

Investigation of Serum Levels and Activity of Matrix Metalloproteinases 2 and 9 (MMP2, 9) in Opioid and Methamphetamine-Dependent Patients

Khadijeh Najafi¹, Daniel Elich Ali Komi^{2,3}, Habibolah Khazaie^{4,5}, Ali Moini⁶, Asad Vaisi-Raygani⁸, Hamid Reza Ahmadi⁵,
Mohammad Rasoul Ghadami⁴, Amir Kiani^{9,7}

¹ Pharmacy Student Research Committee, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran

² Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

³ Department of Immunology, Tabriz University of Medical Sciences, Tabriz, Iran

⁴ Sleep Disorders Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

⁵ Substance Abuse Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

⁶ Department of Internal Medicine, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran

⁷ Department of Pharmacology and Toxicology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran

⁸ Department of Biochemistry, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

⁹ Regenerative Medicine Research Center (RMRC), Kermanshah University of Medical Sciences, Kermanshah, Iran

Received: 17 Jul. 2018; Accepted: 29 Aug. 2018

Abstract- Matrix metalloproteinases (MMPs) are a group of zinc-dependent proteolytic enzymes that play a role in extracellular matrix (mainly collagen) degradation and remodeling. MMPs are not only causes of the increase rewarding effects of drugs, but also act as pro-addictive agents. In this research, 22 morphine and 20 methamphetamine-dependent patients included and their serum levels and activity of MMP2 and 9 were assessed by ELISA and gelatin zymography and compared with those of 23 healthy individuals as a control group. Our findings showed a significant increase in serum levels and activity of MMP-2 in opium and methamphetamine groups in comparison with the control group. Moreover, unlike MMP-2, serum levels and activity of MMP-9 in both case groups found to be decreased. This study showed that long-term abuse of opium and methamphetamine changes the activity and serum levels of MMP9 and MMP2. The effects of methamphetamines and opium are associated with an increase in extracellular dopamine levels in the brain, achieved by facilitating the release of dopamine from pre-synaptic nerves. Our findings showed that serum levels and activities of MMP-9 and MMP-2 could be considered as alternative valuable biomarkers from those investigated of pro-addictive or anti-addictive factors in dependent patients.

© 2018 Tehran University of Medical Sciences. All rights reserved.

Acta Med Iran 2018;56(9):559-562.

Keywords: Drug dependence; Matrix metalloproteinase; Morphine; Methamphetamine

Introduction

Methamphetamine, an addictive synthetic derivative of amphetamine, is currently one of the most widely abused stimulants (1). Its exposure is associated with adverse effects mainly neurotoxicity, neuropsychological deficits, and cardiotoxicity that are mediated through releasing of monoamine neurotransmitters, including dopamine, norepinephrine, and serotonin (2,3). Opioids are best known for their analgesic, anxiolytic, and euphoric effects leading to widespread use, dependence and addiction. Opioids, such as morphine or heroin, have been shown to

increase forebrain dopamine release and locomotion (4,5). Opiates act in the ventral tegmental area (VTA) via μ -opioid receptor that leads to the release of dopamine into the synaptic cleft (6). Morphine via the activation of dopamine cells in the VTA causes the increase of dopaminergic neurotransmitter in the nucleus accumbent (NA) (7). Amphetamine directly binds to dopamine transporters and interrupts the uptake of dopamine (6). The endogenous modulators of drug dependence are classified into two groups, pro-addictive, and anti-addictive factors. The pro-addictive factors include basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF), tissue

Corresponding Author: A. Kiani

Department of Pharmacology and Toxicology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
Tel: +98 83 34276489, Fax: +98 83 334276493, E-mail address: amir1kiani@yahoo.com

