INVESTIGATING THE INVOLVEMENT OF KAPPA OPIOID RECEPTOR IN MEDIATING RELAPSE RELATED TO MORPHINE/METHAMPHETAMINE (POLY-DRUG) DEPENDENCE USING AN IMMUNOHISTOCHEMISTRY TECHNIQUE

BY

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ABSTRACT

The upregulation of kappa opioid receptor (KOR) may results in dysphoria which could contribute to relapse towards various drugs of abuse. This research work is conducted to further investigate the involvement of KOR system in mediating relapse related to this poly-drug dependence at the brain level (striatum, amygdala, hippocampus, and prefrontal cortex). The reinstatement (relapse) model was initially developed for morphine (7.5 mg/kg), methamphetamine (1.0 mg/kg), and poly-drug (7.5 mg/kg and 1 mg/kg, respectively) using the conditioned place preference (CPP) paradigm. During reinstatement, a combination of 0.3 mg/kg buprenorphine and 1.0 mg/kg naltrexone (BUP/NTX) or saline was administered prior to the drug priming of morphine (2.5 mg/kg), methamphetamine (1.0 mg/kg), and poly-drug (2.5 mg/kg and 1 mg/kg, respectively). The change in KOR expression was quantitatively measured through the immunohistochemistry (IHC) technique by using the rabbit monoclonal antibody (EPR 18881) since it specifically binds at the KOR. Only the poly-drug group was investigated in order to evaluate the potential of this BUP/NTX treatment in IHC. The CPP results showed that the drug dependence models were successfully established in all groups, where the preference at the drug-paired compartment was significantly different ($p < 0.001$) compared to its baseline (23.45 ± 5.24 %, $n = 10$ vs. −8.55 ± 4.82 %, $n = 12$ [morphine]; 42.84 ± 6.83 %, $n = 12$ vs. −7.84 ± 4.31 %, $n = 14$ [methamphetamine]; and 34.91 ± 7.59 %, $n = 10$ vs. −11.16 ± 4.28 %, $n = 13$ [poly-drug]). During reinstatement, the BUP/NTX treatment successfully attenuated reinstatement to morphine (2.05 ± 11.04 %, $n = 11$ vs. −13.50 ± 5.18 %, $n = 13$, $p > 0.05$), but not for methamphetamine (35.03 ± 12.50 %, $n = 10$ vs. −6.75 ± 2.73 %, $n = 14$, $p < 0.05$). This treatment also successfully attenuated the reinstatement to poly-drug in the subgroup of mice that did not develop desensitisation behaviour (e.g., freezing behaviour), where the preference at the drug-paired compartment was not significantly different compared to its own baseline (19.14 ± 16.89 %, $n = 5$ vs. −16.14 ± 4.81 %, $n = 12$, $p > 0.05$). In IHC, only the striatum showed an increment in the KOR expression during reinstatement compared to post-conditioning in the saline group (33.390 ± 5.595 %, $n = 12$ vs. 16.730 ± 5.265 %, $n = 12$, $p < 0.01$). From the CPP results, it is suggested that the concomitant use of morphine and methamphetamine has triggered the opioid receptor system, which was not evidenced when methamphetamine alone was abused at low dose tested (1 mg/kg). Therefore, it is suggested that the KOR receptor system can be used as one of the targets to treat poly-drug dependence that involve opioid and methamphetamine.

