8. Solary E, Betttaieb A, Dubrez-Daloz L, Corcos L. Mitochondria as a target for inducing death of malignant hematopoietic cells. *Leuk Lymphoma.* 2003; 44:563–74. [PubMed: 12769332]
9. Constantini P, Jacotot E, Decaudin D, Kroemer G. Mitochondrioin as a novel target of anticancer chemotherapy. *J Natl Cancer Inst.* 2000; 92:1042–53. [PubMed: 10880547]
10. Zhang BF, Xing L, Cui PF, Wang FZ, Xie RL, Zhang JL, et al. Mitochondria apoptosis pathway synergistically activated by hierarchical targeted nanoparticles co-delivering siRNA and lonidamine. *Biomaterials.* 2015; 61:178–89. [PubMed: 26004233]
11. Li YC, Fung KP, Kwok TT, Lee CY, Suen TK, Kong SK. Mitochondrial targeting drug lonidamine triggered apoptosis in doxorubicin-resistant HepG2 cells. *Life Sci.* 2002; 71:2729–40. [PubMed: 12383880]
12. Calvino E, Estan MC, Simon GP, Sancho P, Boyano-Adanez MC, de Blas E, et al. Increased apoptotic efficacy of lonidamine plus arsenic trioxide combination in human leukemia cells. Reactive oxygen species generation and defensive protein kinase (MEK/ERK, Akt/mTOR) modulation. *Biochem Pharmacol.* 2011; 82(11):1619–29. [PubMed: 21889928]
13. Belzacq AS, El-Hamel C, Vieira HLA, Cohen I, Haouzi D, Metivier D, et al. Adenine nucleotide translocator mediates the mitochondrial membrane permeabilization induced by lonidamine, arsenite and CD437. *Oncogene.* 2001; 20:7579–87. [PubMed: 11753636]
14. Ben-Horin H, Tassini M, Vivi A, Navon G, Kaplan O. Mechanism of action of the antineoplastic drug lonidamine: ^{31}P and ^{13}C nuclear magnetic resonance studies. *Cancer Res.* 1995; 55:2814–21. [PubMed: 7796408]

15. Vivi A, Tassini M, Ben-Horin H, Navon G, Kaplan O. Comparison of action of the anti-neoplastic drug lonidamine on drug-sensitive and drug-resistant human breast cancer cells: ^{31}P and ^{13}C nuclear magnetic resonance studies. *Breast Cancer Res Treat.* 1997; 43:15–25. [PubMed: 9065595]
16. Morais-Santos F, Miranda-Goncalves V, Pinheiro S, Vieira AF, Paredes J, Schmitt FC, et al. Differential sensitivities to lactate transport inhibitors of breast cancer cell lines. *Endocrine-related cancer.* 2014; 21(1):27–38. [PubMed: 24174370]
17. McVicar N, Li AX, Meakin SO, Bartha R. Imaging chemical exchange saturation transfer (CEST) effects following tumor-selective acidification using lonidamine. *NMR in biomedicine.* 2015; 28(5):566–75. [PubMed: 25808190]
18. de Martino C, Malcorni W, Bellocchi M, Floridi A, Marcante M. Effects of AF1312 TS and lonidamine on mammalian testis. A morphological study. *Chemotherapy (Suppl 2).* 1981; 27:27–42. [PubMed: 7285636]
19. Cheng CY, Silvestrini B, Grima J, Mo MY, Zhu LJ, Johansson E, et al. Two new male contraceptives exert their effects by depleting germ cells prematurely from the testis. *Biol Reproduct.* 2001; 65:449–61.
