REVIEW ARTICLES

J. Sci. Soc. Thailand, 10 (1984) 197-206

MOLECULAR MECHANISM OF THE ANTIFERTILITY EFFECTS OF GOSSYPOL: A REVIEW

NONGNUJ TANPHAICHITR[a], M.R. JISNUSON SVASTI[b], AND PRASERT SOBHON[c]

a. Laboratory of Human Reproduction and Reporductive Biology, Harvard Medical School, 45 Shattuck St., Boston, MA 02115, U.S.A.

b. Department of Biochemistry and c. Department of Anatomy, Faculty of Science, Mahidol University, Rama VI Rd., Bangkok 10400, Thailand.

(Received 14 September 1984)

Abstract

Gossypol, a plant disesquiterpene, has been used as a male contraceptive in China. After oral administration, gossypol can be taken up by several tissues, including liver, muscle and testes. Force-feeding of rats with gossypol causes degeneration of the plasma membrane, mitochondria and axial filaments of epididymal sperm, nuclear vacuolation and mitochondrial swelling in spermatocytes and spermatids, and intercellular and intracellular vacuolation of Sertoli cells. Chemically, gossypol has been shown to react with macromolecules, such as proteins, and to form chelates with ferrous ions. It has direct effects on spermatozoa, decreasing the motility very markedly, and inhibiting the activities of several enzymes, including mitochondria-related enzymes. In addition, gossypol adversely affects the proliferation of primary cultures of Sertoli cells and transformed Sertoli cell tumor lines: in the case of the rat tumor cell line, protein synthesis was also shown to be inhibited. Similar to spermatozoa, mitochondrial transmembrane potential is diminished, as assessed by rhodamine-123 incorporation.

Introduction

Gossypol is a compound naturally found in cottonseed extract. Approximately 78,000 tons of gossypol is present in 25 million tons of cottonseed produced each year. The compound is toxic to nonruminant animals taking cottonfeed[1-3]. Gossypol became a

subject of worldwide interest in 1978 when a Chinese publication claimed that the compound had been used successfully as a male contraceptive for 10,000 men[4]. By ingesting gossypol at a much lower level than that causing toxicity, i.e., 20 mg daily for about 2 months, more than 99% of the men became oligospermic with less than 4 million spermatozoa per ml. of semen. Gossypol does not cause any drastic acute side effects, although about 1-7% of these people complained of fatigue, decrease in male libido, dizziness, gastrointestinal symptoms and hypokalemia[4]. The last side effect is of major concern since it leads to paralysis. However, this hypokalemic side effect may be a result of dietary habits, since it was found only in one village and could be alleviated by sufficient intake of potassium[3, 5]. The antifertility effect of gossypol is also reversible. Sperm density of greater than 10 million/ml resumes within one year after discontinuing the drug[4]. Moreover, gossypol is not mutagenic, as assessed by Ames test. Therefore, gossypol is attractive as a male contraceptive due to its effectiveness, minimal side effects, reversibility and inexpensive cost of preparation.

Chemical Properties

The structure of gossypol comprises two modified naphthalene rings connected at 2, 2 positions. Three main functional groups attached to the rings are 1) two aldehyde groups at the 8, 8′ positions; 2) six hydroxyl groups at the 1, 1′, 6, 6′, 7, 7′ positions; and 3) four alkyl groups: two methyl groups at the 3, 3′ positions and two isopropyl groups at the 5, 5′ positions. The aldehyde group can form a Schiff's base with an amine group of proteins or phospholipids[1]. Apart from an aldehyde form, gossypol can exist as other tautomers, i.e., the hemiacetal and the ketonoid (Fig. 1)[1]. The enolic proton of the ketonoid form ionizes with pK_a at 6.0[6]. The compound is yellow, soluble in organic solvents, and has a maximum absorption wavelength at 385 nm and a molar extinction coefficient of 18,000 in ethano[7]. When stored in organic solvents in the dark at -20° C, gossypol is relatively stable. By contrast, it shows severe degradation in aqueous solutions of pH 7.0 or higher[7, 8]

