記錄編號	6313
狀態	NC094FJU00105006
助教查核	
索書號	
學校名稱	輔仁大學
系所名稱	生命科學系
舊系所名稱	
學號	493546077
研究生(中)	黃郁蓁
研究生(英)	Yu-Chen Huang
論文名稱(中)	三氧化二砷抑制小白鼠精蟲之分化及活性
論文名稱(英)	Arsenic trioxide inhibits mouse spermatogenesis and sperm activity
其他題名	
指導教授(中)	曾婉芳 朱善德
指導教授(英)	Woan-Fang Tzeng Sin-Tak Chu
校內全文開放日期	不公開

校外全文開放日期	不公開
全文不開放理由	
電子全文送交國圖.	同意
國圖全文開放日期.	2009.07.07
檔案說明	電子全文
電子全文	01
學位類別	碩士
畢業學年度	94
出版年	
語文別	中文
關鍵字(中)	精蟲 三氧化二砷 荷爾蒙 活動力
關鍵字(英)	sperm arsenic trioxide hormone motility
摘要(中)	三氧化二砷 (As2O3) 為砒霜之主要成份，傳統醫學中利用少量三氧化二砷做為治療疾病的藥物。三氧化二砷可做為肝癌、大腸癌、乳癌等多種癌症的治療藥物。目前並不了解三氧化二砷做為治療藥物是否對病患的生殖系

	統造成影響，故本實驗探討動物施用三氧化二砷後其生殖系統是否受到傷害。利用雄性 ICR 小白鼠為實驗動物進行皮下注射不同濃度之三氧化二砷 (0, 0.15, 0.3, 1.5 及 3.0 mg/g 體重)，以施打 5 天休息 2 天為一周期。三周期後，觀察雄性小白鼠生殖系統的各項變化，以期進一步了解三氧化二砷對人類生殖系統可能產生的影響。由實驗結果得知，注射三氧化二砷後小鼠體重、攝食量、睪丸及副睪的器官重量之變化均無統計上差異。利用 Hematoxylin & Eosin 染色法觀察睪丸組織中精子細胞的發育，發現實驗組的精原母細胞 (spermatogonia) 數量減少，同時可觀察到精蟲之分化也受到抑制。此外，在肝組織切片中觀察到細胞核濃縮 (nuclear condensation) 的現象。透過電腦輔助精子分析系統 (computer-aided sperm analysis, CASA) 分析副睪的精子活動，顯示精子活動力下降，且精蟲數目會隨著注射三氧化二砷劑量的增加而減少。利用螢光顯微鏡以 SYBR14/PI 判別精子細胞存活率時，亦呈現精子存活率降低，同時測得副睪中精子細胞內抗氧化酵素 superoxide dismutase (SOD)， glutathione peroxidase (GP)，glutathione S-transferase (GST) 和 catalase 之活性皆低於控制組，顯示精子的抗氧化能力降低。另一方面利用酵素免疫法 (Enzyme-linked immunosorbent assay, ELISA) 檢測血清中荷爾蒙濃度，結果顯示濾泡刺激激素 (follicle stimulating hormone, FSH) 濃度無明顯變化，黃體刺激激素 (luteinizing hormone, LH) 及血清中的睪固酮 (testosterone) 濃度明顯降低，但睪丸組織中的睪固酮濃度卻無明顯變化。以 Real-Time PCR 分析三氧化二砷處理小白鼠睪丸組織中睪固酮生合成中主要酵素的 mRNA 變化，得知 cytochrome P450 side chain cleavage (P450scc)、3b-hydroxysteroid dehydrogenase (3b-HSD)、cytochrome P450 17-a hydroxylase/C17-20 lyase (Cyp17) 等三種酵素的基因表現均低於控制組。以 RT-PCR 分析與調控睪丸睪固酮濃度相關的基因表現，結果顯示 ABP (androgen binding protein)、AR (androgen receptor) 和 5?-reductase 基因表現均無顯著差異。利用免疫螢光染色觀察睪固酮在睪丸組織中分佈情形，顯示睪固酮累積在間質細胞。此一發現合理解釋睪固酮運往支持細胞之過程受到干擾，而影響生精作用，而所觀察到的各種現象是否會使小白鼠生殖功能受到影響，則有待進一步實驗探討。
摘要 (英)	Arsenic trioxide (As2O3) is an effective therapeutic agent for the treatment of acute promyelocytic leukemia (APL), liver cancer, breast cancer and colorectal cancer. The effect of arsenic trioxide on male reproductive system is unclear. Using male mice as an animal model, we attempt to illustrate the effects of arsenic trioxide on male reproductive system. The mice were administrated with arsenic trioxide in various dosages subcutaneously. The treatments were sustained for 5 days in a week and continued for 3 weeks. Our data showed that no difference in the food consumption, body weight, liver weight, testis weight and epididymis weight. Using histological observation, we showed the decrease of spermatogonia number and inhibition of spermatogenesis. The DNA condensation was found in liver cells. A significant decrease in sperm motility and viability were found in computer assised spermatozoa analysis (CASA) and SYBR14/PI staining assay. The activities of antioxidative enzyme including superoxide dismutase (SOD), glutathione peroxidase (GP), glutathione S-transferase (GST) and catalase (CAT), were significantly decreased in epididymal spermatozoa. Using enzyme-linked immunosorbent assay,

