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Markers of cardiac oxidative stress and altered morphology after intraperitoneal cocaine injection in a rat model

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Abstract This study was designed to assess the parameters of myocardial oxidative stress and related cardiac morphological changes following intraperitoneal cocaine exposure in rats. The cardiac levels of reduced glutathione(GSH), oxidised glutathione(GSSG), ascorbic acid (AA), and the production of malondialdehyde (MDA) were measured, as well as the variations of activity in the enzyme systems involved in cell antioxidant defence, glutathione peroxidase (GSH-Px), glutathione reductase (GR) and superoxide dismutase (SOD). After chronic cocaine administration for 30 days GSH was significantly depleted in the heart from 30 min ($P < 0.001$) to 24 h ($P < 0.001$) after exposure, and GSSG was increased for a similar time ($P < 0.05$ at 30 min and $P < 0.01$ at 24 h). SOD increased during the first hour ($P < 0.001$), GR and GSH-Px both increased from 30 min to 24 h, and these increases were statistically significant ($P < 0.01$ and $P < 0.001$ at 30 min and $P < 0.01$ and $P < 0.001$ at 24 h, respectively). The AA levels increased after 1 h ($P < 0.01$), remaining significantly so for 24 h ($P < 0.001$) and MDA increased from 30 min to 24 h, all values being highly significant ($P < 0.001$). The body weight was significantly ($P < 0.001$) reduced in both cocaine groups (40 mg/kg × 30 days and 40 mg/kg × 10 days + 60 mg/kg × 20 days). The heart weight ($P < 0.01$) and its percentage of the body weight ($P < 0.001$) were significantly higher in these two groups than in the controls. Similarly, in the noradrenaline 4 mg/kg × 30 days group, the body weight was significantly ($P < 0.001$) reduced and the heart weight ($P < 0.01$) and its percentage of body weight ($P < 0.001$) were significantly higher than in the controls. In comparing the cocaine and noradrenaline experiments, the frequency and extent of cardiac lesions obtained with 40 mg/kg × 10 days + 60 mg/kg × 20 days of cocaine were similar to those with 8 mg/kg of noradrenaline at 24 h. In this experimental model, cocaine administration compromised the antioxidant defence system of the heart associated with a significant increase of heart weight and the percentage of body weight.

Keywords Cocaine · Myocardial necrosis · Cardiac oxidative stress · Parameters · Contraction band necrosis

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Introduction

The effects of cocaine on the cardiovascular system have been extensively documented both in animal models and in humans [1, 2]. However, the relationship between cardiac morphological alterations and cardiac disorders in cocaine abusers is still controversial. Many reports have postulated a relationship between cocaine abuse and cardiac abnormalities, particularly of an ischaemic nature [3, 4]. Some of the toxic effects of cocaine are due to a blockage of catecholamine re-uptake, the accumulation of which leads to numerous alterations [5, 6].

In a previous study of 26 cocaine-associated deaths in chronic cocaine abusers, no histological evidence of ischaemic myocardial necrosis was found. The only lesion identified was a microfocal myocardial necrosis, pathognomonic of catecholamine myotoxicity [7].

Animal and clinical studies also suggest that cocaine use is associated with increased concentrations of catecholamine in the circulation and that chronic exposure to high levels of adrenaline and noradrenaline can damage the heart [8]. The oxidative metabolism of catecholamines may have damaging effects due to the generation of reac-

tive oxygen species (ROSs) and the formation of oxidation products [9]. ROSs are the critical mediators of cellular damage, and the biochemical parameters investigated in our study are those involved in cell redox state maintenance [10]. In particular, we studied the levels of reduced glutathione (GSH), oxidised glutathione (GSSG), ascorbic acid (AA), and the production of malondialdehyde (MDA), a product of lipid peroxidation indicative of oxidative stress. We also examined variations in activity of the enzyme systems involved in the cell antioxidant defence, e.g. glutathione peroxidase (GSH-Px), glutathione reductase (GR) and superoxide dismutase (SOD).

We determined the concentrations of cocaine (CC) and its metabolites, benzoylecgonine (BE), ecgonine methyl ester (EM), norcocaine (NC), present in the heart to correlate them with any possible local biochemical alterations.

The present study also defined the types of myocardial necrosis and quantified the frequency and extent. This quantification of the morphological changes of cocaine cardiotoxicity should help in defining its functional significance.

