New Targets for Non-Hormonal Male Contraception

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“Non-Hormonal” Male Contraception

Definition: An approach to reversible male contraception that doesn’t primarily involve the administration of hormones (or hormone blockers)

Advantages:
- Shouldn’t impact testosterone dependent functions (e.g. sexual function, body composition)
- Possibly less stigma due to negative connotations surrounding hormones (esp. testosterone)

Disadvantages:
- Risk of non-reproductive toxicities (e.g. gossypol)
- Lack of long history of clinical use and familiarity to providers
Some Approaches to Non-Hormonal Contraception

- Sperm Motility (e.g. Catsper)
- Fertilization Blockers (e.g. Izumo)
- Ejaculation Inhibitors (e.g. phentolamine)
- Reversible Blockage of the Vas (e.g. RISUG)
- Non-hormonal Inhibition of Spermatogenesis (e.g. retinoic acid inhibitors)
- Blockade of the apical ectoplasmic specialization (e.g. Adjudin, gamendazole)
- Mechanical Methods (e.g. testicular ultrasound)
Which of These is Most Practical?

- Sperm Motility (e.g. Catsper)
- Fertilization Blockers (e.g. Izumo)
- Ejaculation Inhibitors (e.g. phentolamine)
- Mechanical Methods (e.g. RISUG/testicular ultrasound)
- Non-hormonal Inhibition of Spermatogenesis (e.g. retinoic acid receptor antagonists and retinoic acid biosynthesis inhibitors)
- Blockade of the apical ectoplasmic specialization (e.g. Adjudin, gamendazole)
Lonidamine Derivatives

A

Adjudin [1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid]

B

Lonidamine [1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid]

Gamendazole (GMZ)
**Table 1** A summary of the efficacy, toxicity, and sites of action for adjudin versus gamendazole and CDB-4022.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Adjudin</th>
<th>Gamendazole</th>
<th>CDB-4022</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. effective dosage for 100% efficacy</td>
<td>Two doses of 50 mg/kg b.w. (one dose per week)</td>
<td>Single dose of 6 mg/kg b.w.</td>
<td>Single dose of 2 mg/kg b.w. for rats Seven daily doses of 12.5 mg/kg b.w. for monkeys</td>
</tr>
<tr>
<td>Reversibility at min. effective dosage</td>
<td>100%</td>
<td>57%</td>
<td>0% in rats 100% in monkeys</td>
</tr>
<tr>
<td>Level of FSH, LH, and testosterone after treatment</td>
<td>FSH (−), LH (−), and testosterone (−)</td>
<td>FSH (NS †), and testosterone (−)</td>
<td>FSH (+), LH (−), and testosterone (−)</td>
</tr>
<tr>
<td>Lethal dosage</td>
<td>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)</td>
<td>200 mg/kg b.w.</td>
<td>Not determined</td>
</tr>
<tr>
<td>Sites of action</td>
<td>Activation of integrin/FAK/ERK signaling pathway</td>
<td>Partial inhibition of eEF1A1</td>
<td>Alteration of expression of apical ES proteins</td>
</tr>
<tr>
<td></td>
<td>Activation of integrin/RhoB/cofilin signaling pathway</td>
<td>Partial inhibition of HSP90</td>
<td>Activation of ERK–MAPK signaling pathway</td>
</tr>
<tr>
<td></td>
<td>Down-regulation of EPS8</td>
<td></td>
<td>Decreasing the ratio of membrane to soluble form SCF</td>
</tr>
<tr>
<td></td>
<td>Causing truncated localization of ARP3</td>
<td></td>
<td>Activation of pro-apoptotic factors</td>
</tr>
<tr>
<td>Effect(s)</td>
<td>Premature spermiation due to disruption of apical ES</td>
<td>Premature spermiation due to disruption of apical ES</td>
<td>Premature spermiation due to disruption of apical ES An increase in germ cell apoptosis</td>
</tr>
</tbody>
</table>