	we found the significant decrease of luteinizing hormone (LH) and testosterone levels in plasma but no difference in testicular testosterone level. Moreover, the mRNA of enzymes which involved in testicular testosterone synthesis, cytochrome P450 side chain cleavage (P450scc), 3?-hydroxysteroid dehydrogenase (3?-HSD) and cytochrome P450 17-? hydroxylase/C17-20 lyase (Cyp17) were significantly decreased. Using immunohistological observation, we showed the accumulation of testosterone in leydig cell. These results suggest that testosterone transportation should be interfered by arsenic trioxide treatment and then disrupt spermatogenesis. Further investigation should be continued to elucidate the fertility of male mice of arsenic trioxide treatment.
論文目次	

參考文獻

Aebi, H. 1984. Catalase in vitro. Methods Enzymol. 105:121-6. Afri, M., A.A. Frimer, and Y. Cohena. 2004. Active oxygen chemistry within the liposomal bilayer Part IV: Locating 2,7-dichlorofluorescein (DCF), 2,7-dichlorodihydrofluorescein (DCFH) and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) in the lipid bilayer. Chemistry and Physics of Lipids. 131:123-133. Bau, D.T., T.S. Wang, C.H. Chung, A.S. Wang, A.S. Wang, and K.Y. Jan. 2002. Oxidative DNA adducts and DNA-protein cross-links are the major DNA lesions induced by arsenite. Environ Health Perspect. 110 Suppl 5:753-6. Blanco-Rodriguez, J., and C. Martinez-Garcia. 1998. Apoptosis precedes detachment of germ cells from the seminiferous epithelium after hormone suppression by short-term oestradiol treatment of rats. Int J Androl. 21:109-15. Buaas, F.W., A.L. Kirsh, M. Sharma, D.J. McLean, J.L. Morris, M.D. Griswold, D.G. de Rooij, and R.E. Braun. 2004. Plzf is required in adult male germ cells for stem cell self-renewal. Nat Genet. 36:647-52. Carter, D.E., H.V. Aposhian, and A.J. Gandolfi. 2003. The metabolism of inorganic arsenic