Material and methods

Laboratory protocol

The study was approved by the animal study subcommittee of the local institutional review board and conformed to the guiding principles of the guide for the care and use of laboratory animals (NIH publication no. 86-23). Male albino rats with a body weight range 145–330 g (Harlan, Italy) were maintained under standard conditions (MIL, Morini diet; water ad libitum, room temperature 25 °C, alternate 12-h cycles of light and dark) for 7 days before beginning the experiment. Animals were assigned at random to the experimental groups as follows:

1. Control group: 23 animals maintained under standard conditions and killed after 30 days and none died spontaneously during this period: 10 rats were used for the biochemical measurements, 10 for histological examination and 3 rats were the negative control for the toxicological analysis.
2. Cocaine group: composed of six subgroups. In subgroups 1 and 2, 40 mg/kg of cocaine-HCl (Sigma, St. Louis, Mo.) dissolved in 0.9% saline was injected into the peritoneal cavity and the animals were killed after 2 h (group 1, 5 rats), and 24 h (group 2, 5 rats). In a third subgroup (group 3), rats ($n = 20$) were exposed to a daily intraperitoneal injection of 40 mg/kg of CC for 30 days. In a fourth subgroup (group 4, 10 rats) a CC injection of 60 mg/kg was administered for 20 days after a daily intraperitoneal injection of 40 mg/kg of CC for 10 days. In these subgroups the heart was removed and fixed in buffered formalin solution (10%). The last two subgroups were treated for toxicological analysis and biochemical measurements, respectively. In the fifth subgroup (group 5) rats were exposed to CC for 30 days (daily intraperitoneal injection of 40 mg/kg), and were then killed at 5, 15, 30, and 45 min and 1, 2, 4, 8, and 24 h (3 rats for each time period) after the last injection. In the sixth subgroup (group 6), the cocaine treatment was the same as in subgroup 5 and the animals were killed at 0.5, 1, 2, 4, 8, and 24 h (5 rats each group) after the last injection. In the subgroups 5 and 6 the hearts were removed and immediately frozen in liquid nitrogen.
3. Noradrenaline group: noradrenaline was injected into the peritoneal cavity in increasing doses (2, 4 and 8 mg/kg) and the animals (5 rats each time) were killed after 2 and 24 h,and 10 rats were treated with 4 mg/kg body weight per day for 30 days.

The animals which did not die during the test period were anaesthetised with choral hydrate (400 mg/kg i.p, Carlo Erba, Italy) and killed.

Biochemical measurements

Gluthatione (GSH and GSSG) and protein assessment. Heart tissue was homogenised in EDTA K^+ phosphate buffer, pH 7.4 (1 : 3, w/v), at 0 °C, and 1-ml aliquots of the samples were added to an equal volume of 25% trichloroacetic acid (TCA). After centrifugation at 2000 *g* for 15 min (0 °C), the supernatant was rinsed with diethylether. Total gluthatione was analysed as described by Tietze [11] and the oxidised gluthatione was assessed according to Griffith's method [12]. In the remaining aliquot, proteins were assayed according to the method of Lowry [13].

Gluthatione peroxidase (GSH-Px) and gluthatione reductase (GR) assessment. In order to measure cytosolic enzyme activity, the heart samples were homogenised according to Whanger and Butler [14] in 6 vols of cold 0.25 M sucrose in 0.1 M potassium phosphate buffer, pH 7. The homogenates were centrifuged at 40,000 *g* for 20 min at 4 °C and the supernatants were used for GSH-Px and GR assays. GSH-Px activity was measured according to Paglia and Valentine [15], using hydrogen peroxide as the substrate, and the rate of disappearance of NADPH was recorded spectrophotometrically (340 nm) at 37 °C.

GR activity was analysed by the method described by Goldberg and Spooner [16]. GR is highly specific for GSSG and NADPH remains the preferred cofactor for analytical purposes. The reaction forming GSH is highly favoured and catalytic activity is measured spectrophotometrically at 340 nm owing to a decrease in absorbance by the oxidation of NADPH.

The cytosolic protein concentration was determined using the Lowry method with BSA as standard [13].

Superoxide dismutase (SOD) assay. Total superoxide dismutase (Cu/Zn superoxide dismutase and Mn superoxide dismutase) was assayed by a spectrophotometric method based on the inhibition of a superoxide-induced NADH oxidation [17]. The decrease in the rate of NADH oxidation is dependent on the enzyme concentration and subsequently saturation levels are attainable. The tissue extract was first prepared by homogenising the heart tissue in 3 vols of 25 mM triethanolamine-diethanolamine buffer, pH 7.4, and then cleared by centrifugation at 40,000 *g* for 60 min at 4 °C. The supernatant was dialysed against a cold homogenisation buffer and then used for enzyme assays.

