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MORPHOLOGICAL AND HISTOPATHOLOGICAL HEART CHANGES IN AUTOPSIES OF HEROIN ABUSERS

Morfološki i patohistološki nalaz na srcu obdukovanih korisnika heroina

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Apstrakt
Uvod/Cilj: Heroin je polusintetička droga iz grupe opioida, koja može uzrokovati morfološke i patohistološke promene na srcu: hipertrofiju komora, fibrozu miokrada, hipertrofiju kardiomiocita, izvijuganost miofibrila, gubitak jedara i poprečne ispruganosti miocita, perivaskularna krvena, zapaljenski infiltrat. Cilj rada je prikazati ove promene kod obdukovanih sa toksikološkim nalazom metabolita heroina, 6-monoacetilmorfina (6-MAM) i morfina.

Metode: Retrospektivna studija je rađena u Institutu za patologiju i sudsku medicinu Vojnomedicinske akademije u Beogradu u periodu od 2010. do 2014. godine na 27 obdukovanih starosti od 18 do 60 godina. Analizirani su debljina zida komora i patohistološki nalaz isećaka miokarda, obrađenih standardnom procedurom i obojenih hematoksilin-eozin (H&E) i trihromnim bojenjem (Masson). U uzorcima krvi i urina analizirane su koncentracije 6-MAM-a i morfina primenom tečne hromatografije sa UV detektorom (HPLC-PDA).

Rezultati: Debljine zida komora srca kod 27/27 (100%) su bile veće od normalne i to leva 1,74±0,17 cm, a desna 0,6±0,09 cm; fibroza miokrada 27/27(100%) i to perivaskularna kod 24/27(88,89%) obdukovanih, a fokalna intersticijalna kod 3/27(11,11%); hipertrofija kardiomiocita 22/27(81,48%); izvijuganost miofibrila 22/27(81,48%); gubitak jedara i poprečne ispruganosti miocita 10/27(37,04%); sveže perivaskularno krvena 23/27(85,19%); fokalni zapaljenski infiltrat 14/27(51,85%). Toksikološkom analizom kod 27/27 (100%) su nađeni 6-MAM i morfin u urinu. 6-MAM i morfin zajedno u krvi nađeni su kod 3/27(11,11%), a samo morfin kod 16/27(59,26%).
Morphological and histopathological heart changes in autopsies of heroin abusers

Abstract
Introduction/Aim: Heroin is a semisynthetic opioid that may cause morphological and histopathological changes in heart: ventricular hypertrophy, myocardial fibrosis, hypertrophy of cardiomyocytes, myofibrils contraction band necrosis, loss of myocytes nuclei and cross-striation, perivascular bleeding, inflammatory cells infiltrate. The aim of the study is to show histopathological heart changes in autopsies of long-time heroin abusers with positive toxicological analysis for 6-monoacetylmorphine (6-MAM) and morphine in blood and urine.

Methods: Retrospective study was done at the Institute of Pathology and Forensic Medicine Military Medical Academy in Belgrade between 2010 and 2014 and included forensic autopsies of 27 examinees aged between 18 and 60. Heart ventricles thicknesses were analysed and histopathological myocard findings from processed material stained by haematoxyline-eosine (H&E) and trichrome stains (Masson) was examined. 6-MAM and morphine concentration in blood and urine using fluid chromatography with UV (HPLC-PDA) was analysed.

Results: Heart ventricles thickness were increased in 27/27 (100%) included left 1.74±0.17 cm and right 0.6±0.09 cm. Myocardial fibrosis had 27/27(100%) examined including perivascular in 24/27(88.89%) and interstitial focal fibrosis in 3/27(11.11%); hypertrophy of cardiomyocytes 22/27(81.48%); myofibril contraction band necrosis 22/27(81.48%); loss of myocytes nuclei and cross-striation 10/27(37.04%); fresh perivascular bleeding 23/27(85.19%); focal inflammatory cells infiltrate 14/27(51.85%). In toxicological findings
of 27/27 (100%) 6-MAM and morphine were found in urine. Both 6-MAM and morphine in blood were found in 3/27(11.11%) and only morphine in blood of 16/27(59.26%).

Conclusion: Our results indicate both morphological (left and right ventricle hypertrophy) and histopathological heart changes (myocardial fibrosis, hypertrophy of cardiomyocytes, contraction-band necrosis, loss of myocytes nuclei and cross-striation, fresh perivascular bleeding and focal inflammatory infiltrate). These changes are non-specific and could be caused either by long-term heroin abuse or by other factors. Having in mind lack of medical histories of examined we couldn’t exclude other factors besides long-term heroin abuse as cause of heart changes.