oxides, gallium arsenide, and arsine: a toxicochemical review. Toxicol Appl Pharmacol. 193:309-34. Chitra, K.C., R. Sujatha, C. Latchoumycandane, and P.P. Mathur. 2001. Effect of lindane on antioxidant enzymes in epididymis and epididymal sperm of adult rats. Asian J Androl. 3:205-8. Cooke, H.J., and P.T.K. Saunders. 2002. Mouse models of male Iinfertility. Nature genetics. 3:790-801. Danzo, B.J., J.H. Black, and B.W. Bell. 1991. Analysis of the oligosaccharides on androgen-binding proteins: implications concerning their role in structure/function relationships. J Steroid Biochem Mol Biol. 40:821-31. Evens, A.W., M.S. Tallman, and R.B. Gartenhaus. 2004. The potential of arsenic trioxide in the treatment of malignant disease: past, present, and future. Leukemia research. 28:891-900. Florea, A.M., E.N. Yamoah, and E. Dopp. 2005. Intracellular calcium disturbances induced by arsenic and its methylated derivatives in relation to genomic damage and apoptosis induction. Environ Health Perspect. 113:659-64. Garner, D.L., and L.A. Johnson. 1995. Viability assessment of mammalian sperm using SYBR-14 and propidium iodide. Biol Reprod. 53:276-84. Habig, W.H., and W.B. Jakoby. 1981. Assay for differentiation of glutathione S-transferase. Methods enzymol. 77:398-405. Hammond, G.L., D.A. Underhill, H.M. Rykse, and C.L. Smith. 1989. The human sex hormone-binding globulin gene contains exons for androgen-binding protein and two other testicular messenger RNAs. Mol Endocrinol. 3:1869-76. Hayakawa, T., Y. Kobayashi, X. Cui, and S. Hirano. 2005. A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19. Arch Toxicol. 79:183-91. Hazelton, A.L.o. 1990. Two generation dietary reproduction study with arsenic acid in mice. Report HLA, Hazelton Laboratories America, Inc., Madison, WI:6120-6138. Heinlein, C.A., and C. Chang. 2002. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol. 16:2181-7. Hess, R.A., D. Bunick, K.H. Lee, J. Bahr, J.A. Taylor, K.S. Korach, and D.B. Lubahn. 1997. A role for oestrogens in the male reproductive system. Nature. 390:509-12. Hill, C.M., M.D. Anway, B.R. Zirkin, and T.R. Brown. 2004. Intratesticular Androgen Levels, Androgen Receptor Localization, and Androgen Receptor Expression in Adult Rat Sertoli Cells1. Biology of Reproduction 71:1348-1358. Hissin, P.J., and R. Hilf. 1976. A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem. 74:214-26. Huang, W.J., J.Y. Yeh, S.F. Kan, L.S. Chang, and P.S. Wang. 2003. Role of testicular interstitial macrophages in regulating testosterone release in hyperprolactinemia. J Cell Biochem. 88:766-73. Hughes, M.F. 2002. Arsenic toxicity and potential mechanisms of action. Toxicology Letters. 133:1-16. IARC. 1980. Some metals and metallic compounds, IARC Monographs on the Evaluation of the Carcinogenic Risk of chemicals to Man International Agency for Research on cancer. 23. Ishibashi, K., M. Kuwahara, Y. Gu, Y. Kageyama, A. Tohsaka, F. Suzuki, F. Marumo, and S. Sasaki. 1997. Cloning and functional expression of a new water channel abundantly expressed in the testis permeable to water, glycerol, and urea. J Biol Chem. 272:20782-6. Jana, K., S. Jana, and P.K. Samanta. 2006. Effects of chronic exposure to sodium arsenite on hypothalamo-pituitarytesticular activities in adult rats: possible an estrogenic mode

