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Abstract

Context: Although opiate abuse is known to affect MMPs, data on these enzymes and their tissue inhibitors in heroin addicts are scarce.

Objective: In the present study we determined serum concentrations of MMP-2, MMP-9, TIMP-1 and TIMP-2 in heroin users, and compared them with healthy individuals. We evaluated whether 21 days of abstinence are adequate to reverse the effect of opiates and we compared seropositive with seronegative, for anti-HCV antibodies, heroin users.

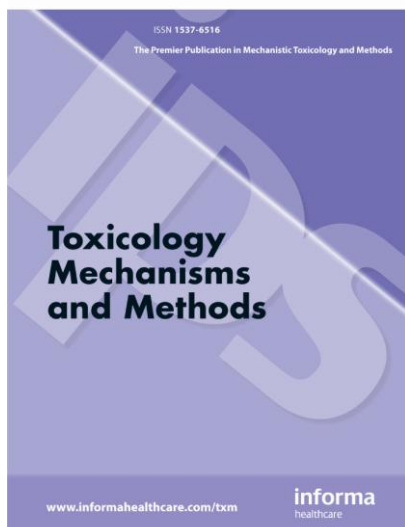
Materials and Methods: Twenty six heroin-dependent male volunteers and an equal number of healthy individuals participated in this study. ELISA was used to assess the serum levels of MMP-2, MMP-9, TIMP-1 and

TIMP-2. Heroin users were assessed both upon admission, as well as upon completion of a 21-day detoxification program.

Results: Serum TIMP-1 concentrations were significantly lower and the ratios MMP-2/TIMP-1, MMP-9/TIMP-1 and MMP-2/TIMP-2 were significantly higher in heroin users compared to healthy individuals. Heroin users who were seropositive, had lower MMP concentrations, as well as lower MMP/TIMP ratios compared to those who were seronegative.

Discussion: Our results showed that in heroin addicted individuals, and especially those who are positive for anti-HCV antibodies, the balance between MMPs and TIMPs in serum is disrupted and this disruption cannot be restored within 21 days of abstinence.

Conclusion: Chronic heroin abuse disrupts the balance between MMPs and TIMPs in serum and this effect is not reversible within 21 days of abstinence.



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Keywords

heroin, morphine, MMP, TIMP, metalloproteinases

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Discussion: Our results showed that in heroin addicted individuals, and especially those who are positive for anti-HCV antibodies, the balance between MMPs and TIMPs in serum is disrupted and this disruption cannot be restored within 21 days of abstinence.

Conclusion: Chronic heroin abuse disrupts the balance between MMPs and TIMPs in serum and this effect is not reversible within 21 days of abstinence.

Introduction

Matrix metalloproteinases (MMPs) represent a class of zinc-dependent endopeptidases, which regulate extracellular matrix (ECM) remodeling via cellular inflammation, extracellular matrix deposition, and tissue reorganization. They are therefore involved in a great number of both physiological and pathological processes, including normal development, wound healing and cancer (Stetler-Stevenson et al., 1996). Most MMPs are secreted as zymogens and become active following proteolytic activation, while their transcription, translation and pro-enzyme activity are regulated by growth factors, cytokines and tissue inhibitors of metalloproteinases (TIMPs) (Clark et al., 2008). Thus, the disruption of the balance between MMPs and TIMPs is linked to various diseases and is a constant finding in tissue fibrosis of many organs, such as the lungs (Lemjabbar et al., 1999; Madala et al., 2010), the heart (Robert et al., 1997; Li et al., 2000), the liver (Consolo et al., 2009) and the kidneys (Rysz et al., 2011).

Numerous researchers have demonstrated that opiate abuse affects the expression and enzyme activity of multiple members of the MMP family. Nevertheless, the results of these studies are still on debate and current data have not been efficient in verifying a strong positive or negative correlation between drug abuse and MMP expression. It is nowadays accepted that MMP-2 is under nitric oxide (NO) control (Pfeilschifter et al., 2001). On the other hand, opiates, and especially morphine, inhibit nitric oxide synthases (NOS), which are responsible for NO production (Kampa et al., 2001). Therefore, morphine was speculated to decrease MMPs' activity via the NO/NOS system (Sharifabrizi et al., 2006). The suppression of the gelatinolytic activity of MMPs by morphine was additionally shown to lead to collagen accumulation and mesangium expansion (Sagar et al., 1994). On the other hand, recent data have demonstrated that mice treated with morphine, exhibit an increase in MMP-2 expression (Chang et al., 2010). The

effect of opiates on MMPs has been further proven by studies on tumor cells, which showed that morphine inhibits tumor metastasis via suppression of the invasion, migration, adhesion and ECM degradation and that the underlying mechanism is the inhibited production of MMP-2 and MMP-9 (Harimaya et al., 2002). These findings were very recently reinforced by Gach and her colleagues (Gach et al., 2011), who demonstrated that morphine inhibits the expression and secretion of gelatinases (MMP-2 and -9) in the MCF-7 breast cancer cell line. Finally, it has been recently shown in an animal model that chronic morphine exposure increases the activity of spinal MMP-9, leading to the development of physical dependence on morphine (Liu et al., 2010).