Key words: heroin, myocardium, heart, heart hypertrophy, fibrosis, haemorrhage, 6-MAM, morphine

Introduction
In European population 0.6 % of individuals aged between 15 and 64 are heroin abusers 1. Heroin (3, 6-diacetylmorphine) is a semisynthetic opioid, morphine derivative synthetized by acetylation of two hydroxyl groups of morphine. It is taken into body intravenously, intramuscularly, intranasally, subcutaneously and by smoking. Maximal blood concentration is reached one to five minutes after intravenous intake and smoking or five minutes after intranasal and intramuscular application of heroin 2. Heroin is metabolized fast in the body to 6-monoacetyl morphine (6-MAM) which is converted to morphine by 6-acetyl group hydroxylation. The metabolic path of morphine includes glucuronidation to morphin-3-glucuronide and morphin-6-glucuronide in liver 3. Heroin conversion into 6-MAM lasts 10 to 15 minutes and further conversion to morphine lasts few hours. 6-MAM half-life in urine is 0.6 hours and it can be detected in urine 2 to 8 hours after the intake 4. Morphine is detected in urine up to 24 hours after heroin intake 5. Histopathological changes in heart, lungs, liver, brain and other organs may appear due to heroin abuse 6. Some of the common heart changes are myocardial fibrosis, ventricular hypertrophy and inflammatory cells infiltrate in myocardium 13. Heart muscle changes in long-term opioid abusers increases the risk of sudden cardiac death after intravenous drug injection 7. The aim of the study is to show the presence of morphological and histopathological heart changes observed in autopsies of long-term heroin abusers, who have positive toxicological analysis for heroin metabolites, 6-MAM and morphine in body fluids (blood and urine).
Methods
Retrospective study was done at the Institute of Pathology and Forensic Medicine Military Medical Academy Belgrade between 2010 and 2014 and included 27 forensic autopsies of examinees aged between 18 and 60. Heteroanamestic data from family members showed heroin abuse lasting more than two years. An average age of examinees was 35.11±10.78 years old. Most of examined persons were male 25/27 (92.59%), and female included 2/27 (7.41%). Forensic autopsies with positive toxicological analysis for heroin metabolites, 6-MAM and morphine, but without presence of other drugs, alcohol and other elements of abuse in body fluids (blood and urine) were analysed. In external autopsy examination fresh injection marks, scars and tattoos have been searched as characteristic findings for the population of drug abusers. During internal examination left and right heart ventricle thickness were measured and parts of heart muscle were taken as material for further histopathological examination. The material was processed by standard procedure, stained by hematoxylin-eosine (H&E) and trichrome stains by Masson. Light microscope Olympus BX 50 (x40) was used for histopathological examination of stained microscopic slides. Body fluids (blood and urine) were taken during the autopsy and the concentrations of 6-MAM and morphine were searched. Toxicological analyses were done using fluid chromatography with UV detection (HPLC-PDA) in the National Poison Control Centre of Military Medical Academy Belgrade and compared with standard library of spectrophotometry. Results were statistically analysed using descriptive statistic methods and non-parametrical test by statistical software package IBM SPSS Statistics 20. Non-parametrical test included Wilcoxon signet rank test, with confidence level p<0.05.

Results
Fresh injection marks were found during external examination in 17/27 (62.96%), tattoos in 14/27 (51.85%), scars in 18/27 (66.67%) included both linear and circular scars in 4/27 (14.81%), only linear in 11/27 (40.74%), and only circular in 3/27 (11.11%). Neither scars and tattoos nor fresh injection marks were examined in 1/27 (3.70%) autopsies. All the 27/27 (100%) examined had increased both left and right heart ventricles thicknesses in comparison to normal thickness’ values (normal value ranges from 1.0 to 1.5 cm for left and 0.25 to 0.5 cm for right ventricle thickness) 8. An average left ventricle thickness of examined was 1.74±0.17 cm, and right 0.6±0.09 cm.
Findings of the histopathological examination of heart muscle material were: myocardial fibrosis in 27/27 (100%), hypertrophy of cardiomyocytes in 22/27 (81.48%), contraction band necrosis of myofibrils in 22/27 (81.48%), loss of myocytes nuclei and cross-striation in 10/27 (37.04%), fresh perivascular bleeding in 23/27 (85.19%), focal inflammatory cells infiltrate in 14/27 (51.85%) (Fig. 1). Perivascular myocardial fibrosis was found in 24/27 (88.89%) examined (Fig. 2) and interstitial focal fibrosis in 3/27 (11.11%) (Fig. 3). All the six findings mentioned above had 2/27 (7.41%) examined, five findings had 11/27 (40.74%), four 7/27 (25.93%), three 6/27 (22.22%) and 1/27 (3.70%) had two histopathological change.

All the examined persons 27/27 (100%) had 6-MAM and morphine in urine found during toxicological analysis. Both 6-MAM and morphine were found in blood of 3/27 (11.11%) examined, only morphine in blood of 16/27 (59.26%). 6-MAM concentrations were 0.001-3.9 mg/L in urine, and 0.006-0.5 mg/L in blood. Morphine concentrations range was 0.015-12.72 mg/L in urine, and 0.004-0.8 mg/L in blood. Statistically significant difference was not found between concentrations of 6-MAM in blood and urine (p=0.109), but there was between concentrations of morphine in blood and urine (p=0.002). There was no statistically significant difference between concentrations of 6-MAM and morphine in blood (p=0.717), but there was in urine (p=0.023).