Metabolism of Gossypol in Animals

Gossypol's antifertility effect has been observed also in experimental animals such as rats, mice, hamsters, stumptail monkeys and cynomolgus monkeys. Conversely, rabbits, dogs, rhesus monkeys and ruminant are less responsive[5]. Studies on the metabolism of gossypol may give some clues to this preferential susceptibility in certain animals, as well as on the mechanism of the drug's action. However, earlier observations were directed toward defining the drug's toxic effects, rather than to discern the relationship between the drug's metabolic fate and its contraceptive properties. More than 70% of the(^{14}C) gossypol administered to rats, chickens and swine is excreted in the feces during the first three to four days[4, 9-11]. In rats, the $t_{1/2}$ for the retention of(^{14}C)

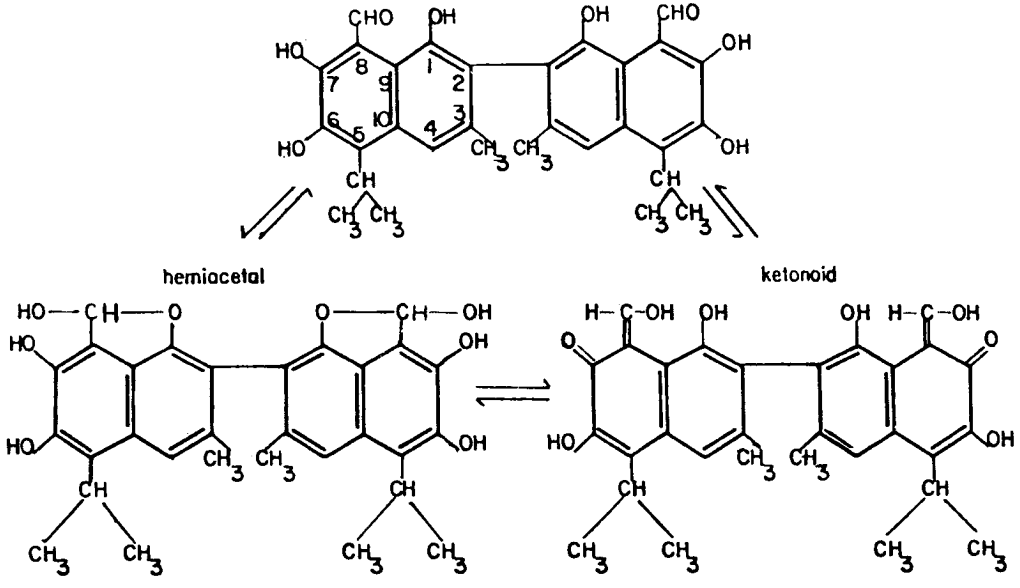

Fig. 1:
Structure and tautomerism of gossypol (1, 1, 6, 6, 7, 7, hexahydroxy-3, 3, dimethyl-5, 5, diisopropyl-2,2,, binaphthyl-8, 8 dialdehyde).

gossypol is 48 to 72 hr[4, 11]. The amount of the drug remaining after the first few days of administration is distributed in the blood and various organs, primarily in liver and muscle. The next highest concentrations of gossypol are found in intestine, kidney and spleen[4]. However, the testes can also take up gossypol, and this uptake only becomes maximal after 9 days, so that testes might conceivably be able to retain the drug better than other organs. In rats treated for two days, (^{14}C)gossypol present in the liver is distributed primarily in the microsomes, plasma membrane and mitochondria, whereas in testicular cells at 9 days, 50% of the drug is recovered in the mitochondria[12]

Gossypol exists in both free (ether soluble) and bound (ether insoluble) forms, which vary in tissue distribution from species to species. Thus, 24 hr after drug administration, rat liver and testis contain 13.8% and 35% respectively of the total gossypol in free form[11]. By contrast, equal amounts of free and bound gossypol are found in pig liver[13]. Presumably the ether-insoluble fraction represents gossypol bound by ionic, hydrophobic and covalent interactions to macromolecules such as proteins and phospholipids. Such interactions are likely to change the properties of the respective molecules. For example, the binding of gossypol to bovine serum albumin[14, 15] alters the protein's sedimentation, electrophoretic and fluorescence spectral properties[14]. Model studies indicate that the carbonyl groups of gossypol form Schiff's bases with amine groups of various free amino acids, thereby broadening the drug's major absorption peak at 392 nm to give two less intense peaks at 365 and 402 nm[16]. At pH 7.0-7.5, lysine and arginine react with gossypol at higher rates than do other amino acids. Covalent gossypol-protein complexes may form in sea-urchin sperm on exposure to the drug[17]. In addition, phospholipids containing amine groups, such as phosphatidylserine and phosphatidylethanolamine, may react with the drug to form gossypol-cephalin complexes, which have been found in hens fed with cottonseeds[18]. Besides its interaction with macromolecules, gossypol can form an ether-insoluble complex with ferrous ions[10].

