APPENDIX

ประกาศบัณฑิตวิทยาลัย มหาวิทยาลัยนเรศวร

เรื่อง อนุมัติให้นิสิตระดับปริญญาโทดำเนินการทำวิจัย

ครั้งที่ 219/2555

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บัณฑิตวิทยาลัยอนุมัติให้ นางสาวนารีลักษณ์ ตั้งศรีศักดา รหัสประจำตัว 53060858 นิสิตระดับปริญญาโท หลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต สาขาวิชากายวิภาคศาสตร์ ดำเนินการทำวิจัยตามโครงร่างวิทยานิพนธ์ที่เสนอ

เรื่อง **ภาษาไทย** "ผลกระทบของการได้รับสารเสพติดเมทแอมเฟตามีนต่อกระบวนการสร้างเซลล์อสุจิในหนูแรทเพศผู้"

ภาษาอังกฤษ "EFFECTS OF METHAMPHETAMINE DEPENDENCE ON SPERMATOGENESIS IN MALE RAT TESTIS"

โดยมี รองศาสตราจารย์ ดร.เสมอ ถาน้อย เป็นประธานที่ปรึกษาวิทยานิพนธ์

จึงประกาศมาให้ทราบโดยทั่วกัน

ประกาศ ณ วันที่ 19 พฤศจิกายน พ.ศ.2555

(ผู้ช่วยศาสตราจารย์ ดร.คนึงนิจ ภู่พัฒน์วิบูลย์)

คณบดีบัณฑิตวิทยาลัย มหาวิทยาลัยนเรศวร

เอกสารรับรองโครงการ
คณะกรรมการกำกับการเลี้ยงและการใช้สัตว์ มหาวิทยาลัยนเรศวร

ชื่อโครงการ	ผลกระทบของการได้รับสารเสพติดเมทแอมเฟตามีนต่อกระบวนการสร้างเซลล์อสุจิในหนูแรทเพศผู้ Effects of Methamphetamine dependence on spermatogenesis in male rat testis
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วันที่รับรอง	วันที่ 26 มีนาคม 2555

ขอรับรองว่าโครงการวิจัยนี้ ได้รับการรับรองด้านจรรยาบรรณการใช้สัตว์ จากคณะกรรมการกำกับการเลี้ยงและการใช้สัตว์ มหาวิทยาลัยนเรศวร

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(รองศาสตราจารย์ ดร.รัตติมา จีนาพงษา)

ประธานคณะกรรมการกำกับการเลี้ยงและการใช้สัตว์ มหาวิทยาลัยนเรศวร

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Effect of Methamphetamine Administration on Alteration of Sperm Quality

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Abstract

Methamphetamine (METH) is an illicit psychostimulant drug. It is known as neurotoxicant because it can degenerate neurons in the brain and induce euphoria or psychosis. Moreover, METH can cause reproductive toxicity as it can induce apoptosis in seminiferous tubule and changes of plasma testosterone concentration. The present study was carried out to investigate the effect of METH that imitates human addiction on rat sperm quality. The percentages of normal sperm motility and normal sperm morphology were significantly decreased in animals treated with METH when compared with control group, especially in escalating dose (ED) METH and ED-binge METH groups. However, sperm concentration in ED METH group was numerically decreased when compared with the control group but it just failed to reach significant. The results of this study indicate that METH can induce alteration of sperm quality. These changes of sperm quality may relate to fertilizing abilities of sperm.

Keywords methamphetamine, sperm quality, sperm concentration, sperm morphology, sperm motility

Background

Methamphetamine (METH) is an illicit drug which has a primary action in the brain. METH is synthesized from ephedrine and pseudoephedrine (1). It can uptake into body by smoking, injection, snorting and ingestion (2). Several studies have reported that METH is a neurotoxicant (1). METH can induce euphoria, alertness (3), anxiety, hallucination and psychosis (4). In addition, METH also has effects in reproductive system. It can induce apoptosis in seminiferous tubules (6, 7) and change serum testosterone concentration in male mice (5). Moreover, male rats treated with METH indicate a decrease of cell proliferation (6).

Furthermore, METH that imitates human addiction has been reported to cause degeneration of pyramidal neuron and interneuron in the neocortex and limbic system in male rats. These results may be related to the cognitive alteration in METH users (8). However, study of METH addiction with acute binge and escalating dose has not been reported in reproductive system. Therefore, the objective of this study was to investigate the effect of METH addiction on the alteration of sperm quality in male rat.