خلاصة البحث

التنظيم الرفعي للمُستقبلات الأفيونية من نوع كابا (KOR) قد ينتج عنه حالة الديسفوريا أو الانزعاج والتي بإمكانها المساهمة في حالات الإنتكاس وتعاطي العديد من المخدرات. تم إجراء هذه الدراسة لمواصلة التحقيق في ارتباط KOR في التوسط في الانتكاسات المتعلقة بهذا الإدمان المتعدد المخدرات على مستوى الدماغ (المخطَّط، واللوزة، والحصين، وقشرة الفص الجبهي). تم تطوير نموذج الإرجاع أو الإنتكاسة مسبقا لعقار المورفين (7.5 ملغم/كغم)، والميثامفيتامين (1 ملغم/كغم)، والعقارات المتعددة (7.5 ملغم/كغم و 1 ملغم/كغم، على التوالي) باستخدام نموذج المكان المكيف المفضل (CPP). أثناء عملية الإرجاع، تم إعطاء مزيج من 0.3 ملغم/كغم من البوبرينورفين و 1 ملغم/كغم من النالتريكسون (BUP/NTX) أو محلول ملحي قبل الشروع بإعطاء المورفين (2.5 ملغم/كغم)، والميثامفيتامين (1 ملغم/كغم)، والعقارات المتعددة 2.5 مغ/كغ و 1 مغ/كغ، على التوالي). تم قياس التغير في تعبير المستقبلات الأفيونية من نوع كابا كميا من خلال التصوير الكيميائي الهيستولوجي المناعي باستخدام الأجسام المضادة الأحادية النسيلة للأرانب (EPR 18881) لارتباطها التحديدي على KOR. تم التحقيق فقط في مجموعة العقارات المتعددة من أجل تقييم إمكانية علاج (BUP/NTX) من خلال الطريقة الكيميائية الهيستولوجية المناعية. أظهرت النتائج أنه تم إنشاء نماذج الإدمان على المخدرات بنجاح في جميع المجموعات، حيث كان التفضيل في القسم المرتبط بالمخدرات مختلفا بشكل ملحوظ (p <0.001) مقارنة مع مجموعة خط الأساس (23.45±5.24% ، n = 10 مقابل 55.8- ± 4.82% ، n = 12 [مورفين] ; 42.84 ± 6.83% ، n = 12 مقابل 7.84- ± 4.31% ، n = 14 [ميثامفيتامين] ; و 34.91 ± 7.59% ، n = 10 مقابل 11.16- ± 4.28% ، n = 13 [عقارات متعددة]). تم تخفيف الإرجاع للمورفين بنجاح بواسطة علاج الـ BUP/NTX (2.05 ± 11.04% ، n = 11 مقابل 13.50 ± 5.18% ، n = 13، p > 0.05) ، على خلاف الميثافيتامين (35.03 ± 12.50 % ، n = 10 مقابل 6.75- ± 2.73% ، n = 14 ، p < 0.05). أدى هذا العلاج أيضا إلى تخفيف الإرجاع إلى العقارات المتعددة في المجموعة الفرعية من الفئران التي لم تطور سلوك نزع الحساسية (على سبيل المثال، سلوك التجمد)، حيث التفضيل في المقصورة المخدرات يقترن لا تختلف اختلافا كبيرا بالمقارنة مع خط الأساس الخاص بها (19.14 ± 16.89٪، ن = 5 مقابل 16.14- ± 4.81٪، ن = 12، p> 0.05). أشارت نتائج التحليل الكيميائي الهيستولوجي المناعي أن مخطط الدماغ وحده أظهر زيادة في تعبير KOR أثناء الإرجاع مقارنة مع حالة ما بعد التكييف في المجموعة المعالجة بالأملاح (33.390 ± 5.595٪، n = 12 مقابل 16.730 ± 5.265٪، n = 12، p <0.01). اقترح من نتائج الـ CPP أن الاستخدام الملازم للمورفين والميثامفيتامين أثار نظام مستقبلات الأفيونيات، والذي لم يكن واضحا عندما تعاطي الميثامفيتامين وحده في اختبار جرعة منخفضة (1 ملغ/كلغ). ولذلك يقترح أنه بإمكان نظام مستقبلات KOR أن تستخدم كأحد الأهداف في علاج الإدمان على العقارات العديدة المشتملة على الأفيون والميثامفيتامين.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmacology).