Discussion

Injection marks found during external examination were due to intravenous heroin intake. Skin scars are made by self-infliction 9. Tattoos are usually covering injection marks and scars as a try of covering the needle track of injections 10.

The frequency of heart diseases due to opioid consumption is still unknown. There is published in one study that only 10% examined have heart disease (endocarditis), but also the other one where is reported that there is heart damage in 100% examined people 2. Long-time heroin or morphine abuse causes hypoxia that leads to myocardial hypertrophy and myocardial fibrosis 11, 12. Respiratory centre depression and hard breathing as a consequence of lowered neuron sensibility in respiratory centre in brainstem after intravenous drug application, is one of possible causes of myocardial hypoxia 1.

Morphological change in heart that is presented in all examined during autopsies in our study is left and right ventricle myocardial hypertrophy. Left ventricle myocardial hypertrophy is described also in other studies of abusers’ autopsies as the most frequent among cardiac changes in opioid abusers 13. There is shown in other studies’ results that
the frequency of this change increases with the age of abusers, leading to 2.1% between the age of 15 and 24 years old and 10.7% in older than 44 years old 13. Myocardial hypertrophy increases the risk for hypoxia, which can cause arrhythmias and cardiac arrest 11. Hypoxia leads to myocytes’ apoptosis similarly to myocardial infarction, where is formed fibrosis by remodelling in the region of death myocytes 12. Multiplied fibrous tissue in heart muscle is present in all examined in this study. Some authors report that fibrosis is a response to chronic hypertension that causes myocardial hypertrophy, and other authors add the cellular growth factors as the reason of fibrosis 11, 7, 14. There is shown in experimental studies that the absence of fibroblast growth factor 21 leads to myocardial hypertrophy and ischemia by activation of proinflammatory paths and oxidative stress and also by fibrosis and heart metabolism disruption 14. Collagen accumulation in heart muscle leads to elasticity decrease, thickening and solidification of ventricle wall that complicates contractility. The other study shows that heroin and morphine intoxication causes myocardial contraction depression 15, 7.

Intravenous heroin abusers are exposed to many cardio-toxic factors that lead frequently to heart damage 11. Coronary blood vessels vasoconstriction is caused by increased catecholamine level, especially noradrenaline and dopamine 16, detectable in blood and urine during the first day after heroin intake 17. Nowadays investigations on experimental animals confirm previous theories of early heroin metabolism connection with catecholamine concentration increase 18. Heroin has systemic and direct effect on heart 19, 20, 21. Heart muscle rhabdomyolysis, hypoxia, acidosis and vasoconstriction lead to muscle necrosis and hypersensitive reaction to heroin 22. Heroin has direct effect to coronary arteries causing its spasm or inflammation that may lead to occlusion 23. There is written in studies that heroin has direct effect to vasomotor centre with increased parasympathetic activation, decreased sympathetic activation and histamine production stimulation in mastocytes, with bradycardia and hypotension that may then cause myocardial infarction 24. Increased parasympathetic activity may also play role in coronary artery spasm initiation 25. Bradycardia, tachycardia and atrial fibrillation are noted after heroin application. There is shown in experimental studies that morphine perfusion in sinoatrial node cause first tachycardia, and then bradycardia, which is explained as a consequence of vagal stimulation 11. Fresh lesions, as perivascular bleeding in heart muscle and myofibril contraction band necrosis, are not specific but can appear due to
directly toxic and hypoxic heroin effect and can cause heart rhythm changes and sudden cardiac death.

Presence of heroin metabolites may initiate histamine releasing. Fresh perivascular bleeding in heart muscle is caused by histamine-induced increased blood vessel wall permeability 26.

Conclusion
Our results indicate left and right ventricle hypertrophy as morphological heart change as well as histopathological heart changes - myocardial fibrosis, hypertrophy of cardiomyocytes, contraction-band necrosis, loss of myocytes nuclei and cross-striation, fresh perivascular bleeding and focal inflammatory infiltrate. These changes are non-specific and could be caused either by long-term heroin abuse or by many other factors for example arterial hypertension and other drugs (stimulants) abuse. Having in mind lack of medical histories of examined and the fact that all the information we collected are based on heteroanamnestic data from family members, we could not exclude other factors besides long-term heroin abuse as a cause of heart changes.

References


Fig. 1. Histopathological changes in heart
Fig. 2. Perivascular myocardial fibrosis (trichrome staining by Masson, magnification x40)
Fig. 3. Interstitial focal myocardial fibrosis (trichrome staining by Masson, magnification x40)