plasminogen activator (tPA), matrix metalloproteinase (MMP)-2 and MMP-9 that act to potentiate the rewarding effects of drugs. In contrast, anti-addictive factors such as tumor necrosis factor- α (TNF α) and glial cell line-derived neurotrophic factor reduce the reward (7). MMPs are zinc-dependent endopeptidase that belongs to the metzincin superfamily. They contribute to turnover of ECM in homeostasis and disease through acting on their substrates mainly extracellular proteins in the extracellular space as transmembrane, membrane-anchored, or released enzymes (8-10). MMP activity is tightly regulated in different levels including gene expression, proenzyme activation, and enzyme inhibition by endogenous inhibitors, mainly tissue inhibitors of MMPs (TIMPs) (11). MMP-2 and MMP-9 owing to their capability of cleaving collagen IV and V, laminin, and chondroitin sulfate proteoglycan, play a role in both pathologic conditions including cerebral ischemia, kainate-induced neuronal injuries, multiple sclerosis and also physiological processes including wound healing (3,12-15). MMP-9 also known as gelatinase B, is involved in the degradation of ECM and inducing the accumulation of neutrophils as a neutrophil chemotactic factor (16). Role of the MMP/TIMP system in Methamphetamine-induced behavioral sensitization and reward has been studied on animal models. Mizoguchi *et al.* studied methamphetamine-induced behavioral sensitization and conditioned place preference attenuated in MMP-2 homozygous knock-out MMP-2^{-/-} and MMP-9^{-/-} mice compared with wild-type mice. Their results suggest that both MMP-2 and MMP-9 may play a role in methamphetamine-induced behavioral sensitization and reward through regulating methamphetamine-induced dopamine release and uptake in the nucleus accumbens (NAc) (17). Mizoguchi *et al.* reported that administration of Methamphetamine results in behavioral sensitization accompanied by the induction of MMP-2, MMP-9, and TIMP-2 expression in the brain including the frontal cortex (Fc) and NAc (3). In the present study, we investigated serum levels and activity of MMP-9 and MMP-2 in patients who abuse opium or methamphetamine, for at least two years.

Materials and Methods

Sample, data collection

A total of 22 opium and 20 methamphetamine abusers participated freely in this study. Moreover, 23 healthy non-drug abuser individuals, were recruited as a control group. This study was approved by the ethical committee of Kermanshah University of Medical

Sciences (KUMS). The informed consent was obtained from each subject involved in the research. Our criteria for selection of patients were DSM IV guidelines. Patients with a mental or non-mental disease, those with impertinent drug abusing history, or using medicines such as hypnotic medicines, benzodiazepines, antipsychotics, antidepressants, and sedative medicines or using two or more drugs for abuse were excluded. Peripheral blood samples of individuals of both case and control groups were collected after overnight fasting then serum of each individual was separated, aliquoted, labeled and kept frozen at -80 °C until laboratory measurements.

Measuring serum concentration of MMPs

Human, MMP-2, MMP-9, Quantikine® ELISA kits (R&D systems, Minneapolis, USA) were used to measure serum MMPs.

Protein assay

Total serum protein of each sample was measured by Bradford method, and 30 μ g of each sample was loaded in zymography gels.

Gelatin zymography

Gelatin zymography was performed according to the method previously described (La Rocca *et al.*, 2004) for all sera. We prepared the gel using stacking gel (SDS-PAGE, 4%), resolution gel (SDS-PAGE, 8%) and copolymerizing with gelatin (1 mg/ml) (Merck). For each sample, 30 μ g of total serum protein was loaded. Electrophoresis was performed using the mini gel slab apparatus vertical slab model VS 3000 (Akhtariyan-Iran) by gradient program as the following steps: 30min with 50 V voltage, following by 150 V voltage until the dye reached the bottom of the gel. Following electrophoresis, gels were washed in 100 ml renaturation buffer comprising of 2.5% Triton X-100 in 50 mM Tris-HCl (pH 7.5). Then, the buffer was renewed every 20 min., which was repeated three times in an orbital shaker. Subsequently, the gels were incubated for 18h at 37°C in incubation buffer (0.15 M NaCl, 10 mM CaCl₂, 0.02% NaN₃ in 50 mM Tris-HCl (pH 7.5)). Gels were then stained with Coomassie blue and destained with 7% methanol and 5% acetic acid (Figure 1). Areas of bands were digested to be quantified by Image J software, using a high-resolution digital image of gel against the amount of MMPs standard in each gel.