20. Mok KW, Mruk DD, Lee WM, Cheng CY. Spermatogonial stem cells alone are not sufficient to re-initiate spermatogenesis in the rat testis following adjuvin-induced infertility. *Int J Androl.* 2012; 35:86–101. [PubMed: 21696392]
21. Cheng CY, Grima J, Stahler MS, Lockshin RA, Bardin CW. Testins are two structurally related Sertoli cell proteins whose secretion is tightly coupled to the presence of germ cells. *J Biol Chem.* 1989; 264:21386–93. [PubMed: 2592382]
22. Cheng CY, Mruk DD, Silvestrini B, Bonanomi M, Wong CH, Siu MKY, et al. AF-2364 [1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carbohydrazide] is a potential male contraceptive: A review of recent data. *Contraception.* 2005; 72:251–61. [PubMed: 16181968]
23. Cheng CY, Mo MY, Grima J, Saso L, Tita B, Mruk DD, et al. Indazole carboxylic acids in male contraception. *Contraception.* 2002; 65:265–8. [PubMed: 12020774]
24. Grima J, Silvestrini B, Cheng CY. Reversible inhibition of spermatogenesis in rats using a new male contraceptive, 1-(2,4-dichlorobenzyl)-indazole-3-carbohydrazide. *Biol Reproduct.* 2001; 64:1500–8.
25. Xia W, Mruk DD, Lee WM, Cheng CY. Unraveling the molecular targets pertinent to junction restructuring events during spermatogenesis using the Adjuvin-induced germ cell depletion model. *J Endocrinol.* 2007; 192:563–83. [PubMed: 17332525]
26. Mruk DD, Silvestrini B, Cheng CY. Anchoring junctions as drug targets: Role in contraceptive development. *Pharmacol Rev.* 2008; 60:146–80. [PubMed: 18483144]
27. Mruk DD, Cheng CY. Delivering non-hormonal contraceptives to men: Advances and obstacles. *Trends Biotechnol.* 2008; 26:90–9. [PubMed: 18191256]
28. Cheng CY. Toxicants target cell junctions in the testis-insights from the indazole-carboxylic acid model. *Spermatogenesis.* 2014; 4:e981485.doi: 10.4161/21565562.2014.9814895 [PubMed: 26413399]
29. Vogl AW, Vaid KS, Guttman JA. The Sertoli cell cytoskeleton. *Adv Exp Med Biol.* 2008; 636:186–211. [PubMed: 19856169]
30. Qian X, Mruk DD, Cheng YH, Tang EI, Han D, Lee WM, et al. Actin binding proteins, spermatid transport and spermiation. *Semin Cell Dev Biol.* 2014; 30:75–85. [PubMed: 24735648]
31. Wolski KM, Perrault C, Tran-Son-Tay R, Cameron DF. Strength measurement of the Sertoli-spermatid junctional complex. *J Androl.* 2005; 26:354–9. [PubMed: 15867003]
32. Green KJ, Simpson CL. Desmosomes: New perspectives on a classic. *J Invest Dermatol.* 2007; 127:2499–515. [PubMed: 17934502]
33. Green KJ, Getsios S, Troyanovsky S, Godsel LM. Intercellular junction assembly, dynamics, and homeostasis. *Cold Spring Harb Perspect Biol.* 2010; 2:a000125. [PubMed: 20182611]
34. O'Donnell L, Nicholls PK, O'Bryan MK, McLachlan RI, Stanton PG. Spermiation: the process of sperm release. *Spermatogenesis.* 2011; 1:14–35. [PubMed: 21866274]

35. Cheng CY, Mruk DD. Regulation of spermiogenesis, spermiation and blood-testis barrier dynamics: Novel insights from studies on Eps8 and Arp3. *Biochem J.* 2011; 435:553–62. [PubMed: 21486226]
36. Cheng CY, Mruk DD. Actin binding proteins and spermiogenesis. Some unexpected findings. *Spermatogenesis.* 2011; 1:99–104. [PubMed: 22319657]
37. Gungor-Ordueri NE, Celik-Ozenci C, Cheng CY. Ezrin: A regulator of actin microfilaments in cell junctions of the rat testis. *Asian J Androl.* 2015; 17:653–8. DOI: 10.4103/1008-682X.146103 [PubMed: 25652626]