Malondialdehyde (MDA) assessment. The extent of lipid peroxidation in the rat hearts was estimated by calculation of MDA levels. Samples were homogenised in a 0.04 M K^+-phosphate buffer (pH 7.4) containing 0.01% butyl hydroxytoluene (BHT) (1 : 5 w/v, 0 °C) to prevent the artificial oxidation of polyunsaturated free fatty acids during the assay. This homogenate was deproteinised with acetonitrile (1 : 1) and then centrifuged at 3000 *g* for 15 min The supernatant was utilised for MDA HPLC analysis according to Shara et al. [18].

Ascorbic acid (AA) assay. Tissues were homogenised in EDTA-K^+ phosphate buffer pH 7.4 (1 : 4, w/v) at 0 °C and 0.6 ml aliquots of the samples were added to an equal volume of 10% (w/v) metaphosphoric acid. The samples were immediately centrifuged at 2000 *g* and 0 °C for 10 min. The supernatants were filtered (Anotop 0.2 µm, Merck) and 20 µl was injected into HPLC column and analysed as described by Ross [19].

Toxicokinetics analysis

Heart samples were homogenised in ice-cold 0.5% NaF and centrifuged at 2000 *g* for 10 min. The supernatants were utilised for the CC and metabolite assays according to Cardenas et al. [20].

Table 1 Effect of repeated cocaine exposure on cardiac antioxidant non-enzymatic systems (*GSH* reduced glutathione, *GSSG* oxidised glutathione, *MDA* malondialdehyde, *AA* ascorbic acid)[a]

Group	Time (min)	GSH (nmol/mg protein)	GSSG (nmol/mg protein)	MDA (nmol/g tissue)	AA (nmol/g tissue)
Cocaine	30	6.5 ± 0.55***	0.58 ± 0.111*	1.16 ± 0.025***	181.9 ± 40.65
	60	7.1 ± 0.47***	0.55 ± 0.099*	1.69 ± 0.190***	210.5 ± 20.1**
	120	7.7 ± 0.46***	0.59 ± 0.040***	1.41 ± 0.146***	261.4 ± 21.58***
	240	8.2 ± 1.00**	0.59 ± 0.027***	1.26 ± 0.060***	257.7 ± 53.52***
	480	7.3 ± 0.12***	0.57 ± 0.014***	1.32 ± 0.103***	235.0 ± 26.66***
	1440	7.1 ± 0.71***	0.57 ± 0.075**	1.18 ± 0.034***	308.4 ± 49.62***
Control		10.5 ± 1.23	0.45 ± 0.06	1.04 ± 0.011	184.3 ± 10.51

*$P < 0.05$ compared with control group
**$P < 0.01$ compared with control group
***$P < 0.001$ compared with control group
[a] Values are expressed as means ± SD (n = 10 rats for control group and 5 rats for cocaine groups)

The method involved a solid-phase extraction (SPE) based on the retention properties of cocaine and its metabolites on a reversed-phase C18 column, pre-concentration (evaporation under nitrogen), and derivatisation of CC and its metabolites in supernatant samples for subsequent analysis by gas chromatography and mass spectrometry (GC-MS).

Histological examination

The heart was removed and fixed in a buffered formalin solution (10%), and then sliced in three whole-heart sections. The central section was processed for histological examination and stained with haematoxylin-eosin and Masson trichrome.

Quantitative analysis

A randomly selected histological section was measured by an image analyser (Vidas, Zeiss) and the total area calculated in pixels and converted to square millimeters through a calibration procedure utilising a reference system. The number of foci and the number of necrotic cardiomyocytes were normalised at 100 mm^2. The heart weight index corresponds to the heart weight compared to body weight (in %).

Statistics

Data are expressed as mean values ± standard deviation. Student's *t*-test for unpaired data or non-parametric Mann-Whitney or Wilcoxon tests for skewed variables or one-way analysis of variance and post hoc Scheffe's test for continuous variables and χ^2-test for discrete variables were used to assess the significance of differences statistically. Linear regression analysis was used to determine the presence of a correlation between continuous variables. A probability of $P < 0.05$ was considered as significantly different.

