行政院國家科學委員會補助專題研究計畫成果報告

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（計畫名稱）以活體試驗探討 YC-1(一種能刺激 soluble guanylate cyclase 的藥物)對白兔陰莖勃起的影響

In vivo evaluation of the effects of YC-1, a soluable guaylate cyclase stimulator, on rabbit penile erection

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□國際合作研究計畫國外研究報告書一份

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In-vivo evaluation of the effects of YC-1, a soluable guaylate cyclase stimulator, on rabbit penile erection

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Abstract

Objective In the previous study, the effects of YC-1, a novel nitric oxide (NO) independent soluble guanylate cyclase (sGC) stimulator on isolated rabbit cavernous tissue had been investigated. The results showed YC-1 induced cavernous smooth muscle relaxation and enhanced the relaxant effects of nitroprusside. The mechanisms of the effects are through sGC stimulation and possibly phospodiesterase (PDE) inhibition. The current study was designed to perform in vivo evaluation of the effects of YC-1 on rabbit penile erection.

Materials and methods Mature male New Zealand White rabbits were used in this study. The right external carotid artery was cannulated for continuous blood pressure monitoring. Two 25-gauge butterfly needles were inserted into each corpus cavernosum for intracavernous injection of drugs and intracavernous pressure measurement. Increasing doses of YC-1, papaverine and prostaglandin E1 were injected into the corpus cavernosum and the change of intracavernous pressure was detected and recorded. The dose-response curve of each drug was then achieved. After pretreatment of a low dose of YC-1 for 20 minutes, the dose-response curves of papaverine and prostaglandin E1 were determined again.

Results 1) Intracavernous YC-1 injection increased the intracavernous pressue of the rabbits' penises in a dose-dependent manner but to only mild to moderate extent. 2)

Intracavernous PGE_1 injection increased the intracavernous pressue of the rabbits' penises mildly and the effects were enhanced by the pretreatment of YC-1. 3) Intracavernous papaverine injection significantly and remarkably increased the intracavernous pressue of the rabbits' penises in a dose-dependent manner. With the pretreatment of a low dose of YC-1, intracavernous pressure of the rabbits increased significantly more and the dose-response curve was shifted to the left.

Conclusions Intracavernous YC-1 injection increased the intracavernous pressue of the rabbits' penises mildly to moderately resulting in penile tumescence. Intracavernous papaverine injection increased the intracavernous pressue of the rabbits' penises significantly and remarkably resulting in fully rigid penile erection and its effect was enhanced by the pretreatment of YC-1.

Keywords: YC-1, soluble guanylate cyclase, phosphodiesterase

中文摘要

研究目的： YC-1 是國內自行研發出的一種不需經由 NO 作用即可刺激 soluble guanylate cyclase 使得細胞內 cGMP 增加的新藥物。我們在 88 年度國科會專題計畫"一種不經 NO 誘導即能刺激的藥物 YC-1 對白兔陰莖海綿體平滑肌的影響"中，發現 YC-1 能使 phenylephrine precontracted 白兔 cavernous strips 產生 dose-dependent 的放鬆，且其放鬆效果會被 sGC inhibitor ODQ 所抑制；YC-1 也能明顯地加強 sodium nitroprusside 的放鬆效果。進一步的實驗則証明 YC-1 會使白兔陰莖海綿體平滑肌內的 cGMP 明顯地增加。體外試驗的具體結果引發了我們進行活體試驗的動機。

研究對象與方法：我們將使用性成熟的雄性紐西蘭白兔（體重約 3 至 4 公斤）進行實驗。在麻醉後把一支 polyethylene catheter (PE60)放置於 external carotid artery 來監視血壓；將白兔陰莖皮膚切開後，把兩支 25 號蝴蝶針插入白兔陰莖海綿體內，一支用以進行海綿體內藥物注射，另一支則連結至 pressure transducer 用以監視海綿體內壓力變化。將不同劑量的 YC-1，papaverine，及 prostaglandin E1 注入白兔陰莖海綿體內後，監視其海綿體內壓力變化以得到劑量-反應曲線。再於先施以低劑量的 YC-1 20 分鐘後，再次求得 papaverine，及 prostaglandin E1 的劑量-反應曲線，並與先前結果相比較。實驗中將持續對白兔的血壓變化進行監視。