38. Lie PPY, Mruk DD, Lee WM, Cheng CY. Epidermal growth factor receptor pathway substrate 8 (Eps8) is a novel regulator of cell adhesion and the blood-testis barrier integrity in the seminiferous epithelium. *FASEB J.* 2009; 23:2555–67. [PubMed: 19293393]
39. Lie PPY, Chan AYN, Mruk DD, Lee WM, Cheng CY. Restricted Arp3 expression in the testis prevents blood-testis barrier disruption during junction restructuring at spermatogenesis. *Proc Natl Acad Sci USA.* 2010; 107:11411–6. [PubMed: 20534520]
40. Cheng CY, Lie PPY, Wong EWP, Mruk DD, Silvestrini B. Adjudin disrupts spermatogenesis via the action of some unlikely partners: Eps8, Arp2/3 complex, drebrin E, PAR6 and 14-3-3. *Spermatogenesis.* 2011; 1:291–7. [PubMed: 22332112]
41. Lie PPY, Mruk DD, Mok KW, Su L, Lee WM, Cheng CY, et al. Focal adhesion kinase-Tyr⁴⁰⁷ and -Tyr³⁹⁷ exhibit antagonistic effects on blood-testis barrier dynamics in the rat. *Proc Natl Acad Sci USA.* 2012; 109:12562–7. [PubMed: 22797892]
42. Wan HT, Mruk DD, Li SYT, Mok KW, Lee WM, Wong CKC, et al. p-FAK-Tyr³⁹⁷ regulates spermatid adhesion in the rat testis via its effects on F-actin organization at the ectoplasmic specialization. *Am J Physiol Endocrinol Metab.* 2013; 305:E687–99. [PubMed: 23880313]
43. Mohindra P, Sinha RN, Andrews RJ, Khuntia D. Non-cytotoxic radiosensitizers in brain radiotehrapy: Journey till the first decade of this millenium. *Curr Cancer Drug Targets.* 2012; 12:260–78. [PubMed: 22268387]
44. Nardini S. Non small cell lung cancer and chemotherapy. *Monaldi Arch Chest Dis.* 1994; 49:177–8. [PubMed: 8049706]
45. Costantini P, Jacotot E, Decaudin D, Kroemer G. Mitochondrion as a novel target of anticancer chemotherapy. *J Natl Cancer Inst.* 2000; 92:1042–53. [PubMed: 10880547]
46. Caputo A, Silvestrini B. Lonidamine, a new approach to cancer therapy. *Oncology.* 1984; 41(Suppl 1):2–6. [PubMed: 6371644]
47. Pedersen PL. Voltage dependent anion channels (VDACs): A brief introduction with a focus on the outer mitochondrial compartment's roles together with hexokinase-2 in the “Warburg effect” in cancer. *J Bioenerg Biomembr.* 2008; 40:123–6. [PubMed: 18780167]
48. Floridi A, Lehninger AL. Action of the antitumor and antispermatogenic agent lonidamine on electron transport in Ehrlich ascites tumor mitochondria. *Arch Biochem Biophys.* 1983; 26:73–83.
49. Floridi A, Paggi MG, Mercante ML, Silvestrini B, Caputo A, de Martino C. Lonidamine, a selective inhibitor of aerobic glycolysis of murine tumor cells. *J Natl Cacer Inst.* 1981; 66:497–9.
50. Floridi A, Maarcante ML, D'Atri S, Feriozzi R, Menichini R, Citro G, et al. Energy metabolism of normal and lonidamine-treated Sertoli cells of rats. *Exp Mol Pathol.* 1983; 38:137–47. [PubMed: 6832337]
51. De Martino C, Floridi A, Marcante ML, Malorni W, Scorza Barcellona P, Bellocci M, et al. Morphological, histochemical and biochemical studies on germ cell mitochondria of normal rats. *Cell Tissue Res.* 1979; 196:1–22. [PubMed: 421242]
52. Jutte NHPM, Grootegoed JA, Rommerts FFG, van der Molen HJ. Exogenous lactate is essential for metabolic activities in isolated rat spermatocytes and spermatids. *J Reprod Fertil.* 1981; 62:399–405. [PubMed: 7252921]
53. Boussouar F, Benahmed M. Lactate and energy metabolism in male germ cells. *Trends Endocrinol Metab.* 2004; 15:345–50. [PubMed: 15350607]