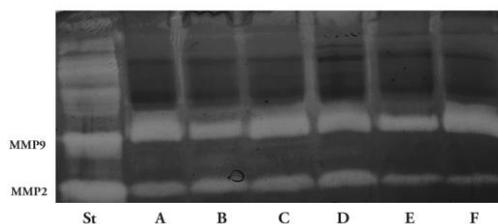


Figure 1. Panel of representative gelatin zymograms of sera from opium and methamphetamine-dependent patient and control groups
Lanes (A,B) sera from opium users; (C,D) sera from methamphetamine users; (E,F) sera from control users; (St) standards of MMP-2 (72 kDa) and MMP-9 (92 kDa)

Statistical analysis

Data analysis was performed with SPSS software version 16.0. Data were presented as Mean \pm SEM to compare serum levels and activity of MMP-2 and

MMP-9 in both groups using One-way analysis of variance (ANOVA) and Tukey's test for quantitative and normal data. The non-parametric Kruskal–Wallis test was used for the comparison of multiple groups (Control–opioid dependent–Methamphetamine dependent). *P* of less than 0.05 was considered as statistically significant.

Results

A significant increase in the serum levels and activity of MMP-2 in the opium and methamphetamine group comparing to that of the control group was seen. Unlike MMP-2, serum levels and activity of MMP-9 in both case groups decreased when compared to the activity of MMP-9 in the control group (Table 1).

Table 1. Comparison of the serum levels and activity of MMPs in case and control groups. Serum levels and activity of MMP-2 and MMP-9 were assessed in both groups. A comparison was made between each of the cases and control groups. Depicted data are Mean \pm SEM. P-value: Comparison of the opium and methamphetamine-dependent patients and control groups

Group	Control(mean)	Opium(mean)	Methamphetamine(mean)
Number	22	22	20
MMP2 activity	507.92 \pm 39.67	877.03 \pm 74.02	952.28 \pm 86.34
<i>P</i>		0.000	0.000
MMP9 activity	566.14 \pm 44.25	265.61 \pm 20.24	239.23 \pm 21.34
<i>P</i>		0.000	0.000
MMP2 conc (ng/ml)	82.7833 \pm 4.24	173.00 \pm 16.23	163.43 \pm 15.47
<i>P</i>		0.000	0.000
MMP9 conc (ng/ml)	717.61 \pm 73.14	460.50 \pm 34.20	374.38 \pm 21.74
<i>P</i>		0.003	0.000

Discussion

Previous studies were mainly performed on animal models and often examined the acute and sub-acute effects of the drug. Our findings show that serum levels and activities of MMP-9 and MMP-2 can be considered in dependent patients as valuable biomarkers for the investigation of pro-addictive or anti-addictive factors. Our results were consistent with results reported from several animal studies on the effects of opium and methamphetamine on MMPs. Martins *et al.* investigated the effect of an acute 30 mg/kg dose of Methamphetamine on the permeability of blood-brain barrier (BBB) in mice. They studied the role of MMP-9 in altering the permeability of BBB due to its potency in degrading the neurovascular matrix and tight junction proteins. Their gelatine zymography analysis revealed that after Methamphetamine treatment, the active form of MMP-9 in the hippocampus was increased. Additionally, they observed that at 1 h post

Methamphetamine injection, there are no changes in the MMP-9 immunoreactivity. However, they reported a significant increase in the MMP-9 immunoreactivity after 24 h (18). Although previous studies show that acute administration of low doses of methamphetamine has positive subjective effects on mood and cognitive performance (7), long-term repeated high doses have harmful symptoms and are correlated with cognitive impairments and mood interference (19,20). Thiennu *et al.* showed MMP9 could inhibit other MMPs (21). Current findings show that methamphetamine and opioid reduce the MMP9, which then leads to the removal of the MMP2 inhibition and occurs as an effect of increased serum level and activity of MMP2. It appears that MMP-2 and MMP-9 can be classified as pro-addictive, whereas TIMP-2 may be anti-addictive. Moreover, it is conceivable that inhibition of MMP-2 enzyme may be a therapeutic option for the treatment of drug dependency induced by methamphetamine or opium.

Acknowledgments

This work was performed in partial fulfillment of the requirements for, Pharm. Dr. of Kadijeh Najafi, in the faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran. The authors gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (Grant Number: 92450) for the financial support.

