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Acute Hemorrhagic Myocardial Necrosis and Sudden Death of Rats Exposed to a Combination of Ephedrine and Caffeine

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Because of possible side effects of herbal medicines containing ephedrine and guarana-derived caffeine, including increased risk of stroke, myocardial infarction, and sudden death, the Food and Drug Administration recently banned the sale of ephedra-containing products, specifically over-the-counter dietary supplements. We report cardiac in 7- and 14-week-old male F344 rats exposed by gavage to ephedrine (25 mg/kg) and caffeine (30 mg/kg) administered in combination for one or two days. The ephedrine-caffeine dosage was approximately 12- and 1.4-fold, respectively, above average human exposure, based on a mg/m^2 body surface-area comparison. Several (5/7) of the exposed 14-week-old rats died or were sacrificed *in extremis* 4–5 h after the first dosing. In these hearts, changes were observed chiefly in the interventricular septum but also left and right ventricular walls. Massive interstitial hemorrhage, with degeneration of myofibers, occurred at the subendocardial myocardium of the left ventricle and interventricular septum. Immunostaining for cleaved caspase-3 and hyperphosphorylated H2A.X, a histone variant that becomes hyperphosphorylated during apoptosis, indicated multifocal generalized positive staining of degenerating myofibers and fragmenting nuclei, respectively. The Barbeito-Lopez trichrome stain revealed generalized patchy yellow myofibers consistent with degeneration and/or coagulative necrosis. In ephedrine-caffeine-treated animals terminated after the second dosing, foci of myocardial degeneration and necrosis were already infiltrated by mixed inflammatory cells. The myocardial necrosis may occur secondarily to intense diffuse vasoconstriction of the coronary arterial system with decreased myocardial perfusion. Our work shows the direct relationship between combined ephedrine and caffeine exposure and cardiac pathology.

Key Words: **ephedrine; caffeine; cardiotoxicity; apoptosis; coagulative necrosis; ischemia.**

Many studies in the literature suggest that the dietary supplements containing ephedra alkaloids derived from the plant *Ephedra sinica* are associated with cardiotoxicity. One study reviewing such adverse events found that 47% of those taking these supplements exhibit cardiovascular symptoms including hypertension, palpitations, and/or tachycardia (Haller and Benowitz, 2000). The Food and Drug Administration (FDA) banned the sale of ephedra-containing over-the-counter dietary supplements because of the risk for serious side effects, including a rise in blood pressure and an increase in heart rate (United States Food and Drug Administration, 2004).

Pharmacologic activity of ephedrine may be mediated by its ability to cause the release of norepinephrine and epinephrine from adrenergic nerve terminals and thereby produce indirect stimulation of adrenoreceptors (Weiner, 1980). The cardiovascular effects of ephedrine are similar to those of epinephrine, but they persist for a longer period of time.

Ephedrine is both an α- and β-adrenergic agonist that enhances the release of catecholamines, such as norepinephrine, from sympathetic neurons. Ephedrine stimulates heart rate and cardiac output and causes systemic vasoconstriction (Tong and Eisenach, 1992); as a result, it usually increases blood pressure (Persky *et al*., 2004). The myocardial ischemia and myocardial cell death are thought to be due to vasoconstriction and cardiac stimulation induced by epinephrine (Weiner, 1980). Single case reports (Enders *et al*., 2003; Foxford *et al*., 2003; Krome and Tucker, 2003; Kumar and Jugdutt, 2003; Lustik *et al*., 1997; Miller and Waite, 2003; Naka *et al*., 2003), reviews of adverse-events data from 140 (Haller *et al*., 2002) and 926 cases (Samenuk *et al*., 2002), and placebo-controlled studies (McBride *et al*., 2004) showed that the risk of hypertension, cardiac arrhythmia, coronary-artery constriction, and reduction in myocardial oxygen supply was associated with the intake of ephedrine-containing herbal supplements. Coronary-artery vasospasms have been reported with ephedrine administration during spinal cord surgery or abusive injection (Cockings and Brown, 1997; Hirabayashi *et al*., 1996).