54. Robinson R, Fritz I. Metabolism of glucose by Sertoli cells in culture. *Biol Reproduct.* 1981; 24:1032–41.

55. Martins AD, Alves MG, Simoes VL, Dias TR, Rato L, Moreira PI, et al. Control of Sertoli cell metabolism by sex steroid hormones is mediated through modulation in glycolysis-related transporters and enzymes. *Cell Tissue Res.* 2013; 354(3):861–8. [PubMed: 24057877]
56. Oliveira PF, Martins AD, Moreira AC, Cheng CY, Alves MG. The Warburg effect revisited - lesson from the Sertoli cell. *Med Res Rev.* 2015; 35:126–51. [PubMed: 25043918]
57. Floridi A, De Martino C, Marcante ML, Apollonj C, Scorza BP, Silvestrini B. Morphological and biochemical modifications of rat germ cell mitochondria induced by new antispermatogenic compounds: Studies *in vivo* and *in vitro*. *Exp Mol Pathol.* 1981; 35:314–31. [PubMed: 7308411]
58. Grootegeod JA, Jansen R, Van der Molen HJ. The role of glucose, pyruvate and lactate in ATP production by rat spermatocytes and spermatids. *Biochim Biophys Acta.* 1984; 767:248–56. [PubMed: 6498180]
59. Gottschalk S, Anderson N, Hainz C, Eckhardt SG, Serkova NJ. Imatinib (STI571)-mediated changes in glucose metabolism in human leukemia BCR-ABL-positive cells. *Clin Cancer Res.* 2004; 10:6661–8. [PubMed: 15475456]
60. Elstrom RL, Bauer De, Buzzai M, Karnauskas R, Harris MH, Plas Dr, et al. Akt stimulates aerobic glycolysis in cancer cells. *Cancer Res.* 2004; 64:3892–9. [PubMed: 15172999]
61. Warburg O. On the origin of cancer cells. *Science.* 1956; 123:309–14. [PubMed: 13298683]
62. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. *J Gen Physiol.* 1927; 8:519–30. [PubMed: 19872213]
63. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nat Rev Cancer.* 2004; 4:891–9. [PubMed: 15516961]
64. Weber JE, Russell LD, Wong V, Peterson RN. Three dimensional reconstruction of a rat stage V Sertoli cell: II. Morphometry of Sertoli-Sertoli and Sertoli-germ cell relationships. *Am J Anat.* 1983; 167:163–79. [PubMed: 6613902]
65. Galardo MN, Regueira M, Riera MF, Pellizzari EH, Cigorraga SB, Meroni SB. Lactate regulates rat male germ cells function through reactive oxygen species. *PLoS One.* 2014; 9:e88024. [PubMed: 24498241]
66. Goldberg E, Eddy EM, Duan C, Odet F. LDHC: The ultimate testis-specific gene. *J Androl.* 2010; 31:86–94. [PubMed: 19875487]
67. Milane L, Duan Z, Amiji M. Therapeutic efficacy and safety of paclitaxel/Ionidamine loaded EGFR-targeted nanoparticles for the treatment of multi-drug resistant cancer. *PLoS One.* 2011; 6:e24075. [PubMed: 21931642]
68. Xie QR, Liu Y, Shao J, Yang J, Liu TI, Zhang T, et al. Male contraceptive adjuvin is a potential anti-cancer drug. *Biochem Pharmacol.* 2013; 85:345–55. [PubMed: 23178657]
69. Shao J, Liu T, Xie QR, Zhang T, Yu H, Wang B, et al. Adjuvin attenuates lipopolysaccharide (LPS)- and ischemia-induced microglial activation. *J Neuroimmunol.* 2013; 254:83–90. [PubMed: 23084372]
70. Mruk DD, Wong CH, Silvestrini B, Cheng CY. A male contraceptive targeting germ cell adhesion. *Nature Med.* 2006; 12:1323–8. [PubMed: 17072312]
71. Vane JR, Botting RM. The mechanism of action of aspirin. *Thromb Res.* 2003; 110:255–8. [PubMed: 14592543]