Materials and Methods

D-methamphetamine hydrochloride (Lipomed AG, Arlesheim, Switzerland) with the consent from the Ministry of Public health was used in this experiment.

Twenty four male Sprague-dawley rats (National Animal Center, Salaya, Nakorn Pathom, Thailand) weighing between 250-300 g were housed one per cage at 24 ± 1 C and dark/light cycle 12 hours. The protocol for this study was approved by the Animal Research Committee of Naresuan University, Thailand. After that, animals were divided into 4 groups. Each group contains 6 animals. All animals were treated intraperitoneally (i.p.) either saline or METH. In control group, rats were treated with 0.9% saline 3 times per day for 14 days and 4 times at 2 hours intervals on day 15. In acute binge (AB) METH group, rats were treated with 0.9% saline 3 times per day for 14 days and 6 mg/kg METH 4 times at 2 hours intervals on day 15. In escalating dose (ED) METH group, rats were treated with gradually increased doses of METH 3 times per day for 13 days and 4.0 mg/kg METH 3 times on day 14 then followed with 0.9% saline 4 times at 2 hours intervals on day 15 (table 1). In ED-binge METH group, rats were treated in the same protocol as in ED-METH group, except using 6 mg/kg METH instead of 0.9 % saline. After treatments, rats were sacrificed by cervical dislocation and cauda epididymis was removed to release spermatozoa in phosphate buffer saline (PBS).

Sperm motility was immediately evaluated under bright field microscope (40x). The spermatozoa were loaded in the Makler counting chamber. Motile and non-motile sperm were expressed in percentage of normal sperm motility. After that, spermatozoa were fixed in 10% formaldehyde and stained with eosin solution to evaluate sperm morphology. Two hundred spermatozoa per animal were evaluated under bright field microscope. The sperm morphology was expressed in percentage of normal sperm morphology. To evaluate sperm concentration, spermatozoa in 10% formaldehyde were counted (as 10^6cells/ml) under bright field microscope by using Neubauer's counting chamber.

Table 1. Schedule of METH administration for escalating dose on days 1-14 and a high dose binge on day 15 (9)

Days	Methamphetamine dose (mg/kg)			
	7.30	10.30	13.30	
1	0.1	0.2	0.3	
2	0.4	0.5	0.6	
3	0.7	0.8	0.9	
4	1.0	1.1	1.2	
5	1.3	1.4	1.5	
6	1.6	1.7	1.8	
7	1.9	2.0	2.1	
8	2.2	2.3	2.4	
9	2.5	2.6	2.7	
10	2.8	2.9	3.0	
11	3.1	3.2	3.3	
12	3.4	3.5	3.6	
13	3.7	3.8	3.9	
14	4.0	4.0	4.0	
Day	**7.30**	**9.30**	**11.30**	**13.30**
15	6	6	6	6

Results

The percentages of normal sperm motility and normal sperm morphology were significantly decreased in all groups of animals treated with METH. However, the sperm concentration was not significantly decreased in all animal groups treated with METH.

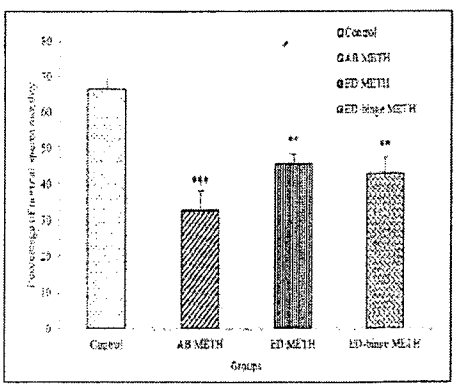

Fig 1. Percentage of normal sperm motility of male rats after treated with METH compared with control group. Data are presented as mean±SEM, n = 6, ** $P<0.01$ vs control group and *** $P<0.001$ vs control group.

Percentage of normal sperm motility was significantly decreased in AB METH group (32.79±5.35%), in ED METH group (45.50±2.83%), and in ED-binge METH group (42.66±4.71%) when compared with control group (66.50±2.79%) (Fig 1). Percentage of normal sperm morphology was significantly decreased in ED METH group (49.20±1.39%), and in ED-binge METH group (44.08±4.53%) when compared with control group. (68.33±7.41%) (Fig. 2). The sperm concentrations were numerically decreased in ED METH group when compared with control group, but it just failed to reach significant (Fig 3).