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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"In the name of Allah, the Most Compassionate, the Most Merciful. Praise be to Allah, Lord of the universe, and peace and prayers be upon His final Prophet and Messenger"

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LIST OF ABBREVIATIONS

5-HT	Serotonin
AMG	Amygdala
AR	Antigen Retrieval
ATS	Amphetamine-Type Stimulants
BA	Basal
BNST	Bed Nucleus of the Stria Terminalis
BUP	Buprenorphine
CA	*cornu ammonis*
CaMKIIα	Calcium/calmodulin-dependent Protein Kinase II α Isoform
cAMP	cyclic Adenosine Monophosphate
CE	Central
CeA	Central Nucleus of the Amygdala
CNS	Central Nervous System
CPP	Conditioned Place Preference
CREB	cAMP Response Element-Binding
CRF	Corticotropin-Releasing Factor
DA	Dopamine
DAB	3,3'-Diaminobenzidine
DAT	Dopamine Transporter
DOR	Delta Opioid Receptor
DPX	Distyrene Plasticizer Xylene
DS	Dorsal Striatum
FDA	Food and Drug Administration
GABA	γ-Aminobutyric Acid
GDP	Guanosine Diphosphate
GPCR	G-Protein Coupled Receptor
GTP	Guanosine Triphosphate
HIV	Human Immunodeficiency Virus
HPC	Hippocampus
IHC	Immunohistochemistry

IL	Infralimbic
Ip	Intraperitoneal Injection
ITC	Intercalated
K^+	Potassium Ion
KOR	Kappa Opioid Receptor
LA	Lateral
LTD	Lateral Dorsal Tegmentum
MDMA	3,4-Methylenedioxy-N-Methyl-Amphetamine (Ecstasy)
MMT	Methadone Maintenance Treatment
MOR	Mu Opioid Receptor
mPFC	medial Prefrontal Cortex
NAc	Nucleus Accumbens
NAc-Sh	Transition zone in the medial shell subregion of the Nucleus Accumbens
NADA	National Anti-Drug Agency
NE	Norepinephrine
NOP	Nociceptin Opioid Peptide
nor-BNI	Norbinaltorphimine
NTX	Naltrexone
oPFC	orbital Prefrontal Cortex
PCR	Polymerase Chain Reaction
PFC	Prefrontal Cortex
PL	Prelimbic
PPT	Pedubcular Pontine Tegmentum
rCMgIC	regional Cerebral Metabolic Rate for Glucose
SEM	Standard Error of the Mean
UNODC	United Nations Office on Drug Crime
VMAT-2	Vesicular Monoamine Transporter-2
VTA	Ventral Tegmental Area

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Drug addiction is a chronic and relapsing brain disorder that can cause an uncontrolled compulsion to drug seeking behaviour despite of its negative consequences such as negative emotional state (e.g., dysphoria and anxiety) and withdrawal syndrome (Koob & Volkow, 2010; Trigo, Martin-García, Berrendero, Robledo, & Maldonado, 2010). The commonly abused drugs include alcohol, heroin, methamphetamine, cannabis (marijuana), ketamine, tobacco (nicotine), and inhalants. All these drugs can to lead harmful risks such as addiction, drugged driving, and infectious diseases (NIDA, 2016b).

Based on the latest worldwide statistics from the United Nations Office on Crime (UNODC), the amphetamine-type stimulants (ATS) is the highest drug seised by the authority since 2009. It also reported that methamphetamine was the main drug from ATS class that being seised, with the South and East Asia, as well as North America being the leading countries (UNODC, 2016). This was followed by opioids, cocaine, and cannabis (Figure 1.1) (UNODC, 2016).

In Malaysia, the National Anti-Drug Agency (NADA) in 2016 reported that opioids and methamphetamine are ranked first and second for the mostly abused drugs from the year of 2010 to 2016, with the recent usage percentage of 53.47 % and 31.82 % in 2016, respectively. It was followed by other amphetamine-type stimulants (10.69 %) and cannabis (3.89 %) as shown in Figure 1.2 (NADA, 2016). However, NADA has

separately classified methamphetamine from ATS, unlike UNODC, for the drug classification.