Results

Cardiac oxidative stress parameters

In the rats exposed to CC for 30 days (40 mg/kg), we observed the following results (Tables 1 and 2):

a. GSH was significantly depleted in the heart from 30 min ($P < 0.001$) to 24 h ($P < 0.001$) and GSSG was increased at similar time periods ($P < 0.05$ at 30 min and $P < 0.01$ at 24 h).

Table 2 Effect of repeated cocaine exposure on cardiac antioxidant-related enzymes (*SOD* superoxide dismutase, *GR* glutathione reductase, *GSH-Px* glutathione peroxidase)[a]

Groups	Time (min)	SOD (U/mg protein)	GR (U/mg protein)	GSH-Px (U/mg protein)
Cocaine	30	3.09 ± 0.516***	22.4 ± 8.53**	221.2 ± 34.65***
	60	3.54 ± 0.800***	22.9 ± 5.03***	225.1 ± 50.36***
	120	2.18 ± 1.277	22.5 ± 7.34***	209.1 ± 90.97***
	240	1.97 ± 0.827	21.7 ± 5.33***	225.1 ± 52.30***
	480	1.88 ± 0.108	18.4 ± 5.27**	205.9 ± 99.49*
	1440	1.60 ± 0.245	17.1 ± 4.92**	298.0 ± 74.85***
Control		1.77 ± 0.451	12.3 ± 1.28	128.4 ± 22.18

*$P < 0.05$ compared with control group
**$P < 0.01$ compared with control group
***$P < 0.001$ compared with control group
[a] Values are expressed as means ± SD (n = 10 rats for control group and 5 rats for cocaine groups)

b. SOD increased during the first hour ($P < 0.001$) and GR and GSH-Px both increased from 30 min to 24 h ($P < 0.01$ and $P < 0.001$ at 30 min or $P < 0.01$ and $P < 0.001$ at 24 h, respectively).
c. AA levels increased after 1 h ($P < 0.01$) remaining significant for 24 h ($P < 0.001$).
d. MDA increased from 30 min to 24 h, all values being highly significant ($P < 0.001$).

Toxicokinetic results

Figure 1 illustrates concentrations of CC, EM, BE and NC in the heart as a function of time. The t_{max} for CC was 5 min, which was still detectable for 8 h, EM t_{max} was 15 min, which was still detectable in the heart after 24 h (0.02 µg/ml), BE in the heart had a t_{max} of 45 min and was no longer detectable after 8 h, and NC had a t_{max} of 5 min, detectable for 2 h.

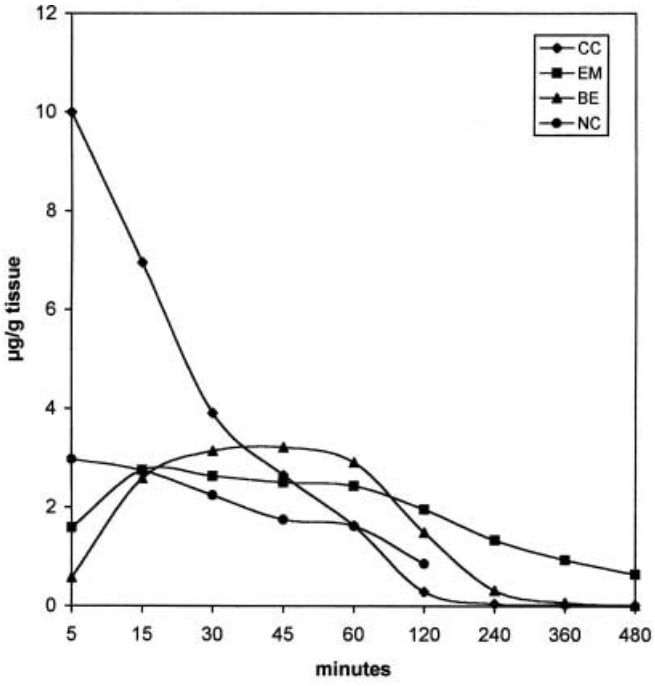

Fig. 1 Concentration-time profile for cocaine and its metabolites in the heart after administration of 40 mg/kg cocaine × 30 days

Animal survival and the ratio of the heart weight to body weight

A CC dose of 40 mg/kg did not result in spontaneous death or myocardial lesions within 24 h. Only 55% of the animals survived up to 30 days after a dose of 40 mg/kg of CC while 45% died spontaneously after 26 ± 6 days. With a CC dose of 40 mg/kg, followed by 60 mg/kg after 10 days, 90% of the animals died spontaneously (survival 20 ± 5 days).