of action. Reproductive Biology and Endocrinology. Jing B.Y., Dai J.,Chalmers-Redman. R. M. E., Tatton W.G., Waman S. 1999. Arsenic trioxide selectively induces acute promyelocytic leukemia cell apoptosis via a hydrogen peroxide-dependent pathway.Blood. 94:2102-2111. Lin, C.J., M.H. Wu, Y.M. Hsueh, S.S. Sun, and A.L. Cheng. 2005. Tissue distribution of arsenic species in rabbits after single and multiple parenteral administration of arsenic trioxide: tissue accumulation and the reversibility after washout are tissue-selective. Cancer Chemother Pharmacol. 55:170-8. Ling, Y.H., J.D. Jiang, J.F. Holland, and R. Perez-Soler. 2002. Arsenic trioxide produces polymerization of microtubules and mitotic arrest before apoptosis in human tumor cell lines. Mol Pharmacol. 62:529-38. Liu, Z., J. Shen, J.M. Carbrey, R. Mukhopadhyay, P. Agre, and B.P. Rosen. 2002. Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP9. Proc Natl Acad Sci U S A. 99:6053-8. Luna, M.T., L. Rozo, and A. Roy. 2006. Pro-proliferative function of the long isoform of PML-RARa involved in acute promyelocytic leukemia. In Oncogene 1-12. Mannervik, B. 1985. Glutathione peroxidase. Methods Enzymol. 113:490-5. McCords, J.M., and I. Fridovich. 1969. Superoxide dismutase. Journal of biolooical chemistry. 244:6049-6055. Miller, W.H., Jr, H.M. Schipper, J.S. Lee, J. Singer, and S. Waxman. 2002a. Mechanisms of action of arsenic trioxide. Cancer research. 62:3893-3903. Miller, W.H., Jr., H.M. Schipper, J.S. Lee, J. Singer, and S. Waxman. 2002b. Mechanisms of Action of Arsenic Trioxide. Cancer research. 62:3893-3903. Munell, F., C.A. Suarez-Quian, D.M. Selva, O.M. Tirado, and J. Reventos. 2002. Androgen-binding protein and reproduction: where do we stand? J Androl. 23:598-609. Navas-Acien, A., A.R. Sharrett, E.K. Silbergeld, B.S. Schwartz, K.E. Nachman, T.A. Burke, and E. Guallar. 2005. Arsenic exposure and cardiovascular disease: a systematic review of the epidemiologic evidence. Am J Epidemiol. 162:1037-49. Naz, R.K., and P.B. Rajesh. 2004. Role of tyrosine phosphorylation in sperm capacitation / acrosome reaction. Reproductive Biology and Endocrinology 2(75):1-12. NRC. 1999. Arsenic in Drinking Water. Subcommittee on Arsenic in Drinking Water Report. National Research Council, National Academy of Sciences. Washington, DC: National Academy Press. O’ Shaughnessy, P.J., P. Baker, U. Sohnius, A.-M. Haavisto, H.M. Charlton, and I. Huhtaniemi. 2003. Fetal Development of Leydig Cell Activity in the Mouse Is Independent of Pituitary Gonadotroph Function. Endocrinology. 130(3):1141-1146. Pant, N., R. Kumar, R.C. Murthy, and S.P. Srivastava. 2001. Male reproductive effect of arsenic in mice. Biometals. 14:113-7. Pant, N., R. Murthy, and S. Srivastava. 2004. Male reproductive toxicity of sodiumarsenite in mice. Human & Experimental Toxicology 23:399-403. Pardridge, W.M. 1986. Serum bioavailability of sex steroid hormones. Clin Endocrinol Metab. 15:259-78. Park, J.W., Y.J. Choi, M.A. Jang, S.H. Baek, J.H. Lim, T. Passaniti, and T.K. Kwon. 2001. Arsenic trioxide induces G2/M growth arrest and apoptosis after caspase-3 activation and bcl-2 phosphorylation in promonocytic U937 cells. Biochem Biophys Res Commun. 286:726-34. Rahman, I., S.K. Biswas, and A. Kode. 2006. Oxidant and antioxidant balance in the airways and airway diseases. Eur J Pharmacol. 533:222-39. Rousselot, P., J. Larghero, B. Arnulf, J. Poupon, B. Royer, A. Tibi, I. Madelaine-