Such gossypol conjugates may have significant pharmacological effects. Albumin can alleviate the adverse effects of gossypol on the proliferation of cultured Sertoli cells[19] and murine erythroleukemia cells[14]. The binding of the drug to the proenzyme pepsinogen inhibits its transformation to pepsin[20], and gossypol-bound enzymes such as glutathione-S-transferase and LDH-C_4 exhibit reduced activities[22].

Effects on Semen

Gossypol adversely affects spermatozoa *in vitro*. Mitochondria appear to be a primary site of gossypol action, as suggested by the decreased activities of mitochondrial-related enzymes such as sperm-specific LDH-C_4[23-27], malate dehydrogenase, glutathione-S-transferase, fumarase, pyruvate dehydrogenase and Mg^{++}-and Na^+-, K^+-dependent ATPases[28]. Fructose also is converted to CO_2 at a reduced rate in human sperm exposed to gossypol.[29, 30] Moreover, sea urchin sperm, incubated with gossypol, exhibit perforations

of the plasma membrane overlying the head and midpiece[31]. In addition, gossypol may affect the motility apparatus by interacting with dynein ATPase in rat[32] and sea urchin sperm[33]. Together, these various effects could reduce sperm motility markedly[29, 34-39] Other reported responses include an increased production of superoxide, a substance toxic to sperm[40], a reduction in acrosin activity[26, 41], and an inhibition of sperm capacitation and fertilizing capability[40-43] In addition to direct effects on spermatozoa, components of seminal plasma such as acidic and neutral proteases are affected by gossypol[44]. The proenzyme of acidic protease has been shown to form covalent conjugates with gossypol. Thus, the clearance of ejaculate in the vagina, a possible function of this protease, would be decreased. By contrast, inhibition of seminal plasma neutral protease causes a delay in semen liquefaction[44]

Effects on Differentiating Spermatogenic Cells

Daily administration of gossypol to rats (20 mg/kg body wt), by either gavage or subcutaneous injection, for three weeks causes morphological damage to spermatogenic cells and epididymal sperm[45, 46] The initial effects on motile, epididymal sperm include a degeneration of the plasma membrane and mitochondria, and a derangement and/or absence of one or more outer dense fibers[35, 45-48]. Longer periods of treatment cause more adverse effects on the cell, including: a) separation of the axial fibers, which subsequently bulge from the tail structure[45, 46, 49]; and b) damage to the sperm head in the form of fragmented and distorted acrosomal and nuclear membranes. By contrast to sperm, the Epithelial cells of the rat epididymides appear to be insensitive to the drug[35, 45].

Testicular damage is evident after three or more weeks of gossypol treatment (20-30 mg/kg body wt). The Chinese group has reported detrimental effects on pachytene spermatocytes and spermatids at steps 7 to 9 of spermiogenesis (Stage VII)[4, 12, 50], while Hoffer[46], and Oko and Hrudka[49] have noted an apparent sensitivity of steps-18 and 19 spermatids, which all occur at Stage VII of the cycle of the seminiferous epithelium. By contrast, Hadley *et al.* did not report any marked changes in germ cell number or their associations in the seminiferous epithelium. The observed deleterious effects on spermatogenic cells include nuclear vacuolation, swelling of the mitochondria, demembranation and, in maturing spermatids, detachment of the acrosome. The most injured organelles are probably mitochondria, which appear as distended structures after being isolated from the testes of rats receiving gossypol.[12] The greater susceptibility of germ cell mitochondria may be due to their enhanced ability of the cells or their mitochondria, themselves, to accumulate gossypol. Among rat spermatogenic cells, the mitochondria of spermatids at steps 18 and 19 are the first[46, 49, 51] to show a deleterious response to *in vivo* treatment with gossypol. Prolonged exposure to gossypol also causes extensive damage at other stages of spermiogenesis, including the formation of multinuleated cells, cytolysis, pycnosis, asynchronous cellular associations, and

premature exfoliation of germ cells into the tubular lumen. Thus, abnormal germ cells with morphologically deranged nuclei, mitochondria, acrosomes, and plasma membranes gradually accumulate in the epididymis. After chronic treatment with gossypol only Leydig cells, Sertoli cells and spermatogonia may remain in the testes[4, 50, 51]