The body weight was significantly ($P < 0.001$) reduced in both CC 40 mg/kg × 30 days and 40 mg/kg × 10 days + 60 mg/kg × 20 days. The heart weight ($P < 0.01$) and its percentage of the body weight ($P < 0.001$) were significantly higher in these two groups than in the controls (Table 3). Similarly, in the noradrenaline 4 mg/kg × 30 days group, the body weight was significantly ($P < 0.001$) reduced and the heart weight ($P < 0.01$) and its percentage of body weight ($P < 0.001$) were significantly higher than in controls.

Histopathology of the heart

After chronic CC exposure rats showed contraction band necrosis with the maximal extent obtained with 40 mg/kg

Table 3 Body weight, heart weight and cardiac index in different groups of rats

Source	Number of rats	Body weight (g)	Heart weight (g)	Cardiac index[a]
Cocaine				
40 mg/kg × 30 days	20	257 ± 35	0.78 ± 0.107	0.31 ± 0.037
40 mg/kg × 10 days + 60 mg/kg × 20 days	10	239 ± 23	0.80 ± 0.103	0.34 ± 0.050
Noradrenaline				
4 mg/kg × 30 days	10	188 ± 17	0.80 ± 0.050	0.43 ± 0.010
Controls	10	284 ± 26	0.68 ± 0.030	0.24 ± 0.020

[a] Cardiac index = heart weight/total body weight × 100

Table 4 Frequency and extent of contraction band necrosis in cocaine versus noradrenaline-induced myocardial toxicity (*K* killed, *D* died spontaneously)

Groups	Rats			Survival days	Contraction band necrosis		
	No	K	D		Present	Foci	Cardiomyocytes
Cocaine (mg/kg)							
40 × 2 h	5	5					
40 × 24 h	5	5					
40 × 30 days	20	11	9	26 ± 6	5	36 ± 54	62 ± 103
40 × 10 days + 60 × 20 days	10	1	9	20 ± 5	7	146 ± 222	543 ± 893
Noradrenaline (mg/kg)							
2 × 2 h	5	5			3	32 ± 41	51 ± 74
2 × 24 h	5	5			3	104 ± 60	187 ± 101
4 × 2 h	5	5			2	83 ± 24	242 ± 129
4 × 24 h	5	3	2		4	37 ± 43	64 ± 93
4 × 30 days	10	5	5		6	96 ± 186	161 ± 327
8 × 2 h	5	5			5	130 ± 113	230 ± 227
8 × 24 h	5	1	4	4 ± 1	5	221 ± 104	426 ± 269
Controls	10	10		30	1	3	8