結果：一）海綿體內 YC-1 注射會使白兔陰莖海綿體內壓力發生 dose-dependent 方式的上昇，然其上昇效果並不顯著。二）海綿體內 PGE_1 注射同樣會使白兔陰莖海綿體內壓力發生 dose-dependent 方式的上昇，而其上昇效果也是並不顯

著。在少量 YC-1 的存在下，其上昇效果會被增強。。三）海綿體內 papaverine 注射會使白兔陰莖海綿體內壓力發生 dose-dependent 方式的明顯上昇，其最大反應壓力高過血管收縮壓而使白兔陰莖產生堅硬的勃起。

結論：YC-1 這種國內新合成的藥物注射入白兔陰莖海綿體時，會使陰莖海綿體內壓力上昇而發生陰莖膨脹的現象。該藥物也會加強 papaverine 引發白兔陰莖勃起的效果。因此本實驗更進一步証實其可能用以治療男性勃起功能障礙的潛力。

關鍵詞： YC-1， guanylate cyclase， phosphodiesterase

Introduction

In the previous study, we have investigated the effects of YC-1, a novel nitric oxide (NO) independent soluble guanylate cyclase (sGC) stimulator, on isolated rabbit cavernous tissue. The results are summarized as follows: 1) YC-1 relaxed the phenylephrine precontracted cavernous strips in a dose-dependent manner. 2) YC-1 significantly enhanced the relaxing effect of nitroprusside. 3) YC-1 increased the intracellular cGMP. 4) YC-1 potentiated both nitroprusside and IBMX in increasing the intracellular cGMP. The mechanisms of the effects are through sGC stimulation and possibly phospodiesterase (PDE) inhibition.

The current study was designed to perform in vivo evaluation of the effects of YC-1 on rabbit penile erection in order to determine its therapeutic potential for clinical application.

Materials and methods

Animal preparation

Mature male New Zealand White rabbits (3 to 4 kg) were used in this study. Each rabbit was sedated with an intramuscular injection of ketamine/xylazine (25mg ketamine, 6mg xylazine/kg) and placed in the supine position. Anesthesia was maintained by intravenous injection of pentobarbital sodium. After cannulating the trachea, the right external carotid artery was cannulated for continuous blood pressure monitoring via a pressure transducer on Gould RS3400 polygraphy. The skin overlying the penis was incised till tunica albuginea of corpus cavernousum was exposed, then two 25-gauge butterfly needles were inserted into each corpus cavernosum. Both needles and tubes were filled with heparinized saline. One was for intracavernous injection of drugs and the other for intracavernous pressure

measurement also via a pressure transducer on Gould RS3400 polygraphy.

Intracavernous Drugs Administration

In each groups of rabbits (n = 4-8), increasing doses of YC-1 (o.25-1.5 mg/kg), papaverine (0.25-2.0 mg/kg) and prostaglandin E1 (0.25-2 ug/kg) were injected into the rabbits' corpus cavernosum in a volume less than 0.2ml, followed by flushing of normal saline 0.2ml. The change of intracavernous pressure following the administration of drugs was detected and recorded. Dose-response curves of the drugs were achieved from injections in a cumulative manner and at 3-15minute intervals.

After 20-minute intracavernous pretreatment of a low dose of YC-1, the dose-response curves of intracavernous administration of papaverine and prostaglandin E1, were determined again.

Specific drugs are chosen because papaverine and prostaglandin E1 are commonly used intracavernous therapeutic agents.

Drugs and Chemical Reagents

The following drugs used in the study were purchased from Sigma Chemicals (St. Louis, Mo., USA): papaverine and prostaglandin E1. YC-1 was self-supplied.

Statistics

All values presented in the results represent the mean ± standard error of the mean of the number of animals used.

Student's t test was used for paired or unpaired observations to determine the significance of the difference between the mean values. A probability level of < 0.05 was required for statistical significance.

Results

Figure 1 shows intracavernous pressue of the rabbits increased in response to intracavernous YC-1 injection in a dose-dependent manner. Yet, the maximal increase of intracavernous pressure was only about 40 mm Hg and the rabbits' penises displayed tumescence of inadequate rigidity. Blood pressure of the rabbits dropped mildly but significantly after the injected YC-1 accumulated into larger doses.

Figure 2 shows intracavernous pressue of the rabbits increased in response to intracavernous PGE_1 injection also in a dose-dependent manner. The maximal increase of intracavernous pressure was even less than the effect of YC-1 injection and the rabbits' penises displayed again tumescence of inadequate rigidity. With the

pretreatment of a low dose of YC-1, intracavernous pressure of the rabbits increased significantly more but with limited extent.