References

- Najafi K, Ahmadi S, Rahpeyma M, Khazaie H, Vaisi-Raygani A, Moini A, et al. Study of Serum Malondialdehyde Level in Opioid and Methamphetamine Dependent Patients. *Acta Med Iran* 2017;55:616-20.
- Henry BL, Minassian A, Perry W. Effect of methamphetamine dependence on heart rate variability. *Addict Biol* 2012;17:648-58.
- Mizoguchi H, Yamada K, Nabeshima T. Neuropsychotoxicity of abused drugs: involvement of matrix metalloproteinase-2 and -9 and tissue inhibitor of matrix metalloproteinase-2 in methamphetamine-induced behavioral sensitization and reward in rodents. *J Pharmacol Sci* 2008;106:9-14.
- Liu C, Hua Z, Bai Y. Classification of Opium by UPLC-Q-TOF Analysis of Principal and Minor Alkaloids. *J Forensic Sci* 2016;61:1615-21.
- Steidl S, Wasserman DI, Blaha CD, Yeomans JS. Opioid-induced rewards, locomotion, and dopamine activation: A proposed model for control by mesopontine and rostromedial tegmental neurons. *Neurosci Biobehav Rev* 2017;83:72-82.
- García-Pérez D, Núñez C, Laorden ML, Milanés MV. Regulation of dopaminergic markers expression in response to acute and chronic morphine and to morphine withdrawal. *Addict Biol* 2016;21:374-86.
- Yamada K. Pro-addictive and anti-addictive factors for drug dependence. *Nagoya J Med Sci* 2008;70:67-72.
- Smigiel K. S, Parks W. C. Matrix Metalloproteinases and Leukocyte Activation. *Prog Mol Biol Trans Sci* 2017;147:167-95.
- Nissinen L, Kähäri VM. Matrix metalloproteinases in inflammation. *Biochim Biophys Acta* 2014;1840:2571-80.
- Shariftabrizi A, Nifli AP, Ansari M, Saadat F, Ebrahimkhani MR, Alizadeh N, et al. Matrix metalloproteinase 2 secretion in WEHI 164 fibrosarcoma cells is nitric oxide-related and modified by morphine. *Eur J Pharmacol* 2006;530:33-9.
- Franco C, Patricia HR, Timo S, Claudia B, Marcela H. Matrix Metalloproteinases as Regulators of Periodontal Inflammation. *Int J Mol Sci* 2017;18:E440.
- Javaid MA, Abdallah MN, Ahmed AS, Sheikh Z. Matrix metalloproteinases and their pathological upregulation in multiple sclerosis: an overview. *Acta Neurol Belg* 2013;113:381-90.
- Vandooren J, Van den Steen PE, Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. *Crit Rev Biochem Mol Biol* 2013;48:222-72.
- Elieh-Ali-Komi D, Cao Y. Role of Mast Cells in the Pathogenesis of Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis. *Clin Rev Allergy Immunol* 2017;52:436-45.
- Elieh Ali Komi D, Grauwet K. Role of Mast Cells in Regulation of T Cell Responses in Experimental and Clinical Settings. *Clin Rev Allergy Immunol* 2018;54:432-45.
- Zhang C, Gong W, Liu H, Guo Z, Ge S. Inhibition of matrix metalloproteinase-9 with low-dose doxycycline reduces acute lung injury induced by cardiopulmonary bypass. *Int J Clin Exp Med* 2014;7:4975-82.
- Mizoguchi H, Yamada K, Niwa M, Mouri A, Mizuno T, Noda Y, et al. Reduction of methamphetamine-induced sensitization and reward in matrix metalloproteinase-2 and -9-deficient mice. *J Neurochem* 2007;100:1579-88.
- Martins T, Baptista S, Gonçalves J, Leal E, Milhazes N, Borges F, et al. Methamphetamine transiently increases the blood-brain barrier permeability in the hippocampus: role of tight junction proteins and matrix metalloproteinase-9. *Brain Res* 2011;1411:28-40.
- Hart CL, Haney M, Foltin RW, Fischman MW. Effects of the NMDA antagonist memantine on human methamphetamine discrimination. *Psychopharmacology* 2002;164:376-84.
- Johnson BA, Ait-Daoud N, Wells LT. Effects of isradipine, a dihydropyridine-class calcium channel antagonist, on D-methamphetamine-induced cognitive and physiological changes in humans. *Neuropsychopharmacology* 2000;22:504-12.
- Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev* 2000;14:2123-33.