Myocardial damage in heroin abuse: immunohistochemical investigations with LCA, CD68, and CD45R0

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Abstract: Background. Myocardial insufficiency is suspected to be implicated in fatal lung edema complicating heroin-overdosage. The pathogenetic mechanism is not fully understood, but defects in myocardial contractility were proposed.

Methods. The qualification and quantification of leukocytes, monocytes, and T-lymphocytes was done on the myocardial samples from 13 heroin/morphine related deaths, compared to six controls by histological and immunohistochemical examination. The quantification of cell types was done by counting specific cell type of 30 high power fields in each myocardial sample. Mean values were then tested by Student t-test.

Results. The results showed that the control group had a higher mean number of observed leukocyte common antigen (LCA) and CD45R0 positive white blood cells, without significant difference. The mean number of CD68 positive white blood cells was significantly lower in drug addicts group than in the control group (p=0.013, Cohen’s d=1.41, power 0.86).

Conclusions. Deterioration of immune cell function in heroin abusers will produce in time left ventricular dysfunction and susceptibility to lung edema complicating heroin-overdosage.

Key Words: drug abuse; heroin, myocarditis, immunohistochemistry

Substance abuse and infections are two of the major problems in the world today with an estimated 200 million people abusing illegal drugs regularly [1] and an estimated one billion people living with one or more infections [2]. Both the drugs of abuse and infections such as human immunodeficiency virus and hepatitis C virus affect almost every physiological/biochemical system in the body. Thus, health effects may range between neuropsychiatric complications, anxiety and depressive disorders, cardiomyopathies, immune impairment, metabolic/endocrine disorders (lipodystrophy), and hepatic failure, to name a few. Both drug abuse and drugs for treatment of the health complications may contribute to complexity of health condition of addicts [3, 4].

The effects of drugs, especially cocaine, on cardiovascular system have been extensively studied in humans and animal models [5-11]. Stimulants such as cocaine and methamphetamine (‘met’, ‘speed’, or ‘ice’) increase the heart rate while constricting the blood vessels; in susceptible individuals, these two actions together set the stage for cardiac arrhythmias and strokes. Cocaine use decreases the blood flow to the brain, increases the heart rate, and elevates the blood components that promote clotting-effects that can lead to stroke or heart attack even in those not otherwise at risk for these serious cardiovascular events [12]. Even though the increase in oxidative metabolism of catecholamines is proved to have adverse effects on myocardium [13], it cannot explain large infarcts in other organs, such kidney or spleen including acute myoglobinuric renal...
failure [14,15]. Ischaemic vascular events accompanying cocaine abuse were also shown in the skin [16], the intestine [17], the aorta with dissection [18] and in the spleen [19]. Epidemiologic studies have shown cerebral vascular events followed by cocaine usage as trombotic stroke [20] and even subarachnoidal haemorrhage.

Spongiform leukoencephalopathy is an unusual complication of illicit drug abuse with severe, often fatal, neurological deterioration and lesions of the white matter of the cerebrum, cerebellum, and basal ganglia, most often precipitated by inhalation of vapourised heroin [21]. Opioids are potent histamine liberators and the study shows that commonly abused drugs, morphine and cocaine, release histamine from mast cells in the presence of oxidative enzymes, which is the additional risk factor in heroin and cocaine overdose [22]. The analysis of 48 heroin-related deaths [23] suggested anaphylactoid shock as a possible death cause. Lung edema complicating heroin-overdose is regarded as the major mechanism contributing to death in heroin addicts. The pathogenetic mechanism is not fully understood, but defects in myocardial contractility were proposed. Other mechanisms are also discussed: hypoxia-induced increase of pulmonary capillary permeability, centrally induced respiratory depression, primary toxic effects on the alveolar capillaries and acute anaphylactic shock [24]. Myocardial alterations including mild inflammatory processes in the presence of infections, particularly hepatitis B and C, deserve special interest. This agents may be supposed to lead to heart failure causing final lung edema. Studies by conventional histology did not demonstrate differences in myocardial alterations regarding controls, nor could correlate myocardial fibrosis and myocarditis to morphine or 6-monoacetylmorphine (6-MAM) blood concentrations [13].

Cardiac lesions in drug addicts seem to have a variety of causative factors: infections, toxic influence, hypersensitivity, influence of catecholamines and general hypoxia. The authors feel that the two latter suggested causes appear most regularly and deserve special attention. The significance of these heart lesions seems to vary, but at times they may be determining factors in the fatal outcome of drug abuse [25].