Figure 3 shows intracavernous pressue of the rabbits increased significantly and remarkably in response to intracavernous papaverine injection in a dose-dependent manner. The maximal increase of intracavernous pressure was over the systolic blood pressure and the rabbits' penises reached fully rigid penile erection. With the pretreatment of a low dose of YC-1, intracavernous pressure of the rabbits increased significantly more and the dose-response curve was shifted to the left. Compared with the effects of YC-1 injection, papaverine injection exerted much greater effects in increasing the intracavernous pressure of the rabbits' penises.

Discussion

Penile erection happens when smooth muscle of the penile arteries and sinusoids relax resulting in increase of penile blood flow. There is increasing evidence showin that NO released from the non-adrenergic non-cholinergic neurons and from the endothelium plays an important role in mediating penile erection. By stimulating the sGC and in turn converting GTP to cyclic GMP (cGMP), NO triggers cavernous smooth muscle relaxation thus inducing penile erection. cGMP was hydrolyzed by PDE and phosphodiesterase inhibition (PDEI) will lead to accumulation of cGMP. Theoretically, drugs that enhance the NO-SGC-cGMP-PDEI axis will be beneficial for penile erection.

We had proved previously that YC-1, a novel nitric oxide (NO) independent soluble guanylate cyclase (sGC) stimulator, significantly induced cavernous smooth muscle relaxation and enhanced the relaxant effects of nitroprusside. The current study was designed to perform in vivo evaluation of the effects of YC-1 on rabbit penile erection in order to determine if it could be applied for the treatment of male erectile dysfunction.Before the introduction of sildenafil, intracavernous injection with single or multiple vasoactive agents has been the preferred first line treatment of male erectile dysfunction for a couple of decades. Though high success rates have been reported, there are 10 to 25 patients of patient who will not respond to the injection therapy satifactorily. Novel agents enhancing the NO-SGC-cGMP-PDEI axis may be of benefit to those none or poor responders.

Although the in vitro study showed YC-1 relaxed the precontracted rabbit corporal strips to remarkable exent, the results of the current study demonstrated intracavernou injection of YC-1 increased the intracavernous pressure of the rabbit penises only to mild to moderate extent and the rabbits penises displayed tumescence

with inadquate rigidity. The reasons of the descrepancy between the in vitro and in vivo studies are not clear at the present time, and may not be fully explaned till the pharmacodynamics of YC-1 distribution in the corpus cavernosum is thoroughly investigated. Since the blood pressure of the rabbit was not lowered substantially when even high doses of YC-1 was injected intracavernously, YC-1 is safe for in vivo animal experiments.

In contrast to the well known erection inducing effect of PGE_1 in human, intracavernous injection of PGE_1 increased the intracavernous pressure of the rabbits only to mild extent. Similar results had been reported by Y M. Lin and J S-N Lin. The reason is likely due to species difference. On the other hand, papaverine was shown in the current study to be a very potent erection inducing agent for rabbit penises when administered via intracavernous injection and its effect was significantly enhanced by the pretreatment of YC-1. In regard to this finding, though YC-1 might not be useful as a single intracavernou phamacotherapeutic agent for the treatment of male erectile dysfunction, it still has the potential to be used in combination therapies serving to treatment those possible none or poor responders.

In conclusion, intracavernous YC-1 injection increased the intracavernous pressue of the rabbits' penises mildly to moderately resulting in penile tumescence. Intracavernous papaverine injection increased the intracavernous pressue of the rabbits' penises significantly and remarkably resulting in fully rigid penile erection and its effect was enhanced by the pretreatment of YC-1.

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intracavernous pressure by berberine in rabbit. Br J Pharmacol 1998; 125: 1677-1684.

Legends

Fig.1. Intracavernous pressure and blood pressure change in response to intracavernous injection of YC-1 in a cumulative way. Pressure is presented as mmHg. Each bar is the mean ± SEM of 8 individual rabbits. * $p < 0.05$ compared with control.

Fig.2. Intracavernous pressure in response to intracavernous injection of PGE_1 with and without treatment of a low dose of YC-1 in a cumulative way. Pressure is presented as mmHg. Each bar is the mean ± SEM of 5 individual rabbits. * $p < 0.05$ compared with no pretreatment of YC-1.

Fig.3. Intracavernous pressure in response to intracavernous injection of papaverine with and without treatment of a low dose of YC-1 in a cumulative way. Pressure is presented as mmHg. Each bar is the mean ± SEM of 5-6 individual rabbits. * $p < 0.05$ compared with no pretreatment of YC-1.

