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中文計畫名稱：水溶性碳-60 衍生物對精子活動力的保護作用之研究

英文計畫名稱：The protective effects of water soluble Fullerenols on human sperm motility

一、中英文摘要

活細胞在有氧的狀況下會產生氧自由基，例如過氧化氫，過氧離子和氫氧基。為了抵抗這些氧自由基，細胞具有清除這些氧自由基的能力。許多的酵素系統有中和氧自由基的能力，例如 superoxide dismutase, catalase, 和 glutathione peroxidase/reductase。根據報告，氧自由基對精子具有毒性。精子由於富有不飽和多脂肪酸，因此很容易受到氧自由基引起的脂質過氧化反應所傷害。冷凍保存的精子，在解凍後會失去活動力，也可能與失去 superoxide dismutase 的活性有關。根據實驗證實水溶性含氫氧基碳 60 的衍生物 Fullerenols 具有清除由 Xanthine 和 Xanthine oxidase 產生之過氧自由基離子的能力。因此被認為是生物系統中一種很好的自由基清除劑。

由我們初步的研究發現水溶性碳 60 確實可以提高精子在 Xanthine+ Xanthine oxidase 的氧化環境中的存活率。因此如果進一步證明水溶性碳 60 對精子的活動力具有保護作用且對精子不造成傷害，則將可能用於兩方面用途。一、精子冷凍保存的處理。二、對於不良品質精子的處理，例如離心和清洗的步驟均會使精子更易受到氧自由基的傷害，若水溶性碳 60 可以在精子處理過程中具有清除氧自由基的作用，可以提高精子的存活率。

由於精子生存在精液中，是一種體外的環境系統。使用水溶性碳 60，可以很方便的發揮其自由基清除的功能，而不須擔心體內使用時的眾多安全問題。因此使用自由基清除劑（如水溶性碳 60）於精液處理上是最有希望的模式。

關鍵詞：精子，自由基，水溶性碳 60。

Abstract : Human spermatozoa are especially sensitive to lipid peroxidation induced by reactive oxygen species(ROS), because of high content of polyunsaturated fatty acids in spermatozoa. The cryopreserved sperm losing motility after thawing is associated with loss of superoxide dismutase activity in the post-thaw semen. Because Fullerenols, and SOD show excellent efficiency in eliminating superoxide radicals generated by xanthine and xanthine oxidase, thus this reveals the potential use of these compounds as novel potent free radical scavengers in biological system. We design a Xanthine + Xanthine oxidase free radical generating system to evaluate the potential scavenging roles of Fullerenols. Oxygen radicals generation is measured by the method of chemiluminescence. Luminol is employed as a chemiluminescence probe. Cryo-preserved spermatozoa containing C60 or SOD were used for sperm cryopreservation, in order to evaluate whether the ROS scavenger can exert the protect effect on post-thaw spermatozoa.

In our results, the protection of sperm motility by C60 can not significantly observed in this ROS generating system. But C60 in 100 $\mu\text{g/ml}$ can significantly slow the decreasing trend of other sperm motility parameters such as VAP, VSL, ALH and VCL, ROS generating system. ROS scavenger can not display any beneficial effect on the post-thawed sperm motility, even some detrimental effect were shown. We need further study about the sperm

function parameters with ROS impact, and search for other ROS scavengers.