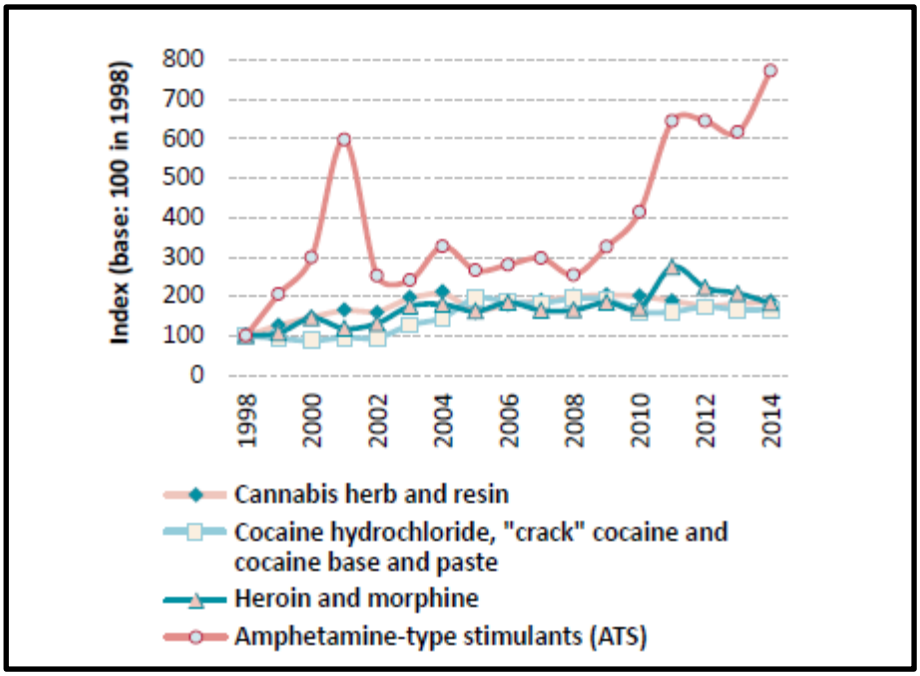

Figure 1.1 World Drug Report 2016 from Year 1998 to 2014 (UNODC, 2016)

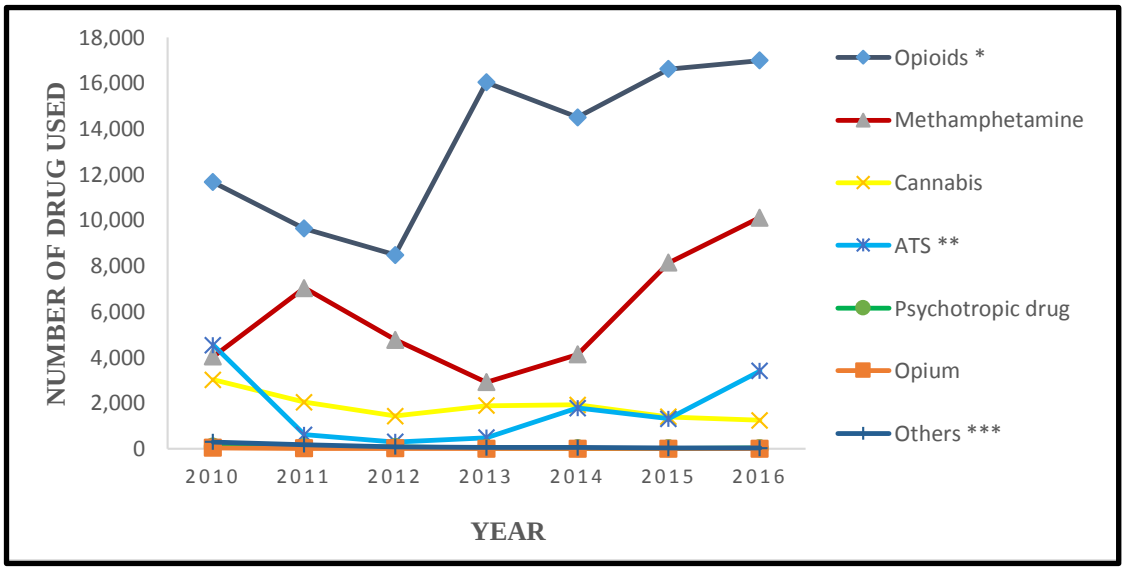

Figure 1.2 Statistic of Drug Usage from Year 2010 to 2016 (NADA, 2016)