Effects on Sertoli Cells

Sertoli cells are sustentacular cells, spanning the seminiferous tubules from the basal membrane to the lumen[52, 53] They adhere to each other, surrounding the tubules. The tight junctions between adjacent Sertoli cells form a blood-testis barrier that create a specific microenvironment for the developing spermatogenic cells in the adluminal compartment[52-54] In response to FSH, Sertoli cells secrete lactate[55, 56], ABP[57, 58], transferrin[59, 60], and plasminogen activator[61] into this compartment. In addition, retinol binding protein[62] and the seminiferous growth factor (SGF)[63, 64] are localized in Sertoli cells. These various substances function in mediating and/or facilitating spermatogenesis. Lactate is known to promote protein and RNA synthesis in round spermatids[55, 56]. Similarly, transferrin[59, 60], which is essential for cells to progress through the G2 phase of the mitotic cycle[65-66], probably enhances the proliferation and differentiation of spermatogenic cells.

Gossypol may also affect Sertoli cells directly. Thus exposure of primary cultures of rat Sertoli cells, tranformed cell lines originating from rat (TR-ST) and mouse (TM-4) Sertoli cell tumors to gossypol causes a decrease in cell proliferation[67, 68]. Total protein synthesis in TR-ST cells treated with gossypol also is inhibited[68]. In primary cultures of rat Sertoli cells, ABP is produced at a diminished rate[67]. In all kinds of Sertoli cells studied, intracellular vacuolation has been observed upon exposure to gossypol. These vacuoles have been shown by electron microscopic and cytochemical studies to be distended mitochondria, possessing cytochrome C oxidase[69]. Concomitant to this morphological damage, mitochondrial transmembrane potential is perturbed significantly in gossypol-treated TR-ST cells[68], as assessed by a decrease in the accumulation of rhodamine-123 into this organelle[70]. In addition to the impairment of mitochondrial structure and function, gossypol causes a change in Sertoli cell shape from epitheloid to stellate then to a rounded conformation[69]

Both *intracellular* and *intercellular* vacuolation also has been observed in 10% of Sertoli cells of rats administered *in vivo* with 10-30 mg gossypol/kg body wt for three weeks[71]. It is not clear whether *intracellular* vacuoles are phagosomes or other distended organelles, and their temporal formation needs to be studied more carefully. By contrast, *intercellular* vacuoles may reflect damage to the Sertoli cell tight junctions. Similar results were observed in immature guinea pigs fed gossypol[72]. However, the permeability of the blood testis barrier apparently is not altered in gossypol-administered rats[71]. In prepuberal guinea pigs fed gossypol, the nuclei of Sertoli cells also assume irregular

shapes and develop increasing areas of heterochromatin. Similar but less severe effects occur in mature, gossypol-treated guinea pigs[72].

Since the metabolism of spermatogenic cells is under the influence of Sertoli cells, the deleterious effects of gossypol on Sertoli cells may also result in the impairment of spermatogenic cells. To discern this possibility temporal changes on the structure and function(s) of Sertoli cells and spermatogenic cells caused by gossypol should be studied more carefully. In addition, with the availability of (^{14}C)gossypol, studies on the accumulation of the drug may give more definite information on the primary site of action of gossypol.

Concluding Remarks

Gossypol may have multiple sites of action on testicular cells. Its effects on Sertoli cells would result in a disruption of spermatogenesis. In addition, gossypol directly affects spermatozoa, allowing a possibility of its usage as a spermicide. However, before implementing gossypol as a male contraceptive or spermicide, it is necessary to define its molecular mechanism of action, its specificity on testicular cells, and the recovery of these cells after discontinuing the drug. The preferential effects of gossypol on Sertoli cells and spermatozoa should be understood in association with 1) the ability of these cells to accumulate the drug and/or 2) specific nature of their membranes, mitochondria or other organelles, rendering these cells more susceptible to gossypol.

Acknowledgments

The authors would like to express their appreciation for the support kindly provided by the Rockefeller Foundation.