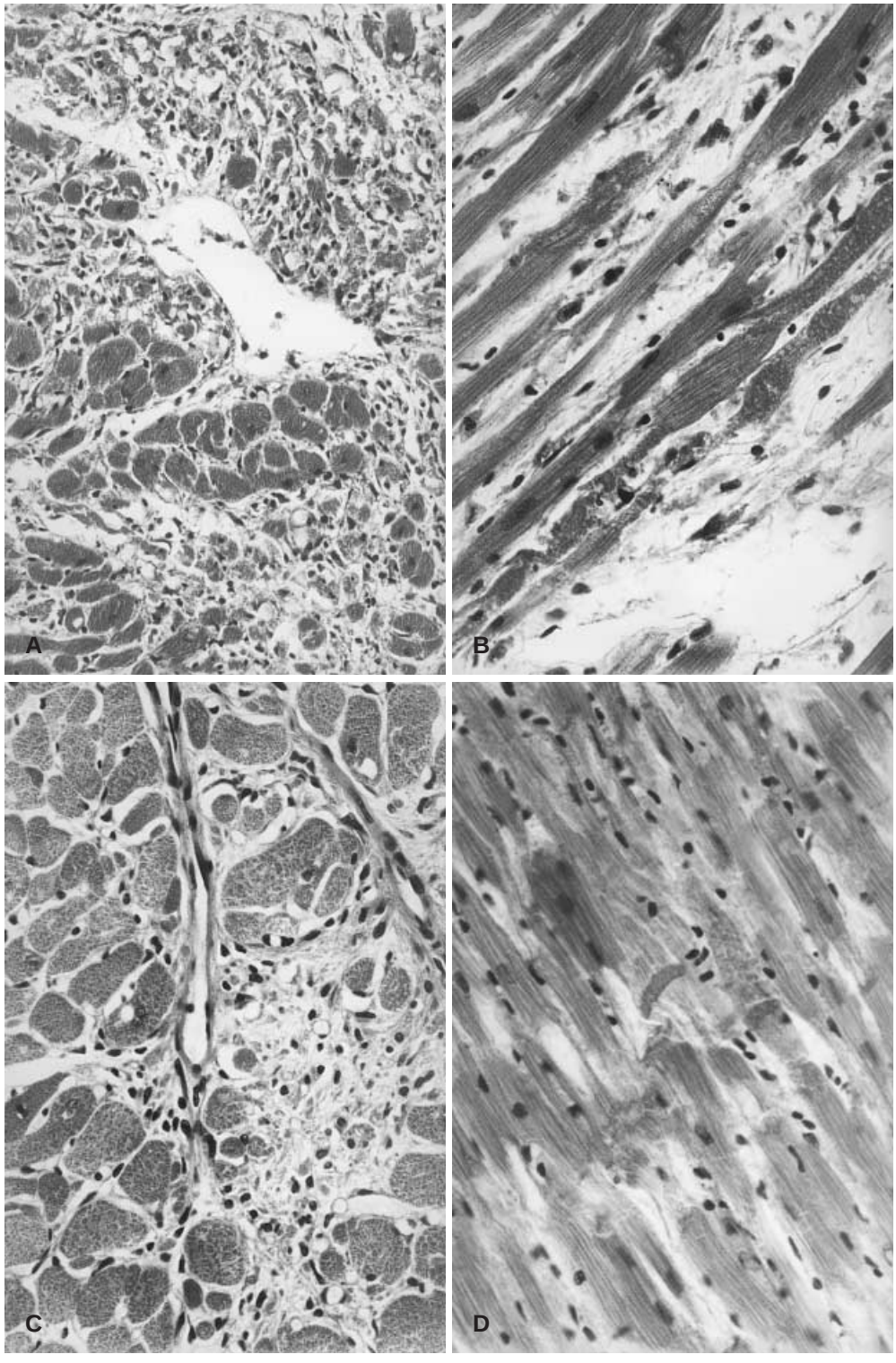

Fig. 2 **A** Confluent foci of advanced contraction band necrosis plus macrophage reaction. Rat treated with cocaine 40 mg/kg × 10 days + 60 mg/kg × 20 days, with a survival time of 29 days (H&E × 250). **B** Early contraction band without macrophage infiltration in a rat treated with 40 mg/kg of cocaine which died at 15 days (H&E × 400). **C** Healing phase of contraction band necrosis in a rat treated with 4 mg/kg of noradrenaline which died spontaneously at 10 days (H&E × 400). **D** Early contraction band in a rat which died after 4 h following 8 mg/kg of noradrenaline (H&E × 400)

× 10 days + 60 mg/kg × 20 days (Table 4). In the noradrenaline group, the majority of animals presented contraction band necrosis at any given dose. In comparing the CC and noradrenaline experiments, the values obtained with 40 mg/kg × 10 days + 60 mg/kg × 20 days were similar to those with 8 mg/kg of noradrenaline at 24 h (Fig. 2). Only one control rat presented three foci of contraction band necrosis with eight necrotic cardiomyocytes on the whole, and none died spontaneously. Myocardial fibrosis, platelet aggregation in coronary vessels, infarct necrosis or other pathological changes were not detected in either the cocaine or noradrenaline group. Healing necrotic foci were seen in rats treated with noradrenaline for more than 10 days.