Although all the above studies demonstrate the existence of a certain degree of correlation between opiate use and differential expression of MMPs, there is, to the best of our knowledge, limited work on the evaluation of these enzymes and their inhibitors in heroin users. In this prospective study, the concentrations of MMP-2, MMP-9, TIMP-1 and TIMP-2, were assessed in the serum of chronic heroin users in relation to an equal number of age- and sex-matched healthy participants. Heroin addicts attended a 21-day detoxification program. The enzymes' serum levels were evaluated both prior, as well as after detoxification treatment, in order to assess whether 21 days of abstinence were enough to reverse the effect of opiates on the serum concentration of MMPs and TIMPs. Furthermore, MMPs' and TIMPs' serum concentrations were compared between seropositive and seronegative, for anti-HCV antibodies, opiate users, since in chronic hepatitis, liver damage and progression of hepatic fibrosis are associated with the disruption of the balance between MMPs and TIMPs (Guido et al., 2006).

Methods

This prospective cohort study comprised of 26 heroin-dependent male volunteers, with a mean age of 31 ± 8 years, ranging from 20 to 54. Fifteen users were tested positive for anti-HCV antibodies and eleven were found negative. All subjects met the criteria for opiate dependence as defined by the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) (American Psychiatric Association, 1994).

All heroin users were admitted to the Addiction Department “Ianos” of the Psychiatric Hospital of Thessaloniki, Greece, which is a residential facility running a 21-day detoxification program. The program provides support and preparation for transfer to continuing care (residential therapeutic community or out-patient services).

Upon admission to the Unit, heroin users were all interviewed by trained personnel using the Addiction Severity Index questionnaire (ASI) (McLellan et al., 1985). Heroin abuse was confirmed by urine drug screening. All users had been injecting heroin for at least 6 months prior to admission, without any abstinence period or detoxification. The average length of heroin abuse was $124.15 (\pm 58.29)$ months and average daily intake ranged from 1.5 to 2.0 g (mean \pm *SD*, 1.67 ± 0.12 g) of street heroin (averagely 17% pure heroin). Previous consumption of drugs of abuse and/or psychotropic agents was accurately evaluated and those who had a history of substance dependence other than heroin were excluded from the study. The study cohort was restricted to heroin users with a self-reported intake of alcohol of < 300 ml/wk and without a history of diabetes, Crohns’ disease, cardiovascular-, renal- or pulmonary-related disorders. Additional exclusion criteria included a history of autoimmune diseases or hypertension, as well as current use of antihypertensive treatment.

Blood samples from heroin users were collected both upon admission to the Unit as well as upon completion of the 21-day detoxification program. Venipuncture was performed in all

participants in the morning, after a 12-hour overnight fast. Following blood collection, serum was isolated by centrifugation and stored at -70°C until analysis.

The control group consisted of an equal number of age- and sex-matched participants. The mean age of the healthy subjects was 30 ± 7 years. All participants were recruited from the University Hospital “AHEPA” in Thessaloniki, Greece, when attending standard pre-scheduled check-ups and were also monitored for drug abuse with urine screens. Apart from the above mentioned exclusion criteria, healthy participants were not included in the study in case of a personal history of DSM-IV Axis I or Axis II disorders (including a history of substance/alcohol abuse or dependence), use of medication 14 days prior to participation in the study, a positive history or excessive alcohol use (>300 ml/wk) and a positive drug screen on the day of admission.

The study was conducted in accordance to the Declaration of Helsinki (World Medical Association Inc., 2009). Written informed consent was obtained from all participants. None of the subjects received financial aid in order to participate in the study.

Serum levels of MMP-2, MMP-9, TIMP-1 and TIMP-2 (ng/ml) were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems Europe, Abingdon, UK), performed according to the manufacturer’s instructions.

The MMP-2 and MMP-9 assays measure total gelatinase concentration (proenzymes and activated forms). The LOQ of the method employed was: MMP-2: 0.16 ng/ml, MMP-9: 0.156 ng/ml, TIMP-1: 0.08 ng/ml and TIMP-2: 0,011 ng/ml. Preliminary investigation established the appropriate sample dilution for each MMP and TIMP as follows: MMP-2, 1:10; MMP-9, 1:40; TIMP-1, 1:100; TIMP-2, 1:50. The optical density was measured at 450 nm (630 nm reference) and all measurements were performed in duplicate.

The SPSS statistical analysis software, version 15.0, (SPSS Inc. Chicago, IL, USA) was used to conduct all statistical analyses. Continuous variables were expressed as mean \pm standard deviation and categorical variables as absolute numbers and percentages. The normality of distribution for each continuous variable was assessed by the Kolmogorov-Smirnov test. Independent samples t-test (two-sided) was used to compare data between the patient and the control group, as well as between subgroups of the patient group. For variables with a non-normal distribution, the Mann-Whitney U-test and the Wilcoxon test were applied. A difference was considered to be statistically significant at $p < 0.05$.