References

1. Abou-Donia, M.B. (1976). *Residue Rev.* **61**, 125.
2. Sang, G.W. (1983). In: *Hormones in Normal and Abnormal Human Tissues* (K. Fotherby and S.B. Pal, eds.), Vol. III, p. 215, Walter de Gruyter & Co., New York.
3. Zatuchni, G.I. and Osborn, C.K. (1981) *PARFR Research Frontiers in Fertility Regulation* **1**, 1.
4. National Coordinating Group on Male Fertility (1978). *Chinese Med. J.* **4**, 417.
5. Prasad, M.R.N. and Diczfalusy, E. (1982). *Int. J. Androl. Suppl.* **5**, 53.
6. Reyes, J., Allen, J., Tanphaichitr, N., Bellve, A.R. and Benos, D.J. (1984). *J. Biol. Chem.* (in press).
7. Lee, C.Y.G. Moon, Y.S., Yuan, J.H. and Chen, A.F. (1982). *Mol. Cell Biochem.* **47**, 65.
8. Nomeir, A.A. and Abou-Donia, M.B. (1984) *J. American Oil Chem. Soc.* **59**, 546.
9. Abou-Donia, M.B. and Lyman, C.M. (1970). *Toxicol. Applied Pharmacol.* **17**, 4473.
10. Abou-Donia, M.B. and Dieckert, J.W. (1970). *Toxicol. Applied Pharmacol.* **31**, 32.
11. Abou-Donia, M.B., Lyman, C.M. and Dieckert, J.W. (1970). *Lipids* **5**, 938.

12. Xue. S., Liang, D., Fei, R., Chen, X., Ye, S., Liu, Y., Yanwan, W., You, M. and Guo, X. (1983). *Sci. Sin.* **26**, 614.
13. Smith, F.H. (1965). *J. Amer. Oil Chem. Soc.* **42**, 145.
14. Haspel, H.C.,Ren, Y.F., Watanabe, K.A., Sonenberg, M. and Corin, R.E. (1984). *J. Pharmacol. Therap.* (in press).
15. Lyman, C.M., Baliga, B.P. and Slay, M. (1959). *Arch. Biochem. Biophys.* **84**, 486.
16. Cater, C.M. and Lyman, C.M. (1969). *J. Amer. Oil Chem. Soc.* **46**, 649.
17. Sato, E., Matsuo, N., Burgos, M.H., Koide, S.S. and Segal, S.J. (1983). *Biol. Bull.* **165**, 516.
18. Worowick, C.L. and Grau, C.R. (1955). *Agric. Food Chem.* **3**, 706.
19. Zhuang, L.A., Phillips, D.M., Gunsalus, G.L., Bardin, C.W. and Mather, J.P. (1983). *J. Androl.* **4**, 336.
20. Tanksley, T.D., Neumann, H., Lyman, C.M., Pace, C.N. and Prescott, J.M. (1970). *J. Biol. Chem.* **245**, 6456.
21. Van der Jagt, D.L., Dean, V.L., Wilson, S.P. and Royer, R.E. (1983). *J. Biol. Chem.* **258**, 5689.
22. Li, S.S.L., Feldmann, R.J., Okabe, M. and Pan, Y.C.E. (1983). *J. Biol. Chem.* **258**, 7017.
23. Eliasson, R. and Virji, N. (1983). *Int. J. Androl.* **6**, 109.
24. Lee, C.Y. and Malling, H.Y. (1981). *Fed. Proc.* **40**, 718 (abstract).
25. Lee, C.Y. and Malling, H.Y. (1982). *Arch. Androl.* **9**, 34.
26. Tso, W.W. and Lee, C.S. (1982). *Arch. Androl.* **8**, 143.
27. Tso, W.W. and Lee, C.S. (1982). *Int. J. Androl.* **5**, 205.
28. Adeyamo, O., Chang, C.Y., Segal, S.J. and Koide, S.S. (1983). *Arch. Androl.* **9**, 343.
29. Poso, H., Wichmann, K., Janne, J. and Luukkainen, T. (1980). *Lancet* 1980, 885.
30. Wichmann, U., Kapyako, K., Sinervierta, R. and Janne, J. (1983). *J. Reprod. Fert.* **69**, 259.
31. Segal, S.J., Burgos, M. and Koide, S.S. (1983). *Biol. Bull.* **165**, 517.
32. Bacetti, B., Bracci, L., Burrini, A.G., Contorni, M., Mencarelli, C. and Pallini, V. (1984). *J. Submicro. Cytol.* **16**, 85.
33. Mohri, E., Matsuda, K., Koide, S.S. and Segal, S.J. (1982). *Biol. Bull.* **163**, 374 (abstract).
34. Burgos, M.H., Chang, C.Y., Nelson, L. and Segal, S.J. (1980). *Biol. Bull.* **159**, 467 (abstract).
35. Hadley, M.A. and Burgos, M.H. (1982). *Ann. NY. Acad. Sci.* **383**, 458.
36. Kalla, N.R. and Vasudev, M. (1980). *IRCS Med. Sci. Libr. Comp.* **8**, 375.
37. Kalla, N.R. and Vasudev, M. (1981). *Andrologia* **13**, 95.
38. Romrell, L.J., Bellve, A.R. and Fawcett, D.W. (1976). *Dev. Biol.* **49**, 119.
39. Waller, D.P., Zaneveld, L.J.D. and Fong, H.H.S. (1980). *Contraception* **22**, 183.
40. Coburn, M., Sinsheimer, P., Segal, S.J., Burgos, M., Troli, W. (1980). *Biol. Bull.* **159**, 468 (abstract).
41. Van der Ven, H.H., Bhattacharya, A.K., Karninski, J., Benior, Z., Bauer, L. and Zaneveld, L.J.D. (1981). *Biol. Reprod.* **24**, Suppl. 1, 38a.
42. Kennedy, W.P., Van der Ven, H.H., Strauss, J.W., Bhattacharya, A.K., Waller, D.P., Zaneveld, L.J.D. and Polakoski, K.L. (1983). *Biol. Reprod.* **29**, 999.
43. Shi, Q.X. and Friend, D. (1983). *Biol. Reprod.* **29**, 1027.
44. Vongsorasak, L. and Svasti, J. (1983). *Third Congress of the Federation of Asian and Oceanian Biochemists,* p. 96 (abstract).
45. Hoffer, A.P. (1982). *Arch. Androl.* **8**, 233.