計畫結果自評

一）研究內容與原計畫相符
二）已達成預期目標
三）適合學術期刊發表

Fig. 1 INTRACAVERNOUS PRESSURE AND BLOOD PRESSURE CHANGE IN RESPONSE TO YC-1 INJECTION

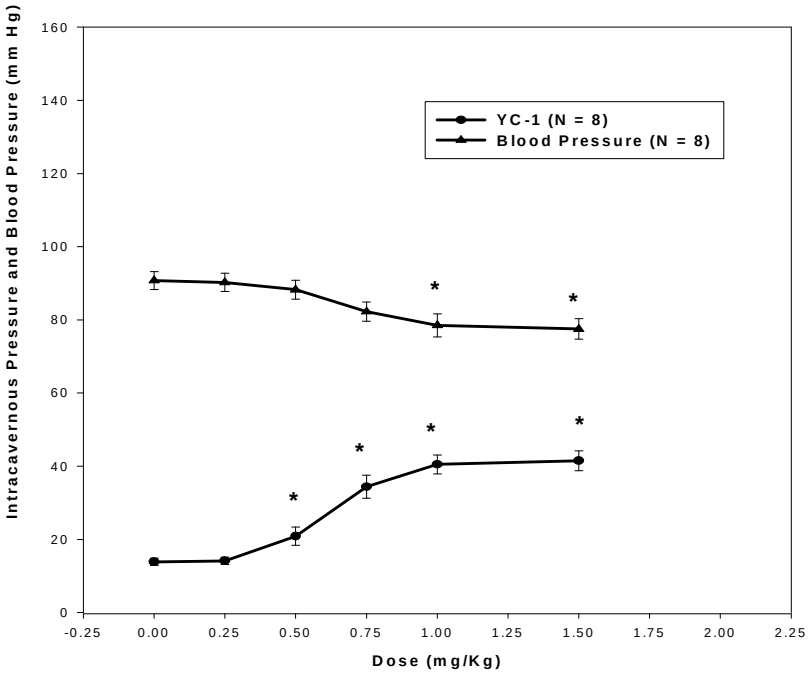

Fig. 2 INTRACAVERNOUS PRESSURE CHANGE IN RESPONSE TO INTRACAVERNOUS PGE1 INJECTION

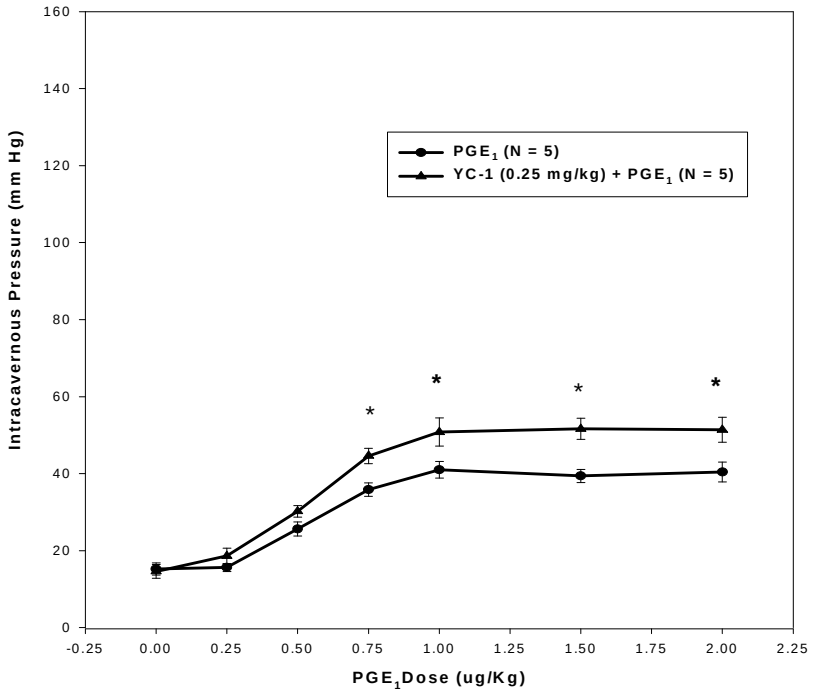

Fig. 3 INTRACAVERNOUS PRESSURE CHANGE IN RESPONSE TO INTRACAVERNOUS PAPAVERINE INJECTION