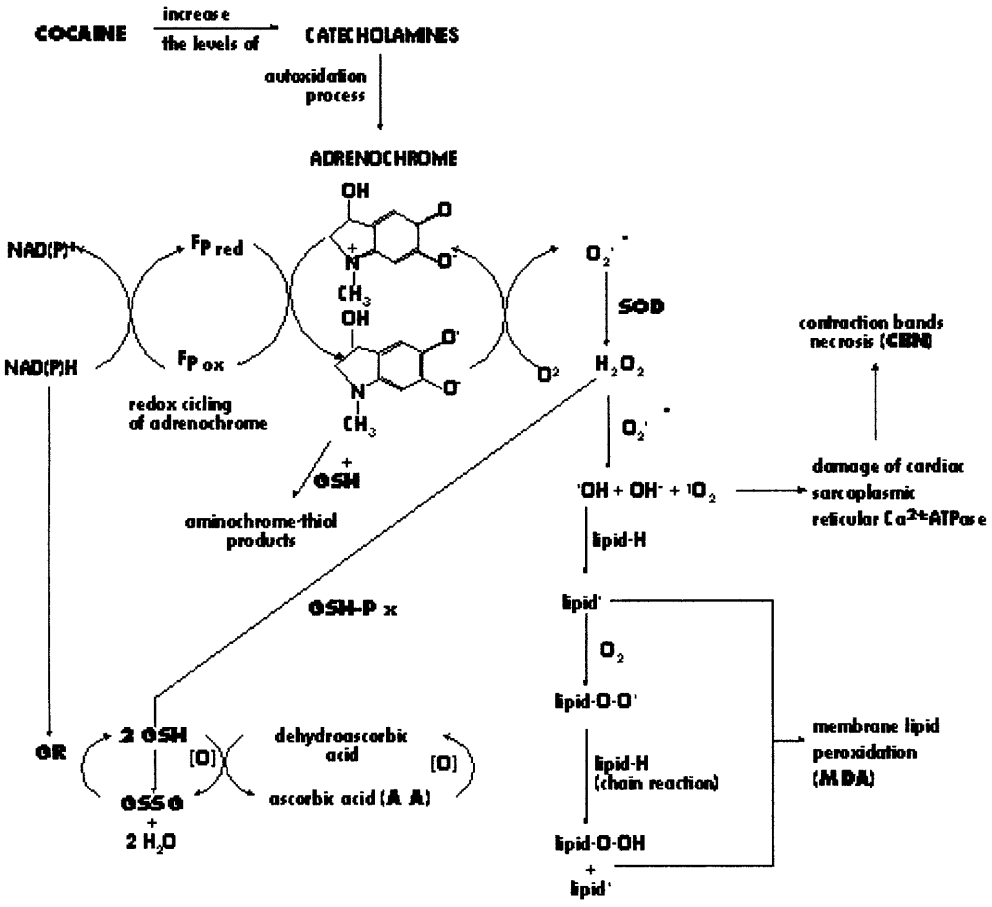

Fig. 3 Proposed mechanism for cocaine-mediated catecholamine cardiotoxicity

Discussion

The role of CC cardiotoxicity and consequent reversible and irreversible pathological changes are well established. Nevertheless, the mechanisms leading to cardiac injury and irreversible myocardial cellular changes remain elusive [10, 21]. Central and peripheral stimulation of the sympathetic and renin-angiotensin systems by CC appears to form a major component of the acute toxicity of this alkaloid, which seems to result in the uncontrolled loss of physiological mechanisms used for normal regulation [22]. Contrary to the general opinion that excess catecholamines produce cardiotoxicity mainly through binding to adrenoceptors, there is increasing evidence that catecholamine-induced deleterious actions may also occur through oxidative mechanisms [23]. Recent studies have shown that oxidation of catecholamines results in the formation of highly toxic substances such as aminochromes (e.g. adrenochrome) and free radicals, and by virtue of the latter's actions on different types of heart membranes, they cause intracellular Ca^{2+} overload and myocardial cell damage (Fig. 3) [24, 25].

Cardiac parameters of oxidative stress

Intracellular concentrations of GSH, GSSG and GSH/GSSG ratios are routinely utilised as indicators of oxidative stress [26]. The GSH depletion that we observed in the rat heart may be due to its interaction with adrenochrome derived from auto-oxidation of catecholamine. In fact, adrenochrome is particularly reactive with several cell nucleophiles, such as GSH, resulting in oxidation, substitution or reduction products. GSH-depletion may also be the result of its utilisation in the ascorbic acid cycle due to the reduction of dehydroascorbate, a reaction catalysed by a GSH-dependent transhydrogenase. Ascorbic acid levels were shown to have increased and this is likely to be due to the increased requirement of antioxidants caused by oxidative stress, unfulfilled as a result of GSH deficiency. Adrenochrome is a likely candidate for such a process of redox cycling, leading to the formation of oxygen free radicals such as the superoxide anion which, after dismutation, gives rise to hydrogen peroxide. Hydrogen peroxide through an iron-catalysed Haber-Weiss mechanism can produce the hydroxyl radical (OH) and singlet oxygen that are particularly damaging to DNA and proteins [25].

The enhanced activity of SOD in the heart after repeated cocaine administration and the increased lipid peroxide measure, MDA, may indicate:

1. A hydrogen peroxide increase as a product of SOD secondary to the increased activity or,
2. The inadequate transformation of hydrogen peroxide into water by GSH-Px.