†, Stimulation; −, no change; min, minimal; NS, not significantly; SCF, stem cell factor.
Changes in Testis Histology After Single Oral Dose of H2-gamendazole

*6mg/kg

Slide Courtesy of Joseph Tash, University of Kansas
Rat Mating Study: Single 6 mg/kg Oral Dose of H2-Gamendazole

Results:
(6 rats per group):
- 100% infertility
- 60% reversibility
- FSH, LH and testosterone normal
- Mating behavior normal
- Normal # of normal conceptuses in recovered fertile males
- F1 offspring are normal
- Toxicology is promising

Current Hypothesis for Gamendazole and Adjudin Mechanism of Action

F-actin filaments which form a vital structural component of the apical ectoplasmic association (aES) are bundled by eEF1A-1.

H2-GMZ binds to eEF1A-1 causing unbundling and instability of the aES, causing premature release of spermatids.

Adjudin binds Testin an actin binding messenger protein.

Slide Courtesy of Joseph Tash, University of Kansas
Lonidamine Derivatives: Gamendazole and Adjudin

- Potent anti-spermatogenic agents in animal models
- Impair integrity of the apical ectoplasmic specialization
- Narrow therapeutic ranges
- Lonidamine has significant toxicities in human trials:
  - Muscular/testicular pain, emesis, liver damage
  - Adjudin and Gamendazole appear less toxic
  - Gamendazole may proceed to human studies
Type A Spermatogonia require retinoic acid for differentiation

Retinol-RBP from Sertoli cells

Differentiation (A→A1 transition)

STRA8
Retinoic Acid-RAR
Retinoic acid
Retinaldehyde
Retinol

Slide courtesy of Michael Griswold
Oral Administration of a Retinoic Acid Receptor Antagonist Reversibly Inhibits Spermatogenesis in Mice

Sanny S. W. Chung, Xiangyuan Wang, Shelby S. Roberts, Stephen M. Griffey, Peter R. Reczek, and Debra J. Wolgemuth

(Endocrinology 152: 2492–2502, 2011)
Inhibition of Testicular Retinoic Acid Biosynthesis

Type A Spermatogonia require retinoic acid for differentiation

Retinol-RBP from Sertoli cells

Differentiation (A→A1 transition)

ALDH1A2

Retinoic Acid-RAR

Retinoic acid

Retinaldehyde

→ Retinol

STRA6

STRA8

Slide courtesy of Michael Griswold
ALDH1A2 is Testes Specific

--The testicular isozyme of aldehyde dehydrogenase (ALDH1A2) is uniquely expressed in the testes

--In the mouse, expression of Aldh1a2 occurs prior to the onset of spermatogenesis

Hsu et al. Biochemica Biophysica Acta 2000; 289-93
Ejaculated Sperm Concentrations with BDAD treatment in Rabbits

Amory et al.

* p<0.05 c.f. baseline
Testicular Histology (100x)

Control Rabbit Testis
With normal spermatogenesis

Rabbit Testis after treatment with the BDAD WIN 18,446 (200 mg/kg orally, daily x 16 weeks)

Testicular Histology (400x)

Control Rabbit Testis with normal spermatogenesis

Rabbit Testis after treatment with the BDAD WIN 18,446 (200 mg/kg orally, daily x 16 weeks)
Testicular Retinoic Acid

Decrease in testicular retinoic acid occurs before drops in ejaculated sperm counts.

Residual testicular retinoic acid during treatment may represent retinoic acid synthesized in testicular interstitium by ALDH1A1, or contamination with blood.

* p<0.05 compared with baseline
** p<0.01 compared with baseline
WIN 18,446 reversibly suppresses spermatogenesis via inhibition of testicular retinoic acid biosynthesis by ALDH1A2

Novel, specific inhibitors of ALDH1A2, may have utility as male contraceptives
Future Work

- Use computational modeling with the solved crystal structure to optimize leads
- Identify compounds with acceptable pharmacokinetic characteristics
- Test impact of compounds on spermatogenesis and fertility in mice
Thank You

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