Methods
The study group of chronic drug abusers consisted of total 13 cases of drug-associated deaths. According to toxicological analyses, in most of the cases, the deaths were the result of mixed intoxications with heroin predominating as a causative factor of overdose induced deaths. The controls comprised 6 subjects without any history of drug abuse and without post-mortem evidence of any pre-existing lethal disease. All autopsies were done in two days after death. The causes of death were polytraumas adopted in traffic accidents. Six myocardial samples were taken from each heart at standardised locations (anterior, posterior and lateral wall of the left ventricle; anterior and posterior wall of the right ventricle, and cranial part of interventricular septum). Samples for toxicological analyses were taken routinely.

Routine histological examination of all body organs were performed by 5 μ tissue sections from paraffin tissue blocks stained by Hematoxylin and eosine (HE) stain. All six myocardial sections from each case were analysed for myocarditis according to Dallas criteria (26).

For immunohistochemical analysis all myocardial sections were deparaffined in xylene and rehydrated with graded alcohols and finally in aqua destillata. The samples were microwave pretreated with citrat buffer pH 6.0 (20 minutes monocular CD45R0, clone UCHL1 and 30 minutes to detect leukocyte common antigen positive leukocytes (LCA), monoclonal LCA, clone PD1/26 and 2B11, and also CD68, monoclonal antibody PG-M1). After cooling at room temperature for about 20 minutes, the slides were washed in distilled water for 5 minutes and treated with 3 % H2O2 to block endogeneous peroxidase. After washing, the slides were transferred into freshly prepared phosphate buffered saline (PBS) which was gently removed after 5 minutes and primary antibodies were applied on sections for 30 minutes in moist chamber at room temperature. The second and the third links were applied (each for 20 minutes and washings with PBS). After that, the samples were treated with diaminobenzidine (DAB) as a chromogen and stained in Hematoxylin for 1 minute, rehydrated and mounted in DPX. Dark brown granules of DAB on the cells were regarded as positive staining. Human tonsilar tissue was used as a positive control. In all tests, a negative control was performed without monoclonal antibody.

The quantification of cell types was done by counting specific cell type of 30 high power fields in each myocardial sample. Mean values were then tested by Student t-test.
Results

Histological examination revealed pronounced lung and brain edema, marked congestion of internal organs and signs of hepatic inflammation in a few cases. The examination of routinely prepared formaldehyde-fixed myocardial specimens revealed acute congestion in almost half of myocardial samples (6 of 13) from the study group. No case in this group met the Dallas criteria for myocarditis. The three cases presented the perivascular fibrosis in the myocardium (Fig. 1).

LCA, as a panleukocyte cell surface marker, has stained all lymphocytes and tissue macrophages in all tested samples of myocardium. Mean counts of myocardial leukocytes (LCA), T-lymphocytes (CD45R0) and macrophages (CD68) were seen using microscopic field under magnification 400 X (Fig. 2), and are shown in table 1. The results for control group are given in table 2.

Student’s T test showed that control group had higher mean number of observed LCA positive and CD45R0 positive white blood cells, but this difference was not significant (p>0.05). The mean number of CD68 positive white blood cells was significantly lower in drug addicts group than in the control group (p=0.013, Cohen’s d =1.41, power 0.86) (table 3).

Toxicological investigations were completely negative in the control group. Fatal toxic blood concentrations were found in every individual of the study group. The concentration of morphine in femoral blood ranged from 0.08 to 2.03 mg/l. The 6-MAM concentration ranged between 0.07 to 0.46 mg/l. The ethanol detected in 38% (5 of 13) cases of the study group. The concentration of ethanol ranged from 0.53 ‰ to 3.05 ‰. The median value for morphine concentration in the subgroup with ethanol was 0.22 mg/l. Codeine was detected in femoral blood in 8 cases (62 %) and diazepam in 3 cases (23 %) together with 6-MAM and morphine, out of which the finding in 2 cases was a combination of diazepam, ethanol and codeine.
Discussion

Drug related myocardial damages are discussed as one possible mechanism causing final lung edema in acute intoxication. The underlying processes still remain unclear, as well as pathophysiological processes that cross-react. There are numerous studies concerning the cocaine. The present study includes 13 cases of deaths primarily induced by heroin. Heroin/morphyne, as well as hepatitis B and C virus infection, commonly found in drug abusers, may also lead to myocardial alterations/damages. Cases from the study group were compared to the control group by qualification and quantification of inflammatory cells by immunohistochemical methods.

Table 1 Mean counts of myocardial leukocytes, T-lymphocytes and macrophages in the study group of chronic drug abusers, (n=13).

<table>
<thead>
<tr>
<th>N</th>
<th>Age</th>
<th>Sex</th>
<th>LCA</th>
<th>CD45R0</th>
<th>CD68</th>
</tr>
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<td>1</td>
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<td>M</td>
<td>3.40</td>
<td>2.20</td>
<td>2.90</td>
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<td>3.10</td>
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<tr>
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<td>31</td>
<td>M</td>
<td>4.23</td>
<td>1.31</td>
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<tr>
<td>6</td>
<td>26</td>
<td>M</td>
<td>7.10</td>
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<tr>
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LCA-myocardial leukocytes; memory T-lymphocyte (CD45R0+); macrophage (CD68).

