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Case report

A case of fatal ephedra intake associated with lipofuscin accumulation, caspase activation and cleavage of myofibrillary proteins

Carol Chen-Scarabelli[a,*], Siân E. Hughes[b], Giorgio Landon[b], Peter Rowley[b], Zuhair Allebban[c], Noel Lawson[d], Louis Saravolatz[e], Julius Gardin[c], David Latchman[f], Tiziano M. Scarabelli[c]

[a]*Division of Cardiology, VA Medical Center, University of Michigan, Ann Arbor, MI, USA*
[b]*Department of Histopathology, Royal Free and University College Medical School, University College London, London, UK*
[c]*Divisions of Cardiology, St John Hospital, Wayne State University, Detroit, MI, USA*
[d]*Divisions of Pathology, St John Hospital, Wayne State University, Detroit, MI, USA*
[e]*Divisions of Internal Medicine, St John Hospital, Wayne State University, Detroit, MI, USA*
[f]*Institute of Child Health, University College London, London, UK*

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Abstract

Ephedra, a herb reported to suppress appetite and stimulate the sympathetic nervous system as well as cardiac performance, has recently been related to several adverse events, including seizure, stroke, hypertension, myocardial infarction, and sudden death. Here, we describe the case of a 45-year-old woman who died of cardiovascular collapse while taking ephedra. Tissue analysis revealed non-specific degenerative alterations in the myocardium (lipofuscin accumulation, basophilic degeneration and vacuolation of myocytes, as well as myofibrillary loss), associated with myocyte apoptosis, caspase activation, and extensive cleavage of miofibrillary proteins α-actin, α-actinin, and cardiac troponin T. Healthcare professionals are therefore urged to warn their patients about the risk of serious adverse effects, which may follow ephedra intake.

Keywords: Ephedra; Lipofuscin accumulation; Caspase activation; Cleavage of myofibrillary proteins

Ephedra, also known as “ma huang, ephedra sinica, ephedrine, sida cordifolia, and epitonin”, has been used in China for thousands of years to treat asthma and other respiratory disorders [1–3]. This herb has been reported to stimulate the sympathetic nervous system and the heart, and suppress appetite. The reported effects have resulted in wide marketing of this substance in dietary supplements for weight loss, as well as for enhanced athletic performance. However, supplements containing ephedra or its alkaloid derivative, ephedrine, have been linked to serious adverse events, including seizure, stroke, and cardiovascular effects, ranging from hypertension and myocardial infarction to sudden death [1–4].

1. Case report

We report a case of a 45-year-old woman who died of cardiovascular collapse while taking ephedra.

A 45-year-old woman was admitted to the hospital with sudden cardiovascular collapse after taking aspirin, while using Xenadrin diet supplements. According to the emergency responder report, the patient complained of a headache and took two aspirin tablets. She subsequently vomited and lost consciousness. Emergency medical care was summoned and the patient, found in ventricular fibrillation and apneic, was resuscitated and intubated on the scene, before being transported to the hospital. Her medical history was significant for migraines, a lump in the breast without medical follow-up, and dermatitis. Review of her medications revealed use of *Xenadrin* diet pills (see Table 1) [5], *aspirin, Prozac*, and Nicotrol inhaler. The patient had a 25

* Corresponding author. Tel.: +1 734 769 7100x5425; fax: +1 413 473 9027.
E-mail address: cchensc@med.umich.edu (C. Chen-Scarabelli).

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Table 1
Composition of Xenadrin RFA-1 (Cytodyne Technologies, Lakewood, NJ)

Content in mg of two capsules of Xenadrin:
Citrus auranticum (4% synephrine), 125 mg
Ma Huang (6% ephedrine), 335 mg
Guarana extract (22% caffeine), 910 mg
White willow bark (15% salicin), 105 mg
Acetyl L-carnitine, 100 mg
L-Tyrosine, 80 mg
Ginger root, 50 mg
Vitamin B5, 40 mg

Xenadrin's formula consists of ephedrine, caffeine, and salicin, all chemicals which have been reported to have significant interactions.

pack-year history of smoking, but had quit 8 months ago. There was no history of illicit drug use.