72. Czarny MJ, Nathan AS, Yeh RW, Mauri L. Adherence to dual antiplatelet therapy after coronary stenting: A systematic review. *Clin Cardiol.* 2014; 37:505–13. [PubMed: 24797884]
73. Schramm AM, Clowse ME. Aspirin for prevention of preeclampsia in lupus pregnancy. *Autoimmune Dis.* 2014; 2014:920467. [PubMed: 24778868]
74. Tash JS, Attardi B, Hild SA, Chakrasali R, Jakkaraj SR, Georg GI. A novel potent indazole carboxylic acid derivative blocks spermatogenesis and is contraceptive in rats after a single oral dose. *Biol Reprod.* 2008; 78:1127–38. [PubMed: 18218612]
75. Tash JS, Chakrasali R, Jakkaraj SR, Hughes J, Smith SK, Hornbaker K, et al. Gamendazole, an orally active indazole carboxylic acid male contraceptive agent, targets HSP90AB1 (HSP90BHETA) and EE1A1 (eEF1A), and stimulates IIIa transcription in rat Sertoli cells. *Biol Reprod.* 2008; 78:1139–52.
76. Heneka MT, Golenbock DT, Latz E. Innate immunity in Alzheimer's disease. *Nat Immunol.* 2015; 16(3):229–36. [PubMed: 25689443]

77. Li Y, Tan MS, Jiang T, Tan L. Microglia in Alzheimer's disease. *Biomed Res Int*. 2014; 2014:437483. [PubMed: 25197646]
78. Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, Hellings JA, et al. Maternal immune activation and abnormal brain development across CNS disorders. *Nature Rev Neurol*. 2014; 10(11):643–60. [PubMed: 25311587]
79. Perry VH, Holmes C. Microglial priming in neurodegenerative disease. *Nat Rev Neurol*. 2014; 10(4):217–24. [PubMed: 24638131]
80. Gonzalez-Scarano F, Baltuch G. Microglia as mediators of inflammatory and degenerative diseases. *Annu Rev Neurosci*. 1999; 22:219–40. [PubMed: 10202538]
81. Herrero MT, Estrada C, Maatouk L, Vyas S. Inflammation in Parkinson's disease: Role of glucocorticoids. *Front Neuroanat*. 2015; 9:32. [PubMed: 25883554]
82. Xiang Z, Haroutunian V, Ho L, Purohit D, Pasinetti GM. Microglia activation in the brain as inflammatory biomarker of Alzheimer's disease neuropathology and clinical dementia. *Dis Markers*. 2006; 22:95–102. [PubMed: 16410654]
83. Michell-Robinson MA, Touil H, Healy LM, Owen DR, Durafour BA, Bar-Or A, et al. Roles of microglia in brain development, tissue maintenance and repair. *Brain*. 2015; 138:1138–59. [PubMed: 25823474]
84. Bhatia M, Ramnath RD, Chevali L, Guglielmotti A. Treatment with bindarit, a blocker of MCP-1 synthesis, protects mice against acute pancreatitis. *Am J Physiol Gastrointest Liver Physiol*. 2005; 288:G1259–65. [PubMed: 15691869]
85. Saso L, Silvestrini B, Zwain I, Guglielmotti A, Luparini M, Cioli V, et al. Abnormal glycosylation of hemopexin in arthritic rats can be blocked by bindarit. *J Rheumatol*. 1992; 19:1859–67. [PubMed: 1284132]
86. Steiner JL, Davis JM, McClellan JL, Guglielmotti A, Murphy EA. Effects of the MCP-1 synthesis inhibitor bindarit on tumorigenesis and inflammatory markers in the C3(1)/SV49Taq mouse model of breast cancer. *Cytokine*. 2014; 66:60–8. [PubMed: 24548426]
87. Sironi M, Pozzi P, Polentarutti N, Benigni F, Coletta I, Guglielmotti A, et al. Inhibition of inflammatory cytokine production and protection against endotoxin toxicity by benzydamine. *Cytokine*. 1996; 8:710–6. [PubMed: 8932982]