Keywords: Spermatozoa, Reactive oxygen species, Fullerenols

二、計畫緣由與目的

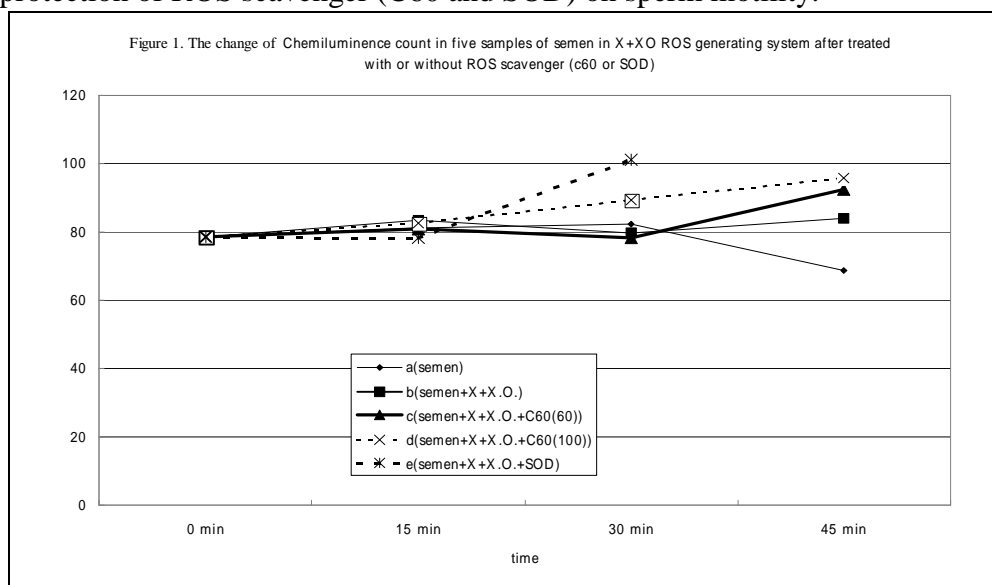
人類的精子由於富含 polyunsaturated fatty acid,因此對於 Reactive oxygen species(ROS) 引起的 lipid peroxidation,特別容易受到傷害。因此一般認為 ROS 對於精子是種有毒物質,而 catalase,superoxide dismutase 則可提供保護。精子生存於含氧的環境,因此容易產生各種 ROS,例如 hydrogen peroxide, superoxide anion and hydroxyl radical.根據現有的報告知道 1. 不良品質的精子 ROS 含量較高。2.精液中的白血球數目增加,ROS 的含量也增加。3. 離心處理精液會增加 ROS 的產生。4.ROS 的產生會影響精子的活動力,但可以是可逆的。5.精液的成分具有清除 ROS 的成分存在。6. 冷凍保存後的精子,在解凍後,活動力會變差,也和 ROS 的產生有關。

因此若能獲得一種良好且無毒性的自由基清除劑,以保護精子,將對男性不孕的精子處理和精子冷凍保存有莫大的助益。尤其在精子冷凍保存上,若能提高其活動力之保存,將可運用於人工生殖科技上,用來保存先天輸精管阻塞的病人的副睪精子,以避免重複的副睪取精手術。水溶性碳六十 (fullerenols; C60)是個由六十個碳原子見結合成的三度空間物質,在 sulfuric acid, citric acid 存在下進行水液酸性反應而得到的分子,其每個分子上含有 18-20 個氫氧基,整個 fullerenols 的結構以在 NMR spectrum, TGA-MS 等儀器分析下有很清楚的認識。最近在實驗室及生體外血液標本之 chemiluminescence 研究,更發現 fullerenols 有很強的自由基清除劑的效果。在我們的初步以 Xanthine + Xanthine oxidase free radical generating system 中測試 Fullerenols 保護精子活動力的實驗發現確實可以提高精子的活動力 因此將進一步以各種不同濃度的 C60 加入精子的生存環境中,觀察精子的活動力,並測量自由基的產生量是否有差別。

本實驗目的在探討 fullerenols 對於保護精子免受自由基傷害之能力,並希望應用於精液處理過程及使用於精子冷凍保存中作為自由基的清除劑。

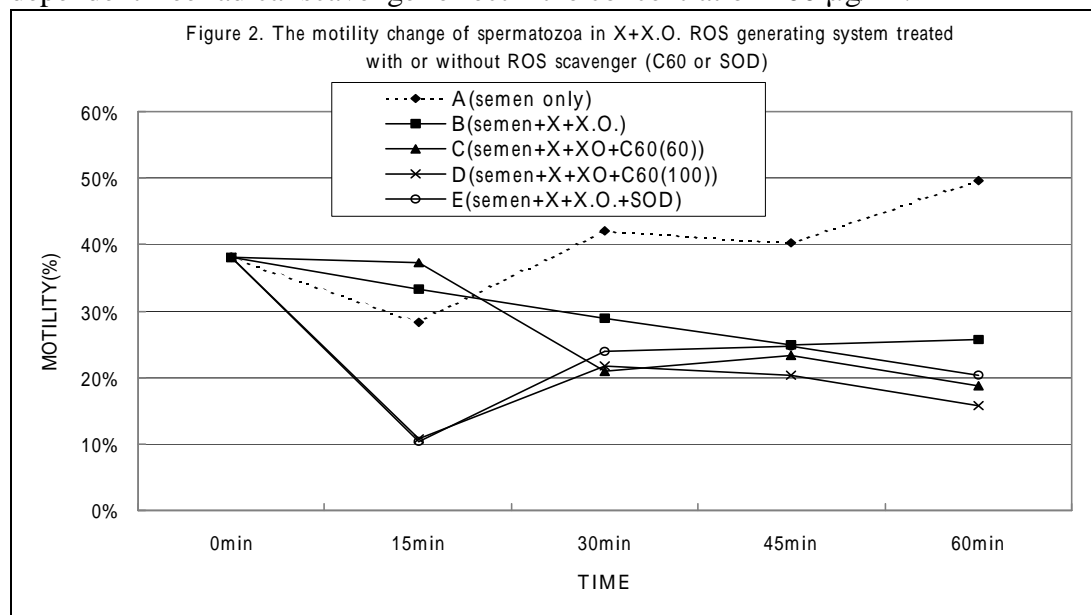
三、結果與討論

A. The protection of ROS scavenger (C60 and SOD) on sperm motility:



From the Figure 1 and 2, we can find when the sperm was preserved in ROS system (Xanthin and Xanthin oxidase), the C60 and SOD are not effective as a ROS scavenger. And also the protection of sperm motility by C60 and SOD can not significantly observed in this ROS generating system. But C60 in 100 $\mu\text{g/ml}$ can significantly slow down the decreasing