46. Oko, R. and Hrudka, F. (1982). *Biol. Reprod.* **26**, 183.

47. Bozek, S.A., Jensen, D.R. and Tone, J.N. (1981). *Cell Tissue Res.* **219**, 659.

48. Nadakavukaren, M.J., Sorensen, R.H., Tone, J.M. (1979). *Cell Tissue Res.* **204**, 293.

49. Oko, R. and Hrudka, F. (1982). *Arch. Androl.* **9**, 39.

50. Xue, S., Zong, S., Shuyun, S., Yanwan, W., Liu, Y., Zhon, Z. and Ma, X. (1980). *Sci. Sin.* **9**, 915.

51. Hoffer, A.P. (1983). *Biol. Reprod.* **23**, 1007.

52. Fawcett, D.W. (1977). In: *Frontiers in Reproduction and Fertility Control: A Review of the Reproductive Sciences and Contraceptive Development* (R.O. Greep and M.A. Koblinsky, eds), p. 302, M.I.T. Press, Cambridge.

53. Russell, L. (1977). *Am. J. Anat.* **148**, 313.

54. Millette, C.F. and Bellve, A.R. (1977). *J. Cell Biol.* **74**, 86.

55. Jutte, N.H.P.M., Grootegoed, J.J.A., Rommerts, F.F.G. and Van der Molen, H.J. (1981). *J. Reprod. Fert.* **62**, 399.

56. Nakamura, M., Hino, A., Yasumasu, I. and Kato, J. (1981). *J. Biochem.* **89**, 1309.

57. Bardin, C.W., Musto, N., Gunsalus, G., Kotite, N., Cheng, S.L., Farrea, F. and Becker, R. (1981). *Ann. Rev. Physiol.* **43**, 189.

58. Hansson, V., Waddington, S.C., Naeso, O., Altramadal, A., French, F.S., Kohle, N., Nayfek, S.M. Ritzen, E.M. and Hagenas, L. (1975). In: *Hormonal Regulation of Spermatogenesis* (F.J. French, V. Hansson, E.M. Ritzen, S.N. Nayfek, eds), p. 323, Plenum Press, New York.