88. Mosti L, Menozzi G, Schenone P, Cervo D, Esposito G, Marmo E. 4-Substituted 1-phenyl-1H-indazoles with analgesic, antiinflammatory, antipyretic and local anesthetic activities. *Farmaco*. 1990; 45:415–29. [PubMed: 2400516]
89. Badr MH, Elbayaa RY, El-Ashmawy IM. Design, synthesis and molecular docking study of some substituted 4,5-dihydro-2H-indazole derivatives as potential anti-inflammatory agents. *Med Chem*. 2013; 9:718–30. [PubMed: 23061568]
90. Rosati O, Curini M, Marcotullio MC, Macchiarulo A, Perfumi M, Mattioli L, et al. Synthesis, docking studies and anti-inflammatory activity of 4,5,6,7-tetrahydro-2H-indazole derivatives. *Bioorg Med Chem*. 2007; 15:3463–73. [PubMed: 17382550]
91. Mora E, Guglielmotti A, Biondi G, Sassone-Corsi P. Bindarit: a small anti-inflammatory molecule that modulates the NFkB pathway. *Cell Cycle*. 2012; 11:159–69. [PubMed: 22189654]
92. Stansley B, Post J, Hensley K. A comparative review of cell culture systems for the study of microglial biology in Alzheimer's disease. *J Neuroinflammation*. 2012; 9:115. [PubMed: 22651808]
93. Yang C, Chan PH, Chen J, Carlson E, Chen SF, Weinstein P, et al. Human copper-zinc superoxide dismutase transgenic mice are highly resistant to reperfusion injury after focal cerebral ischemia. *Stroke*. 1994; 25:165–70. [PubMed: 8266365]
94. Liu T, Zhang T, Yu H, Shen H, Xia W. Adjudin protects against cerebral ischemia reperfusion injury by inhibition of neuroinflammation and blood-brain barrier disruption. *J Neuroinflammation*. 2014; 11:107. [PubMed: 24927761]
95. Hudspeth AJ. Integrating the active process of hair cells with cochlear function. *Nat Rev Neurosci*. 2014; 15:600–14. [PubMed: 25096182]
96. Hudspeth AJ. How the ear's works work: Mechano-electrical transduction and amplification by hair cells. *CR Biol*. 2005; 328:155–62.

97. Mizutari K, Fujioka M, Hosoya M, Bramhall N, Okano HJ, Okano H, et al. Notch inhibition induces cochlear hair cell regeneration and recovery of hearing after acoustic trauma. *Neuron*. 2013; 77:58–69. [PubMed: 23312516]
98. Atkinson PJ, Huarcaya Najarro E, Sayyid ZN, Cheng AG. Sensory hair cell development and regeneration: Similarities and differences. *Development*. 2015; 142(9):1561–71. [PubMed: 25922522]
99. Quan Y, Xia L, Shao J, Yin S, Cheng CY, Xia W, et al. Adjudin protects rodent cochlear hair cells against gentamicin ototoxicity via the SIRT3-ROS pathway. *Scientific reports*. 2015; 5:8181. [PubMed: 25640330]
100. Someya S, Yu W, Hallows WC, Xu J, Vann JM, Leeuwenburgh C, et al. Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. *Cell*. 2010; 143(5):802–12. [PubMed: 21094524]
101. Pauls, HW., Laufer, R., Li, SW., Ng, G. Kinase inhibitors and method of treating cancer with same. US8980934. 2015.
102. Georg, GI., Tash, JS., Chakrasali, R., Jakkaraj, SR., Roby, K. Lonidamine analogues for fertility management. US8377958. 2013.
103. Morrison, JP., Stein, CA., Casebier, DS. Treatment of prostate cancer. US8791094. 2014.
104. Zhi, L. Selective androgen receptor modulators (SARMS) and uses thereof. US8748633. 2014.
105. Turnbull, PS., Cadilla, R., Larkin, A., Zhou, H., Stewart, EL., Stetson, K., McDougald, DL., Randhawa, AS., Ray, JA. Chemical compounds. US8673948. 2015.