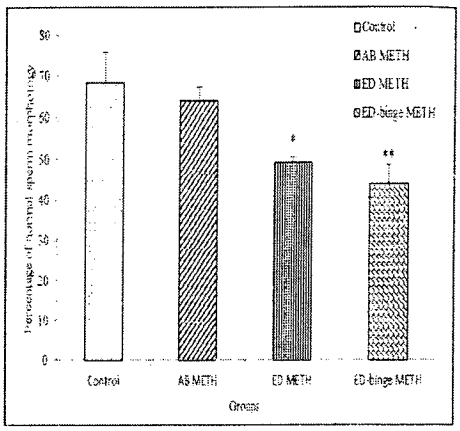

Fig 2. Percentage of normal sperm morphology of male rats after treated with METH compared with control group. Data are presented as mean±SEM, n = 6, * $P<0.05$ vs control group and ** $P<0.01$ vs control group.

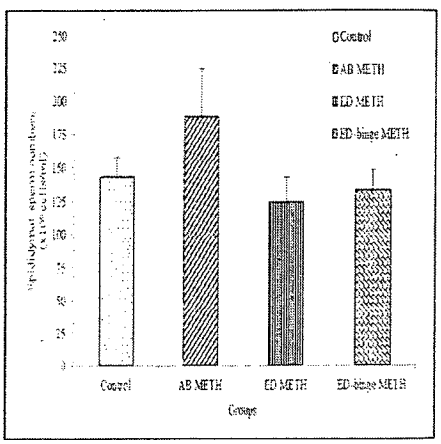

Fig 3. Sperm number of cauda epididymis ($x10^6$/ml) of male rats after treated with METH compared with control group. Data are presented as mean±SEM, n = 6

Discussions

The present study demonstrated that animals treated with METH show decreasing in normal sperm motility and normal sperm morphology involved in effect of METH addiction. Results of this study in ED

METH group and ED-binge METH group showed that gradual increase of METH treatment can induce a significant decrease of normal sperm motility when compared with control group. Moreover, normal sperm motility of AB METH group also significantly decrease compared with control group. A decrease of sperm motility in this study is in agreement with the study of Yamamoto *et al.* who reported a reduction of sperm motility of male mice treated with METH 15 mg/kg at 24 and 48 hours after METH administration. A decrease of normal sperm motility may result from direct effect of METH which it might adversely affect male fertility (10).

The lowest percentage of normal sperm morphology found in animals in ED-binge METH group may be involved the effect of METH-dependence which can induce number of abnormal sperm morphology. In addition, number of normal sperm morphology was also significantly decreased in ED METH group. Decreases of normal sperm morphology in both groups are consistent with the study of Numamud-Thanoi and Thanoi which indicate increase apoptotic cells in seminiferous tubule after exposure to 4 mg/kg and 8 mg/kg METH (7). Moreover, increased apoptotic cells in seminiferous tubule depend on dose-dependent that related to decrease normal sperm morphology (7).

Even though, the results of sperm concentration were not significantly decreased in all groups treated with METH compared with control group, numbers of sperm concentrations were numerically decreased in animals treated with ED METH group. These changes in sperm concentrations may reach significant if animals were exposed to METH for a longer period.

In conclusion, METH administration in animals can induce alteration in sperm quality, especially, in animals treated with ED-binge METH group. Decreases of sperm motility and sperm morphology caused by METH may be involved their fertilizing abilities of sperm leading to reproductive problems in males.