Results

MMP and TIMP values were not significantly altered in the patient group, as compared to healthy participants, with the exception of TIMP-1 which was significantly lower in heroin users (MMP-2, $p=0.094$; MMP-9, $p=0.715$; TIMP-1, $p=0.003$; TIMP-2, $p=0.711$) (Figure 1).

Concerning MMP/TIMP ratios, MMP-2/TIMP-1, MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios were statistically higher in heroin addicts as compared to the control group ($p < 0.001$, $p=0.004$, $p=0.036$ respectively), while the difference in the MMP-9/TIMP-2 ratio was not significant ($p=0.902$) (Figure 2).

The assessment of heroin addicts both prior, as well as following the 21-day detoxification program, yielded no statistically significant alterations in the serum levels of MMPs and TIMPs (MMP-2, $p=0.269$; MMP-9, $p=0.316$; TIMP-1, $p=0.683$; TIMP-2, $p=0.906$). Nevertheless, MMP-2 and TIMP-1 were slightly higher, while MMP-9 and TIMP-2 were slightly lower at the end of the detoxification program, compared to the values upon admission (Figure 3).

The same conclusions were also drawn in relation to the MMP/TIMP ratios found in heroin addicts upon admission and completion of the program, which did not demonstrate a statistically significant alteration (Figure 4).

In relation to the subgroup analysis, concerning the HCV status of heroin addicts, MMP-2 and MMP-9 were significantly higher upon admission in seronegative users, as compared to seropositive participants (MMP-2, $p=0.003$; MMP-9, $p=0.009$). On the other hand, no significant differences were demonstrated in the serum concentrations of TIMP-1 and TIMP-2 (TIMP-1, $p=0.190$; TIMP-2, $p=0.200$) (Figure 5).

Concerning the MMP/TIMP ratios, these were found significantly lower in seropositive heroin addicts when compared to seronegative drug users (MMP-2/TIMP-1, $p=0.029$; MMP-9/TIMP-1, $p=0.004$; MMP-2/TIMP-2, $p=0.011$; MMP-9/TIMP-2, $p=0.004$) (Figure 6).

Discussion

By comparing chronic heroin users upon admission to the detoxification unit with healthy individuals, we showed that chronic heroin abuse disrupts the balance between MMPs and their inhibitors in serum, since serum TIMP-1 concentrations were significantly lower and the ratios MMP-2/TIMP-1, MMP-9/TIMP-1 and MMP-2/TIMP-2 were significantly higher in heroin users compared to healthy individuals.

When heroin users were followed up for 21 days, until they completed the detoxification program, we found that this period of abstinence was not adequate to reverse the action of heroin and its metabolites on the balance between MMPs and their inhibitors. MMPs, TIMPs and all ratios examined had similar values upon admission to the detoxification program and upon completion.

According to our results, serum MMP-2 concentrations were significantly higher in seronegative, compared to seropositive users. The available relevant literature is inconclusive. In a previous study (Koulentaki et al., 2002) it was shown that in acute viral hepatitis, serum MMP-2 concentrations were significantly decreased, while other researchers reported that serum MMP-2 values were similar in healthy individuals and in patients with chronic hepatitis C, but increased in advanced fibrosis and cirrhosis (Boeker et al., 2002; Lichtinghagen et al., 2000, 2003; El-Gindy et al., 2003). Finally, in another study it was reported that MMP-2 protein is elevated even in serum of patients with chronic hepatitis C (Guido et al., 2006). These discrepancies could be attributed to the different composition of the patient groups (different fibrosis stages of the participating patients) included in the different studies.

Our results also showed that serum MMP-9 concentrations were significantly higher in seronegative, compared to seropositive users. This finding is in accordance with previous reports which show that serum MMP-9 concentrations are low in chronic hepatitis patients (Guido et al., 2006; Badra et al., 2010) and are inversely correlated with the fibrosis stages (Leroy et al., 2004; Mangoud et al., 2004).

All the ratios examined were lower in seropositive users, indicating that in these users the balance between MMPs and TIMPs is disrupted in favor of the inhibitors. Our findings are in accordance with previous reports which showed that in chronic hepatitis C patients and in patients with C-induced cirrhosis the serum ratios MMP-2/TIMP-1 and MMP-9/TIMP-1 are lower compared to healthy individuals (Guido et al., 2006; Lichtinghagen et al., 2000).

Conclusions

Although opiate abuse is known to affect MMPs, the available data on these enzymes and their tissue inhibitors in heroin addicts are limited.

We have currently shown that in heroin addicted individuals, and especially those who are positive for anti-HCV antibodies, the balance between MMPs and TIMPs in serum is disrupted. Furthermore, by following-up these individuals, we have shown that this disruption cannot be restored within 21 days of abstinence.

Declaration of interest

The authors report no conflicts of interest.

Just Accepted

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List of abbreviations

ASI: Addiction Severity Index

ECM: Extracellular Matrix

ELISA: Enzyme-Linked Immunosorbent Assay

HCV: Hepatitis C Virus

LOQ: Limit of Quantification

MMPs: Metalloproteinases

NO: Nitric Oxide

NOS: Nitric Oxide Synthase

TIMPs: Tissue Inhibitors of Metalloproteinases