106. Zhu, L., Li, Y. Methods for treating or preventing cancer and neurodegenerative diseases. US9056857. 2015.
107. Soon, HK., Hyoung, JK., Sun, YK., Chul, WC., Sung, BL., Heui, SP., Seung, HY., Hyo, SK., Dong, OS., Eok, P. Indole and indazole compounds as an inhibitor of cellular necrosis. US9000028. 2015.
108. Hood, J., Wallace, M., Sunil, KKC. Indazole inhibitors of the Wnt signal pathway and therapeutic uses thereof. US9090613. 2015.
109. Hood, J., Wallace, M., Sunil, KKC. Indazol inhibitors of the Wnt signaling pathway and therapeutics uses thereof. US8987298. 2015.
110. Gray, N., Zhou, W., Deng, X. Indazole compounds and their uses. US8987275. 2015.
111. Mitchell, CJ., Keeling, SE., Parr, NJ., Jones, PS., Hamblin, JN., Le, J. Oxazole substituted indazoles as PI3-kinase inhibitors. NZ596071. 2013.
112. Georg, IG., Tash, JS., Chakrasali, R., Jakkaraj, SR., Calvet, JP. Lonidamine analogues and treatment of polycystic kidney disease. US8362031. 2013.
113. Amobi, NMI., Smith, C. Male contraceptive. US8927579. 2015.
114. Ballard, TM., Groebke, ZK., Rinard, E., Ryckmans, T., Schaffhauser, H. Indol and indazol derivatives. WO2015044072. 2015.
115. Hanson, RN., Amiji, M., Weissig, V. Precision-guided nanoparticle systems for drug delivery. US9056129. 2015.

Fig. (1).
Structural formulae of adjudin, lonidamine and other related indazole-based compounds.

Fig. (2).

The biological effects of adjudin including its anti-fertility, anti-cancer, and neuroprotective and hearing-protective activities.

Table 1

Some Recent Patents (2013 - Present) on Ionidamine, Lonidamine, Adjudin and Related Indazole-Based Compounds Concerning their Medical Use*.

Patent Number (Date of Approval)	Patent Title	Inventor(s)	Summary of Invention	Reference
US8980934 (03/17/2015)	Kinase inhibitors and method of treating cancer with same	Pauls, H.W., Laufer, R., Li, S.W., Ng, G.	An indazole-based compound or its analogs that shares the structure of lonidamine, which acts as a protein kinase inhibitor for cancer treatment including the pharmaceutical compositions and methods of use	[101]
US8377958 (02/19/2013)	Lonidamine analogues for fertility management	Georg, I.G., Tash, J.S., Chakrasali, R., Jakkaraj, S.R., Roby, K.	Lonidamine analogues in particular H2-gamendazole can be used as a reversible or irreversible male contraceptive. These compounds also affect female ovarian function, and reducing female progesterone serum level	[102]
US8791094 (07/29/2014)	Treatment of prostate cancer	Morrison, J.P., Stein, C.A., Casebier, D.S.	Methods of making, pharmaceutical composition, and methods of using indazole-based compounds that are inhibitors of cytochrome C17 α -hydroxylase/C17,20-lyase to treat androgen receptor mediated diseases or conditions in particular prostate cancer	[103]
US8748633 (06/10/2014)	Selective androgen receptor modulators (SARMS) and uses thereof	Zhi, L.	Indazole-based compounds that bear similar structure as of lonidamine which also bind to androgen receptors and/or modulate activity of androgen receptors. Also included are methods of making and using such compounds	[104]
US8673948 (03/18/2014)	Chemical compounds	Turnbull, P.S., Cadilla, R., Larkin, A., Zhou, H., Stewart, E.L., Stetson, K., McDougald, D.L., Randhawa, A.S., Ray, J.A.	The methods for the making and use of non-steroidal indazole-based compounds as modulators of androgen receptor	[105]
US9056857 (06/16/2015)	Methods for treating or preventing cancer and neurodegenerative diseases	Zhu, L., Li, Y.	Methods of treating or preventing a neurodegenerative disease by treating a subject having a neurodegenerative disease based on an indazole-based compound	[106]