59. Skinner, M.K. and Griswold, M.D. (1980). *J. Biol. Chem.* **255**, 9523.

60. Skinner, M.K. and Griswold, M.D. (1982). *Biol. Reprod.* **27**, 211.

61. LaCroix, M., Parvinen, M. and Fritz, I.B. (1981). *Biol. Reprod.* **25**, 143.

62. Huggenvik, J. and Griswold, M.D. (1981). *J. Reprod. Fert.* **61**, 403.

63. Feig, L.A., Bellve, A.R., Erickson, N.H. and Klagsbrun, M. (1980). *Proc. Nat. Acad. Sci. U.S.A.* **77**, 4774.

64. Feig, L.A., Klagsbrun, M and Bellve, A.R. (1983). *J. Cell Biol.* **97**, 1435.

65. Bottenstein, I., Hayashi, I., Hurchinga, S., Masui, H., Mather, J., McClure, D.B., O'Hara, S., Rossino, A., Sato, G., Serrerq, G., Wulf, R. and Wu, R. (1979). *Methods Enzymol.* **58**, 94.

66. Rudland, P.S., Durbin, H., Clingan, D. and DeAsna, L.J. (1977). *Biochem. Biophys, Res. Commun.* **75**, 556.

67. Zhuang, L.A., Phillips, D.M., Gunsalus G.L., Bardin, C.W. and Mather, J.P. (1983). *J. Androl.* **4**, 336.

68. Tanphaichitr, N., Chen, L.B. and Bellve, A.R. (1984). *Biol. Reprod., (in press.)*

69. Robinson, J., Tanphaichitr, N., Bellve, A.R. and Karnovsky, M. (1984). manuscript in preparation.

70. Johnson, L.V., Walsh, M.L., Bockus, B.J. and Chen, L.B. (1981). *J. Cell Biol.* **88**, 526.

71. Hoffer, A.P. and Hinton, B.T. (1984). *Biol. Reprod.* **30**, 991.

72. Pelletier, R.M. and Friend, D.S. (1982). *J. Cell Biol.* **87**, *1519a.*

บทคัดย่อ

กอสสิพอล (gossypol) เป็นสาร disesquiterpene จากเมล็ดฝ้าย ที่ถูกนำมาใช้เป็นยาคุมกำเนิดเพศชายในสาธารณรัฐประชาชนจีน หลังจากให้สารกอสสิพอล โดยทางปากแก่สัตว์ทดลอง พบว่ามีการดูดซึมของสารชนิดนี้เข้าเนื้อเยื่อหลายแห่ง เช่น ตับ กล้ามเนื้อ รวมทั้งอัณฑะด้วย

ในหนู กอสสิพอลทำให้เกิดการสลายตัวของเยื่อหุ้มเซลล์ ไมโตคอนเดรียและใย axial filaments ของหางในเซลล์อสุจิ และเกิดช่องว่างในนิวเคลียส เกิดการบวมเต่งของไมโตคอนเดรียในเซลล์สืบพันธุ์ สเปอร์มาโตไซต์ (Spermatocytes) และสเปอร์มาติด (Spermatids) นอกจากนี้ ยังทำให้เกิดช่องว่างภายในและภายนอกเซลล์เซอโตลี (Sertoli Cells) อีกด้วย มีผู้พบว่าในเชิงเคมี กอสสิพอลทำปฏิกิริยากับชีวโมเลกุล เช่น โปรตีน และมันยังสามารถรวมตัวกับอิออน เฟอรัส (ferrous ions) ได้ด้วย เนื่องจากกลไกดังกล่าวทำให้กอสสิพอลมีผลกระทบต่อเซลล์อสุจิโดยลดการเคลื่อนไหว และห้ามการออกฤทธิ์ของเอนไซม์หลายตัว รวมทั้งเอนไซม์ในไมโตคอนเดรียด้วย นอกจากนี้กอสสิพอลยังลดอัตราการแบ่งตัวของเซลล์เซอโตลีปกติ และเป็นมะเร็งซึ่งถูกนำมาเลี้ยงในเครื่องเลี้ยงเนื้อเยื่อ ในกรณีของเซลล์เซอโตลีที่เป็นมะเร็ง ปรากฏว่ากอสสิพอลสามารถห้ามการสังเคราะห์โปรตีน และลดความต่างศักย์ข้ามเยื่อหุ้มไมโตคอนเดรีย (Transmembrane potential) เช่นเดียวกันกับที่เกิดในเซลล์อสุจิได้อีกด้วย ทั้งนี้อาจวัดได้โดยการตรวจปริมาณสารโรดามีน (Rhodamine 123) ที่สะสมภายในไมโตคอนเดรีย