Upon admission to the hospital, initial workup included a blood toxicology screen, which was positive for cannabinoids. The cardiac markers were initially normal, but rose significantly five h later (CK-MB was 112, normal <5; and cardiac troponin I level was 33.1, normal 0.0–0.4), and normalized after 5 days. The initial 12-lead electrocardiogram (ECG) on admission showed sinus tachycardia with ST and T wave depressions in the anterior, lateral and inferior leads, and Q waves in the inferior leads. A repeat ECG 4 days later showed T wave inversions in the lateral leads (leads I, AVL, V5-6). A CT scan of the brain showed cerebral edema, but no haemorrhage or masses. A 2D Echocardiogram revealed an ejection fraction of 25%, with global hypokinesis. The patient was subsequently admitted to the Neuro ICU for further management. After 6 days on life support, she was pronounced dead by electroencephalogram, apnea test, and physical exam. Vasopressor support (with Dopamine and Levophed to maintain a systolic blood pressure above 95 mm Hg) was subsequently initiated for organ preservation. The heart, which showed dilation of the left ventricle with no evidence of coronary artery disease or dissection, was explanted by the Organ Procurement Service and preserved in cardioplegia solution for research purposes. The cause of death was listed as respiratory failure and cardiac arrest resulting in anoxic encephalopathy. The causality assessment performed by means of the Naranjo algorithm produced a score of 4, which categorized this sudden cardiovascular event as a "possible" adverse reaction to ephedra.

2. Laboratory assessments

Pathological evaluation revealed non-specific degenerative changes in the myocardium, including widespread accumulation of lipofuscin pigment, basophilic degeneration and vacuolation of myocytes, following extensive myofibrillary loss (Fig. 1). There was no evidence of multifocal and confluent myocyte necrosis, myocarditis, interstitial fibrosis, myocyte disarray, or myocyte hypertrophy, pathological features previously described in

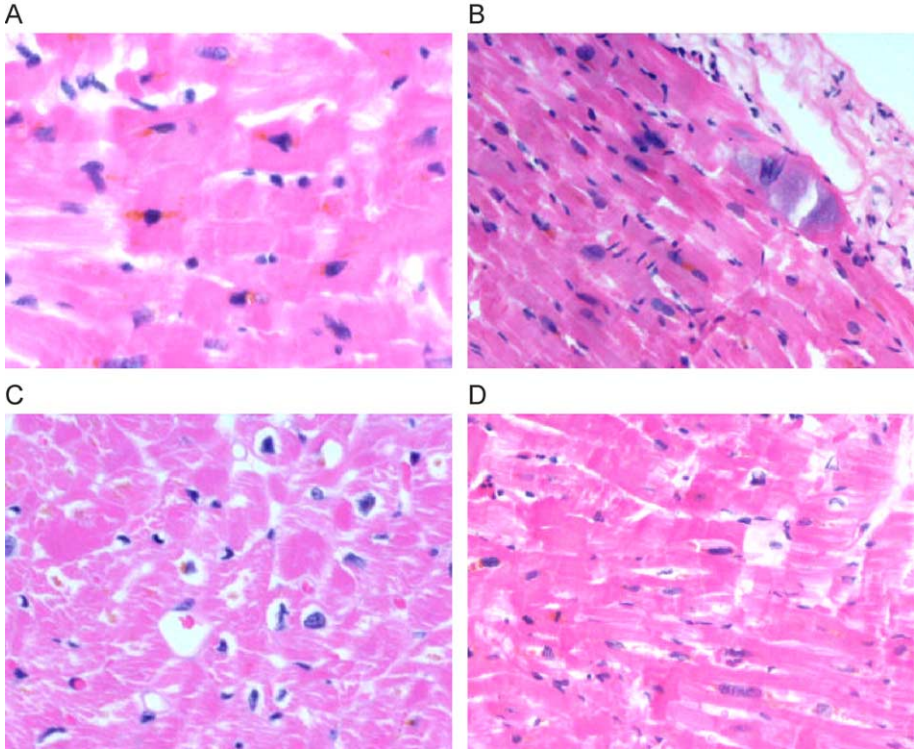

Fig. 1. Haematoxylin and eosin section of myocardium. (a) Myocytes containing lipofucsin pigment as brown granules with a predominantly perinuclear distribution. (b) Basophilic degeneration of a cardiac myocyte exhibiting a blue/grey amorphous material within the cytoplasm. (c) Myocyte vacuolization due to myofibrillary loss. (d) Contraction bands within individual myocytes.

patients who experienced sudden death associated with ephedra intake [6]. However, evidence of acute ischemia in the form of contraction band necrosis was also noted (Fig. 1).