US9000028 (04/07/2015)	Indole and indazole compounds as an inhibitor of cellular necrosis	Kim, S.H., Kim, H.J., Koo, S.Y., Chung, C.W., Lee, S.B., Park, H.S., Yoon, S.H., Kwak, H.S., Seo, D.O., Park, E.	Using pharmaceutically acceptable salts or isomers of indole or indazole compounds for the prevention or treatment of cellular necrosis and necrosis-associated diseases by inhibiting cellular necrosis	[107]
US9090613 (07/28/2015) and US8987298 (03/24/2015)	Indazole inhibitors of the Wnt signal pathway and therapeutic uses thereof	Hood, J., Wallace, M., Sunil, K.K.C.	Use of an indazole compound or analogs of indazole compounds in the treatment of disorders, such as cancer, abnormal cellular proliferation, angiogenesis, Alzheimer's disease and osteoarthritis, characterized by (i) the activation of and/or (ii) modulation of cellular events of Wnt signal pathway, as well as genetic diseases and neurological conditions/disorders/diseases due to mutations in one or more components of Wnt signal pathway or dysregulation of the Wnt signal pathway	[108, 109]
US8987275 (03/24/2015)	Indazole compounds and their uses	Gray, N., Zhou, W., Deng, X.	Therapeutic use of indazole-based compounds including compositions comprising an effect amount of such a	[110]

Patent Number (Date of Approval)	Patent Title	Inventor(s)	Summary of Invention	Reference
			compound, and methods for treating and preventing multiple diseases	
NZ596071 (12/20/2013)	Oxazole substituted indazoles as PI3-kinase inhibitors	Mitchell, C.J., Keeling, S.E., Parr, N.J., Jones, P.S., Hamblin, J.N., Le, J.	Oxazole substituted indazole-based compounds served as PI3-kinase inhibitors for the treatment of diseases mediated by inappropriate PI3-kinase activity, such as asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF) and others	[111]
US8362031 (01/29/2013)	Lonidamine analogues and treatment of polycystic kidney disease	Georg, I.G., Tash, J.S., Chakrasali, R., Jakkaraj, S.R., Calvet, J.P.	Lonidamine derivatives are effective compounds to treat, inhibit, and/or prevent polycystic kidney disease (PKD). These compounds exert their effects by inhibiting ErbB2, Src, Raf-1, B-Raf, MEK, Cdk4 and NKCC1 or a combination of these biomolecules and/or kinases	[112]
US8927579 (01/06/2015)	Male contraceptive	Amobi, N.M.I., Smith, C.	A series of indazole-based compounds for reduction or prevention of the production of sperm and the transmission of viral agents transmitted in seminal fluid	[113]
WO2015044072 (04/02/2015)	Indol and indazol derivatives	Ballard, T.M., Groebke, Z.K., Pinard, E., Ryckmans, T., Schaffhauser, H.	The use of indole and indazole derivatives for the treatment of prophylaxis of Alzheimer's disease, cognitive impairment, schizophrenia, pain or sleep disorders	[114]
US9056129 (06/16/2015)	Precision-guided nanoparticle systems for drug delivery	Hanson, R.N., Amiji, M., Weissig, V.	A method preparing multifunctionalized nanoparticles (applicable to lonidamine or indazole-based compounds) so that drugs in the nanoparticles can be delivered to a desired cellular target to exert their intended effects for treatment of diseases such as neoplastic, infectious and chronic diseases	[115]

* This Table contains information on patents pertinent to lonidamine, adjuvin, and other indazole-based and related compounds, and also concerning their use for male contraception, and for treatment of cancers, inflammatory and chronic diseases, neurodegenerative diseases and kidney disorders. This information also supports recent findings on adjuvin (and lonidamine) in particular other potential health benefits besides adjuvin's male contraceptive activity as discussed in this review.