Chambrin, P. Cimerman, S. Chevret, O. Hermine, H. Dombret, J. Claude Brouet, and J. Paul Fermand. 2004. A clinical and pharmacological study of arsenic trioxide in advanced multiple myeloma patients. Leukemia. 18:1518-21. Sakuraia, T., M. Ochiaia, C. Kojimaa, T. Ohtaa, M.H. Sakuraib, N.O. Takadab, W. Quc, M.P. Waalkesc, S. Himenod, and K. Fujiwaraa. 2005. Preventive mechanism of cellular glutathione in monomethylarsonic acid-induced cytolethality. Toxicology and Applied Pharmacology. 206:54-65. Sanocka, D., and M. Kurpisz. 2004. Reactive oxygen species and sperm cells. Reproductive Biology and Endocrinology 2:1-7. Santti, H., L. Mikkonen, A. Anand, S. Hirvonen-Santti, J. Toppari, M. Panhuysen, F. Vauti, M. Perera, G. Corte, W. Wurst, O.A. J?nne, and J.J. Palvimo. 2005. Disruption of the murine PIASx gene results in reduced testis weight. Journal of Molecular Endocrinology. 24:645-654. Sarkar, M., G.R. Chaudhuri, A. Chattopadhyay, and N.M. Biswas. 2003. Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. Asian J Androl. 5:27-31. Sharpe, R.M., K. Donachie, and I. Cooper. 1988. Re-evaluation of the intratesticular level of testosterone required for quantitative maintenance of spermatogenesis in the rat. J Endocrinol. 117:19-26. Shen, B.Z.-X., G.-Q. Chen, J.-H. Ni, X.-S. Li, S.-M. Xiong, Q.-Y. Qiu, J. Zhu, W. Tang, G.-L. Sun, K.-Q. Yang, Y. Chen, L. Zhou, Z.-W. Fang, Y.-T. Wang, J. Ma, P. Zhang, T.-D. Zhang, S.-J. Chen, Z. Chen, and Z.-Y. Wang. 1997. Use of Arsenic Trioxide (As2O3) in the Treatment of Acute Promyelocytic Leukemia (APL): II. Clinical Efficacy and Pharmacokinetics in Relapsed Patients. Blood. 89(9):3354-3360. Shen, Y., Z.X. Shen, H. Yan, J. Chen, X.Y. Zeng, J.M. Li, X.S. Li, W. Wu, S.M. Xiong, W.L. Zhao, W. Tang, F. Wu, Y.F. Liu, C. Niu, Z.Y. Wang, S.J. Chen, and Z. Chen. 2001. Studies on the clinical efficacy and pharmacokinetics of low-dose arsenic trioxide in the treatment of relapsed acute promyelocytic leukemia: a comparison with conventional dosage. Leukemia. 15:735-41. Steinmaus, C., K. Carrigan, D. Kalman, R. Atallah, Y. Yuan, and A.H. Smith. 2005. Dietary Intake and Arsenic Methylation in a U.S. Population. Environmental Health Perspectives. 113(9):1153-1159. Takamiya, K., A. Yamamoto, K. Furukawa, J. Zhao, S. Fukumoto, S. Yamashiro, M. Okada, M. Haraguchi, M. Shin, M. Kishikawa, H. Shiku, S. Aizawa, and K. Furukawa. 1998. Complex gangliosides are essential in spermatogenesis of mice: possible roles in the transport of testosterone. Proc Natl Acad Sci U S A. 95:12147-52. Tarka-Leeds, D.K., J.D. Suarez, N.L. Roberts, J.M. Rogers, M.P. Hardy, and G.R. Klinefelter. 2003. Gestational exposure to ethane dimethanesulfonate permanently alters reproductive competence in the CD-1 mouse. Biol Reprod. 69:959-67. Urata, K., H. Narahara, Y. Tanaka, T. Egashira, F. Takayama, and I. Miyakawa. 2001. Effect of endotoxin-induced reactive oxygen species on sperm motility. Fertil Steril. 76:163-6. Vernet, P., R.J. Aitken, and J.R. Drevet. 2004. Antioxidant strategies in the epididymis. Molecular and Cellular Endocrinology. 216:31-39. W, R., Holdcraft, R. E, and Braun. 2004. Hormonal regulation of spermatogenesis. international journal of andrology. 27:335-342. W.C.L.Ford. 2004. Regulation of sperm function by reactive oxygen species. Human Reproduction Update 10(5):387-399. Yih, L.H., and T.C. Lee. 2003. Induction of C-anaphase and

	diplochromosome through dysregulation of spindle assembly checkpoint by sodium arsenite in human fibroblasts. Cancer Res. 63:6680-8. Yih, L.H., Y.Y. Tseng, Y.C. Wu, and T.C. Lee. 2006. Induction of centrosome amplification during arsenite-induced mitotic arrest in CGL-2 cells. Cancer Res. 66:2098-106. Zhang, F.-P., T. Pakarainen, M. Poutanen, J. Toppari, and I. Huhtaniemi. 2003. The low gonadotropin-independent constitutive production of testicular testosterone is sufficient to maintain spermatogenesis. PNAS. 100(23):13692-13697.
論文頁數	83
附註	
全文點閱次數	
資料建置時間	
轉檔日期	
全文檔存取記錄	
異動記錄	M admin Y2008.M7.D3 23:18 61.59.161.35