trend of other sperm motility parameters such as VAP, VSL, ALH and VCL, in the ROS generating system. Also in the preliminary study, C60 can show the most strong luminal-dependent free radical scavenger effect in the concentration 100 $\mu\text{g/ml}$.



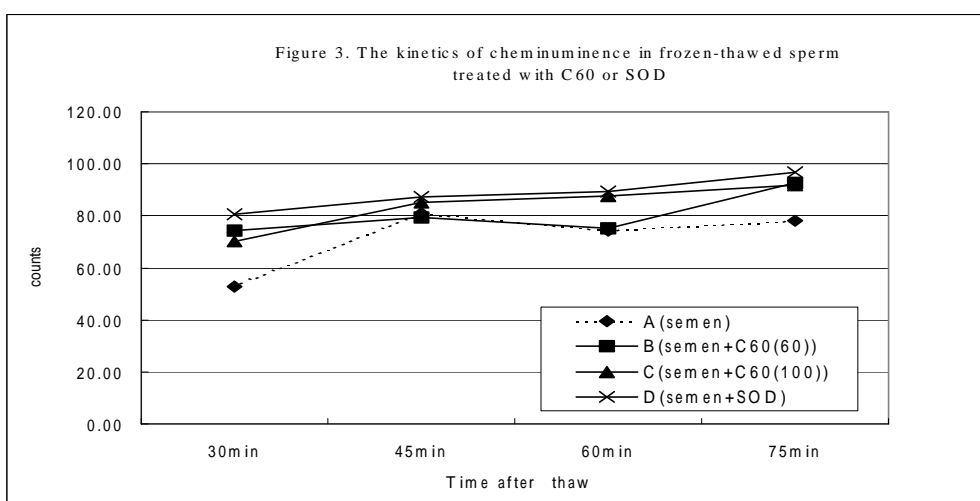
B. The protection effect of C60 on the cryo-thaw-damage to human sperm

Table 1. The post-thawed sperm motility after sperm cryopreserved with ROS scavenger (C60 and SOD). This table showed that sperm motility at 30 minutes after thawing decrease to about 30%. The ROS scavenger can not display any beneficial effect on the post-thawed sperm motility, even also some detrimental effect were shown.

Table 1.

Motility	Before	After thawing (min)							
	Cryo	30	45	60	75	90	105	120	135
semen	80%	35%	24%	32%	33%	24%	52%	30%	30%
semen+C60(60)	80%	13%	43%	33%	22%	27%	24%	23%	19%
semen+C60(100)	80%	9%	30%	25%	20%	10%	17%	34%	24%
semen+SOD	80%	4%	8%	8%	4%	12%	30%	16%	22%

From Figure 3, the luminol-dependent free radical generation at the post-thaw semen cryopreserved at medium with SOD or C60 are higher than sample A without adding ROS scavenger.



Discussion:

Although C60 show excellent efficiency in eliminating superoxide radicals, generated by Xanthine + Xanthine oxidase, there are several enzymes involved in neutralization of ROS, such as SOD, catalase, and the glutathione peroxidase/reductase system. The loss of motility from cryopreserved sperm after thawing is associated with loss of SOD in the post-thaw semen. In our study, we confirmed that C60 can efficiently eradicate the ROS in the X+XO ROS generating system,, and the optimal concentration of 60~100 μ g/ml can exert most strong scavenger effect. However, in biological system of semen, we can not show the same scavenger effect on spermatozoa. The motility of post-thawed sperm also can not be protected by C60 and SOD. But we got a better result in term of other sperm parameters , e.g. VAP, VSL, ALH and VCL, by C60 and SOD. The sperm function is a rather comprehensive category, including motility , acrosomal reaction , fertilization, and morphology. We need further study to know about the ROS impact on the sperm function, and to find out an optimum ROS scavenger.

四、計畫成果自評

本計畫最大困難為於一年內須收集眾多精液樣本, 但符合條件之精液個數不多,這是實驗進展最困難之處. 實驗之結果顯示 C60 是值得繼續研究其做為保護精子的自由基清除劑.

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