The role of singlet oxygen in myocardial cell damage has been studied by Ver Donck et al. [27] who observed ultrastructural injury in isolated myocytes when exposed to singlet oxygen.

The oxidative stress can thus modify the activity of ion translocation proteins in the sarcoplasmic reticulum resulting in intracellular calcium overload, oscillatory release of calcium from the sarcoplasmic reticulum and potential oscillation in membrane potential, as observed by Vandeplassche et al. [28]. Itoh et al. [29] demonstrated that there is an observed reduction of calcium-ATPase activity following ischaemia/reperfusion, and a fivefold increase in free radical production. Furthermore, Kukreja and Hess [30] demonstrated that singlet oxygen is responsible for calcium-ATPase impairment in heart sarcoplasmic reticulum.

The final result of these modifications may be a morphological and functional alteration of myocytes and the formation of contraction band necrosis as a result of impaired intracellular calcium regulation due to free radical production. It has been shown that recovery of cardiac contractility following reperfusion is more frequent in the hearts treated with oxygen radical scavengers.

The hypothesis of CC cardiotoxicity can therefore be related to a catecholamine surplus caused by a CC-produced re-uptake block. It is evident that catecholamines are the most important source of free radicals and can potentiate tissue damage through their reaction with GSH, weakening the intracellular GSH-Px which protects the membranes from lipid peroxidation.

Our experimental data reveal the following observations:

1. A decrease in GSH accompanied by an increase in GSSG in CC-treated hearts indicating that the cellular antioxidant system was overwhelmed by reactive oxygen species.
2. The MDA production as an index of lipid peroxidation induced by hydrogen peroxide surplus from SOD action, and inadequately transformed by GSH-Px into water.

Kinetics of cocaine and the cardiac response

Metabolites of CC have effects on the cardiovascular system of rats and are directly responsible for CC cardiotoxicity. Cocaine is rapidly degraded in the liver once administered where hydrolysis of CC produces BE and EM and is the major metabolic pathway for CC. In particular, unlike CC, BE and EM are slowly excreted by the body and increase blood pressure due to a possible sympathomimetic effect, creating serious cardiovascular complications. The fact that both these compounds increase blood pressure without having a concomitant local anaesthetic effect, may contribute to the toxicity often observed hours after CC administration [2, 31].

Morphological findings

In a previous publication, a definition of the different forms of myocardial cell necrosis was reported [7]. In the present study, the only myocardial lesion found corresponded to “contraction band necrosis” or “coagulative myocytolysis” or “Zenker necrosis”: the two latter terms prove to be more precise due to the presence of different types of contraction bands and they indicate a necrosis of the myocardial cells in a hypercontracted state (tetanic death) characterised by rhexis of the myofibrillar apparatus, anomalous hypereosinophilic cross-bands formed by segments of hypercontracted sarcomeres with extremely thickened *Z* lines, as shown ultrastructurally [32, 33, 34, 35].

Within chronological limits, the present findings confirm the conclusions of the previous human study [7]:

a. Cocaine-related adrenergic overactivity does not induce extensive myocardial necrosis and thus per se is unable to explain the cardiac arrest.
b. This lesion is the histological hallmark of an acute adrenergic stress linked with malignant arrhythmia.
c. The absence of myocardial fibrosis and any other histologically observable myocardial or vascular signs of ischemia exclude morphological or functional coronary obstruction in the genesis of cocaine-related disorders possibly due to an episodic adrenergic crisis in some prone individuals.

A decrease in body weight and an increase of heart weight were observed both in cocaine and noradrenaline-treated rats. It is known that CC potentiates catecholamine action by inhibition of the pre-synaptic uptake carrier and prolonged abuse of CC results in myocardial hypertrophy. A number of human case reports have noted an association of CC abuse with cardiac hypertrophy [36]. Increases in left ventricular mass measured with an echocardiogram were documented in human CC abusers when compared to age-matched control subjects [37]. Cocaine use is also known to cause increased plasma concentration of atrial natriuretic peptide, indicative of circulatory overload and pump failure [38]. In the present experiment a significant increase of heart weight and its percentage in relation to body weight was clearly demonstrated. This may be due to excessive adrenergic tone triggered by CC rather than to a primary action of the latter [39].

In conclusion, our data suggest that cocaine administration in a rat model compromised the antioxidant defence system of the heart through an adrenergic overstimulation, which promotes sensitivity to cathecolamines, resulting in arrhythmogenic dysfunctional disorder [40] and/or typical myocardial damage.

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