By immunohistochemistry, activation of caspase-9 (mitochondrial apoptotic pathway) and caspase-3 was detected in the majority of cardiac cells (56±3.4% and 68±5.1%, respectively), although colocalization of cleaved caspase-3 and TUNEL positive labelling, previously used as a marker of apoptotic cell death [7], was only observed in a small number of myocytes (<5%) (Fig. 2a and b). Caspase-9 and caspase-3 activation was confirmed both by Western blotting analysis and functional evaluation of caspase activity, performed in tissue extracts, as previously described (Fig. 2c) [8]. Conversely, processing of caspase-8 (death receptor-mediated apoptotic pathway) was never detected by any of the aforementioned techniques (Fig. 2). In the same heart, Western blot also documented cleavage of myofibrillary proteins α-actin, α-actinin, and cardiac troponin T (cTnT), with subsequent generation of cleaved fragments (Fig. 3). Conversely, neither caspase activation, nor production of α-actin, α-actinin, and cTnT fragments was observed in three control hearts from subjects deceased from non-cardiac causes (Fig. 3).

3. Discussion

Ephedrine is a sympathomimetic drug (with both α- and β-adrenergic agonist properties) whose cardiovascular toxic effects are postulated to be due to several mechanisms, including coronary artery vasoconstriction, vasospasm, increase in cardiac contractility and automaticity, shortening of the cardiac refractory periods (thereby allowing development of re-entrant cardiac arrhythmias), and myocardial ischemia, due to sudden increase in myocardial oxygen requirements and catecholamine excess, with resultant fibrosis and even death [1–4].

Lipofuscin, an end-product of intracellular lipid peroxidation and a marker of cellular oxidative damage, accumulates over time during the normal aging process [9]. However, accelerated lipofuscin accumulation is associated with numerous adverse effects [10].

Ephedra enhances norepinephrine release with subsequent catecholamine excess and increased sympathetic output. The combination of ephedrine with caffeine (in the form of guarana) and aspirin (in the form of white willow bark or salicin) in the same over-the-counter preparations, such as Xenadrin, is reported to markedly potentiate the effects of ephedrine, by inducing sustained release of norepinephrine [11]. An accelerated basal

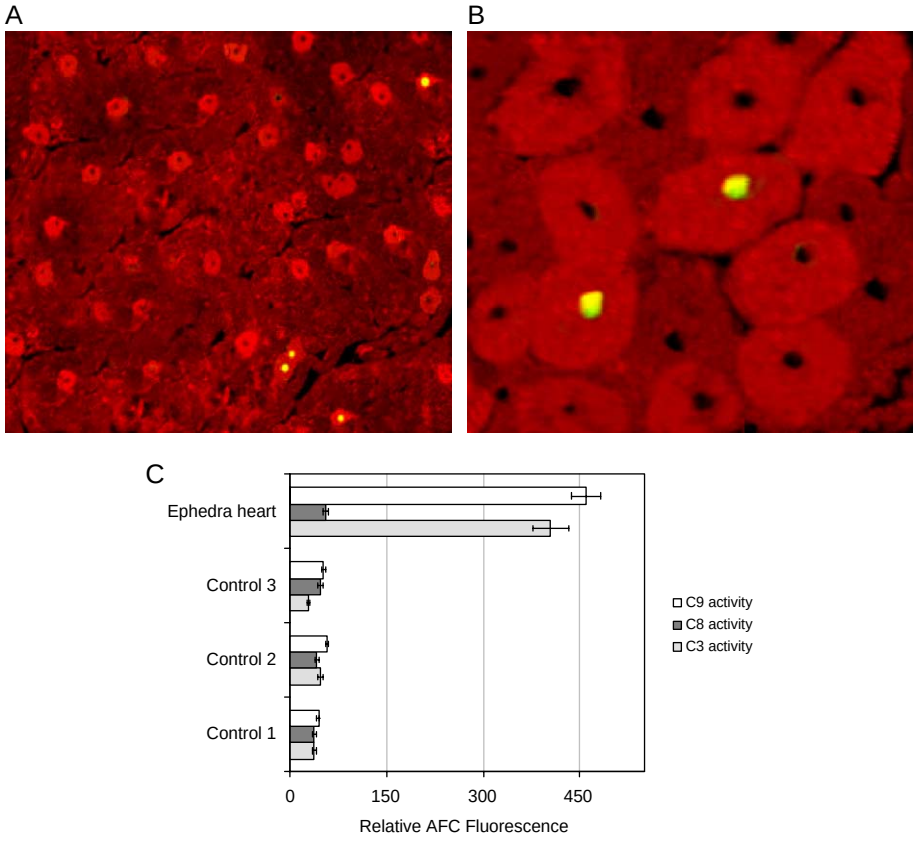

Fig. 2. Low (panel a) and high (panel b) power field of cardiac myocytes showing cytoplasmic anti-active caspase-3 positive staining (bright red) sporadically colocalized with TUNEL positive labeling (yellow/green nuclei). Caspase-3, -8 and -9 enzymatic activity (panel c) in tissue extracts from ephedra heart and control hearts explanted from non-cardiac patients. Data are expressed as mean±S.D. ***$p<0.001$ vs. control hearts.