Table 2 Mean counts of myocardial leukocytes, T-lymphocytes and macrophages in control group

<table>
<thead>
<tr>
<th>N</th>
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<th>Sex</th>
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<th>CD45R0</th>
<th>CD68</th>
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<td>4.53</td>
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LCA-myocardial leukocytes; memory T-lymphocyte (CD45R0+); macrophage (CD68).

Table 3 Comparison of the observed number of CD45R0 positive, LCA positive and CD68 positive white blood cells in the heart of the control group and drug addicts cases (n = 6 control, and 13 drug addict cases)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>t</th>
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<td>50</td>
<td></td>
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<tr>
<td>CD45R0</td>
<td>Control</td>
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<td>50</td>
<td>0.29</td>
<td>17</td>
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<tr>
<td></td>
<td>Drug addicts</td>
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<td>2.43</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD68</td>
<td>Control</td>
<td>6</td>
<td>3.80</td>
<td>31</td>
<td>2.78</td>
<td>17</td>
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<tr>
<td></td>
<td>Drug addicts</td>
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<td>3.96</td>
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</table>

According to the literature, more than 10.0 LCA+leukocytes, 10.0 CD45R0+T-lymphocytes and CD68+-macrophages in summation: more than 6.0 CD68+ macrophages, as well as more than 2.0 up to 5.0 CD45R0+-T-lymphocytes per high power field (400 X) are considered to be suspicious and must be regarded as reliable sign of myocardial inflammatory process, as found in cases with viral myocarditis. At the same time, signs for acute myocarditis according to Dallas criteria do not have to be found [26, 27].

Immunohistochemical qualification and quantification of interstitial inflammatory cells allow the diagnosis of a borderline inflammation, according to Baughman et al. [28]. Leukocytes, T-lymphocytes and macrophages were found to be in diffuse distribution pattern and microfocal accumulations in the interstitium.

We also observed interstitial fibrosis in a few myocardial samples in study group. Regarding the mentioned cut-off values for T-lymphocytes, leukocytes and macrophages, the number of inflammatory cells was not increased as to an extent to verify an inflammatory process comparable to virus-induced myocarditis as in Dettmeyer investigation [13]. In fact, there were lower mean numbers of leukocytes and T-lymphocytes compared to the control group without statistical differences. Mean numbers of inflammatory cells do not reflect the state of myocarditis, because the distribution of inflammatory cells was unequal in topographically different samples of myocardium for the same patient. We believe that criteria for inflammatory reactions must be adapted according to the finding that the earliest myocardial inflammatory abnormalities were evident in
the lateral wall of the left ventricle, and only these sites revealed myocarditis by histological examination [28], as well as according to the fact that drug abusers have altered immunological system [29, 30]. Our results strongly suggest the alteration of the immune system of drug addicts in correlation to the previous observations [28-35] about the most striking effect on macrophages, the number of which was dramatically lower in the study group. If the number of inflammatory cells is the real reflection of myocardial state, then functional capacities must be under ongoing investigations.

One case in the study group showed a severe perivascular and focal interstitial fibrosis, a histological picture of healed myocarditis or acquired cardiomyopathy, inflammatory type, according to a proposed new contemporary and rigorous classification of cardiomyopathies [36]. In the particular case with the initial acquired cardiomyopathy, the inflammatory type may be taken into account due to the virus infection (hepatitis). The myocardial fibrosis in opiate abuse is not clear. The influence of several adulterants also remains unclear.

In agreement with previous studies [37] we observed that the intake of alcohol was one important risk factor, blood ethanol being detected in 38 % cases. Blood ethanol levels exceeding 0,5 ‰ is associated with low morphine levels (in the subgroup with ethanol it was 0,22 mg/l). Apart from pharmacodynamic interaction between ethanol and heroin resulting in potentiated respiratory depression [38], there is a possibility of a metabolic interaction at the degree of glucuronidation.

Conclusions

The results of this investigation, especially the effect on macrophages, allow us to conclude that the deterioration of immune cell function in heroin abusers will produce in time left ventricular dysfunction and susceptibility to lung edema complicating heroin-overdosage.

List of abbreviations

- Leukocyte common antigen (LCA); Phenotypic memory T-lymphocyte marker (CD45R0+); Phenotypic histiocytic anti-gen (CD68); Monoacetylmorphine (6-MAM); Hematoxilin and eosine (HE); Monoclonal antibodies (UCHL1; PD1/26; 2B11; PG-M1); Phosphate buffered saline (PBS); Diaminobenzidine (DAB); Di-n-butyl phthalate in Xylene mounting histochemistry medium (DPX).

References