Therefore, drug addiction remains as a worldwide concern, especially the ATS addiction. The UNODC reported that the prevalence of methamphetamine usage was high in Asia and there was a high demand for methamphetamine addiction treatment (UNODC, 2015). Still, there is no available treatment offered for methamphetamine addiction till today.

In contrast, few treatments have been approved by the FDA for opioid dependence case, mainly methadone and buprenorphine. Due to its cost, methadone is commonly used as the first line treatment for opioids dependence to substitute the illicit opioids (Steketee & Kalivas, 2011; Ward, Bell, Mattick, & Hall, 1996). Although methadone is proven to be safe and effective to treat opioid dependence, this drug still has its own limitation. One of the limitation is high incidence of relapse was found (around 55-80 %) following cessation of substitution therapy with the methadone (full mu-opioid agonist), where it is thought to be due to the kappa overdrive syndrome (Rothman et al., 2000; Tkacz et al., 2011).

The latest finding showed that there is an increasing pattern of methamphetamine addiction among the methadone patients after enrolling into MMT program (Shariatirad, Maarefvand, & Ekhtiari, 2013). These patients started to take methamphetamine while on methadone maintenance therapy in order to feel good, as self-medication for depression, and also to get high by shifting between different classes of drugs (Shaffer & LaSalvia, 1992; Shariatirad et al., 2013). This created another problem where the addicts started abusing more than one class of drugs which leads to poly-drug dependence (Trujillo, Smith, & Guaderrama, 2011).

The most commonly abused drugs by the poly-drug addicts are morphine and methamphetamine (Liu, Lin-Shiau, Chang, & Lan, 2015). Most of the addicts took these

drugs combination due to the greater effect known as “speedball”, as compared to a single drug (Trujillo et al., 2011).

To date, there is no FDA-approved treatment for methamphetamine dependence. Hence, this poly-drug dependence has becoming a serious health problem that needs attention, since its abuse is increasing and there is no available treatment to treat this poly-drug dependence (Pereira et al., 2011).

Buprenorphine is one of the FDA-approved treatments for opioids dependence (Cruciani & Knotkova, 2013). However, the use of buprenorphine is believed to be less than optimal because of its expensive cost. Buprenorphine is a partial mu opioid receptor agonist (MOR), while antagonist at the kappa (KOR) and delta (DOR) opioid receptors (Gerra et al., 2004). It is also a partial agonist at the nociceptin opioid receptor (NOP) (Lutfy & Cowan, 2008). The MOR activity of buprenorphine is the main key in treating opioid dependence, similar to methadone. Meanwhile, the KOR antagonist (the receptor of interest in this study) is strongly believed to counteract with the negative mood state (e.g., dysphoria) that experienced by the addicts due to drug withdrawal (Cruciani & Knotkova, 2013). Few studies had suggested that buprenorphine might be effective in reducing morphine, cocaine, and alcohol dependence (Lutfy et al., 2003; Montoya et al., 2009).

Back to the receptor of interest (the KOR), previous studies showed that there was a link between the drug relapse and KOR activity, including opioids and psychostimulants (Butelman, Yuferov, & Kreek, 2012). The activation of KOR that results in stress and dysphoria was believed contributes to drug relapse (Butelman et al., 2012). Due to the successful of buprenorphine/naltrexone treatment among the cocaine-dependent rats (Cordery et al., 2014), it is believed this treatment might be beneficial to prevent relapse related to methamphetamine dependence as well. Recent study