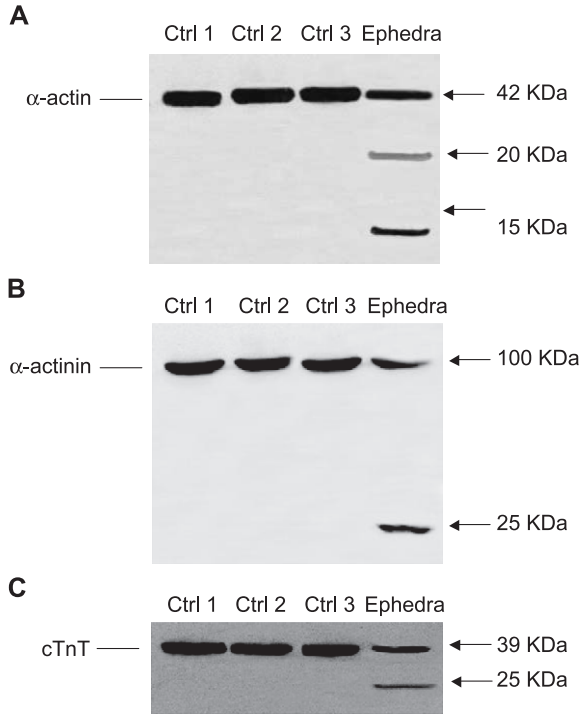

Fig. 3. Western blotting showing reduction of intact α-actin (panel a), α-actinin (panel b), and cardiac troponin T (cTnT; panel c), with subsequent generation of smaller fragments, in the explanted ephedra heart, though not in explanted hearts from patients deceased from non-cardiac reasons.

metabolic rate associated with inanition (such as in the setting of ephedra intake) leads to lipid peroxidation, resulting in increased lipofuscin formation. In this setting, malonaldehyde, a by-product, forms and reacts with nuclear DNA, thereby blocking template activity and reducing protein synthesis ability. As a consequence, mitochondrial dysfunction and limitation in contractile protein replacement occur [10].

Apoptosis has been implicated in the pathogenesis of several cardiovascular disorders, including adrenergic-mediated cardiac toxicity [11]. The apoptotic process is mediated by specialised proteases, called caspases, whose sequential activation is accountable for the cleavage of major cytosolic and nuclear cell components [12]. Caspase activation has also been associated with early myofibrillar protein cleavage, resulting in decreased ATPase activity and contractile dysfunction, before the occurrence of the typical DNA fragmentation detected by TUNEL staining [11,13]. In addition, β-adrenergic stimulation of isolated cardiac myocytes was shown to induce early activation of caspase-9 and caspase-3, via the mitochondria-initiated pathway, as well as cleavage of cardiac troponin I and actin, when TUNEL positive staining was not observed [13]. In line with these findings, we observed broad myofibrillary loss, together with extensive myocyte activation of caspase-9 and caspase-3, which was largely independent from DNA fragmentation. Since this pattern was not observed in previous studies carried out in animal [7] and human [14] models of ischemia/reperfusion injury, it is possible that ephedra toxicity, rather than severe myocardial ischemia secondary to cardiac arrest, is the predominant factor leading to massive caspase activation mainly unrelated to DNA cleavage.

In conclusion, although the human data are necessarily associative, and do not prove causation, our findings seem to suggest that ephedra intake, via enhanced release of sympathetic amines and following β-adrenergic stimulation, may selectively trigger the mitochondria-initiated apoptotic pathway, leading to caspase-9 and following caspase-3 activation. Upon activation, caspase-3 would induce early breakdown of myofilaments, with subsequent functional impairment of cardiac function, before the completion of the apoptotic process, and independently from extensive necrotic cell death. However, although this hypothesis is well supported by experimental data in the rat, further studies are needed to prove such a causative harmful effect of ephedra in the human heart.

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