Acknowledgement

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OG-9

Decreased normal sperm morphology and sperm motility after methamphetamine administration

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Abstract

Methamphetamine (METH) is a psychostimulant drug which often abused and has neurotoxical effects. A few reports have also been reported its effect in reproductive toxicity. There is a study reported acute high dose and sub-acute dose treatments with METH in male rats can induce apoptosis in seminiferous tubules. Therefore the aim of this study was to investigate effect of METH that imitates addiction in human on rat sperm morphology and sperm motility. Male Sprague-Dawley rats were divided into four groups. Control group was treated intraperitoneally (IP) with vehicle three times per day for 14 days and four times on day 15. Escalating binge dose group was gradually increasing treated with METH (IP) three times per day for 13 day and treated 4.0 mg/kg METH (IP) three times on day 14 and treated with 0.6 mg/kg METH (IP) four times at 2 hours intervals on day 15; Escalating dose group was gradually increasing treated with METH (IP) three times per day for 13 day and treated 4.0 mg/kg METH (IP) three times on day 14 and treated with vehicle (IP) four times on day 15; Acute binge group was treated with vehicle (IP) three times per day for 14 days and treated with 0.6 mg/kg METH (IP) four times at 2 hours intervals on day 15. Rats were sacrificed and epididymis was removed to mince by scissor to release spermatozoa. The results of this study indicate that normal sperm morphology was significantly decreased in escalating binge dose group (43.88±2.37%), escalating dose group (29.75±6.50%) and acute binge group (62.69±1.99%) compared with control (75.90±1.02%). Sperm motility was significantly decreased in escalating binge dose group (25.07±6.38%) and escalating dose group (20.84±7.47%) compared with control (49.77±4.03%). Decreased normal sperm morphology and sperm motility may be caused by the effect of METH since our previous study has been reported that METH can induce apoptosis in rat testis.

Keywords: Methamphetamine, spermatogenesis, sperm concentration, sperm morphology, sperm motility

GO-5

Effect of Methamphetamine Administration on Alteration of Sperm Quality

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Abstract

Methamphetamine (METH) is an illicit psychostimulant drug. It is known as neurotoxicant because it can degenerate neurons in the brain and induce euphoria or psychosis. Moreover, METH can cause reproductive toxicity as it can induce apoptosis in seminiferous tubule and changes of plasma testosterone concentration. The present study was carried out to investigate the effect of METH that imitates human addiction on rat sperm quality. 24 male Sprague-dawley rats were divided into 4 groups composed of control group, acute binge (AB) METH group, escalating dose (ED) METH group and ED-binge METH group. All animals were treated intraperitoneally (i.p.) either with saline or METH. After treatment, rats were sacrificed by cervical dislocation and cauda epididymis was removed to release spermatozoa in phosphate buffer saline. Percentages of normal sperm motility were significantly decreased in AB METH group (32.79±5.35%), in ED METH group (45.50±2.83%), and in ED-binge METH group (42.66±4.71%) when compared with control group (66.50±2.79%). Percentages of normal sperm morphology were significantly decreased in ED METH group (49.20±1.39%), and in ED-binge METH group (44.08±4.53%) when compared with control group (68.33±7.41%). However, sperm concentration in ED METH group was numerically decreased when compared with the control group but it just failed to reach significant. The results of this study indicate that METH can induce alteration of sperm quality. These changes of sperm quality may relate to fertilizing abilities of sperm.

Keywords: methamphetamine, sperm quality, sperm concentration, sperm morphology, sperm motility

GO-1

Effect of methamphetamine administration on alteration of androgen receptor on Leydig cells

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Abstract

Methamphetamine (METH) is an illicit drug that abused worldwide. It is used to stimulate central nervous system (CNS) to induce euphoria and increase alertness. In addition, METH has been reported as reproductive toxicity such as it can induce abnormal sperm morphology and decrease sperm motility. The developmental process of germ cell is related to androgen receptor (AR) which it has been detected on Leydig cells. Thus, the aim of this study was carried out to investigate the effect of METH on alteration of AR on Leydig cells. Male Sprague-dawley rats were divided into 4 groups including control group, acute dose-METH binge (AB-METH) group, escalating dose-METH (ED-METH) group and escalating dose-METH binge (ED-METH binge) group. All animals were treated intraperitoneally (i.p.) with saline or METH. After that, the animals were sacrificed and testis was removed to investigate AR expression on Leydig cells by immunohistochemistry study. The percentage of AR on Leydig cells were decreased in ED-METH group (74.54±1.49%) ($P < 0.05$) and ED-METH binge group (70.72±2.15%) ($P = 0.000$) when compared with control group (80.48±1.60%). The result of this study indicated that METH can induce alteration of AR on Leydig cells which may relate to the dysfunction of leydig cells in producing hormone related to sperm production.

Keywords: methamphetamine, androgen receptor, Leydig cell, immunohistochemistry study