Total alkaloid content of these herbal medicines has been estimated at 0.5–2.5%, with ephedrine accounting for 30–90% of the alkaloids (Gurley *et al*., 1998; Lee *et al*., 2000). Many of these ephedrine-containing herbal supplements contain a botanical form of caffeine, referred to as guarana-derived

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caffeine, from the plant *Paullinia cupano* (Haller *et al*., 2004). The ephedrine-containing herbal supplements may also be taken with caffeine from coffee or tea averaging 75–150 mg caffeine per cup (Rose'Meyer *et al*., 2001). Ephedrine and caffeine act synergistically to increase heart rate and diastolic and systolic blood pressure (Astrup *et al*., 1991). The pressor effects of caffeine have been attributed to increased catecholamine and renin release and/or antagonism of endogenous adenosine (Nurminen *et al*., 1999; Rose'Meyer *et al*., 2001; White *et al*., 1997).

Ephedra sinica extract commonly used in dietary supplements consists of ephedrine, pseudoephedrine, methylephedrine, norpseudoephedrine, and norephedrine, of which ephedrine is the principal alkaloid component. A typical ephedrine-alkaloid-containing dietary supplement dose contains ~20 mg ephedrine and ~200 mg of guararna-derived caffeine (Haller *et al*., 2004; Jacob *et al*., 2004). Ephedrine alkaloids are rapidly absorbed, with maximum plasma concentration (t_{max}) occurring within 2.4 h and an ephedrine plasma half-life of ~6.1 h (Haller *et al*., 2002; White *et al*., 1997; Wilkinson and Beckett, 1968). A typical human ephedrine/caffeine dosage is ~0.3 mg ephedrine/kg body weight (11 mg/m^2 body surface area) and ~3 mg caffeine/kg body weight (111 mg/m^2 body surface area) (Table 1).

In this study, we evaluated the cardiac pathology of a combined exposure to ephedrine and caffeine. A previous subchronic study in rats, in which ephedrine alone was administered at doses up to approximately 140 mg/kg, and a chronic study in which doses as high as 18 mg/kg were achieved during the first weeks revealed no evidence of cardiac toxicity (National Toxicology Program, 1986). For the current study, the ephedrine dose selected was in the range of the high dose used in the two-year-male-rat chronic study (National Toxicology Program, 1986), and the caffeine dosage was approximately equal to the amount of guarana-derived caffeine from a typical human ephedra-supplement dosage based on a mg/m^2 body surface-area comparison. Thus, the ephedrine exposure given to rats was ~12× and the caffeine dosage ~1.4× that of an average human exposure based on a mg/m^2 body surface-area comparison (Table 1) (Freireich *et al*., 1966). To characterize the nature of the morphological manifestation of cardiotoxicity and clarify the pathogenesis of the ephedrine and caffeine-induced myocardial damage, we applied histochemical and immunohistochemical stainings for ischemia and apoptosis. Our work shows the direct relationship between combined ephedrine and caffeine exposure and acute myocardial necrosis, acute myocardial hemorrhage, and myocardial inflammation.

TABLE 1
Comparison of Ephedrine and Guarana-derived Caffeine Dosages in Human and Rat

	Human mg/kg	Rat mg/kg	Human mg/m^2*	Rat mg/m^2*
Ephedrine	0.3	25	11	130
Caffeine	3	30	111	156

Note. Comparison of ephedrine and guarana-derived caffeine dosages in a 70-kg human taking two pills of a typical herbal supplement and the rat ephedrine-caffeine dosage used in this experiment.

*Calculation for body surface area dose based on method previously published (Freireich *et al*., 1966); mg/m^2 = K_m × (dose in mg/kg), where K_m is 37 for humans, 5.2 for rats.

MATERIALS AND METHODS

Animals and chemicals. Seven- (n = 8) and 14-week- (n = 9) old male F344 rats (Charles River, Raleigh, NC) used for these studies were housed singly in polycarbonate cages and fed NIH31 feed (Zeigler Brothers, Inc., Gardners, PA) *ad libitum*. Ephedrine hydrochloride (Sigma-Aldrich, St. Louis, Mo; >99% L-ephedrine)-plus-caffeine (Pfaltz and Bauer, Waterbury, CT) solutions were prepared in 0.5% methylcellulose (Fig. 1) (O'Neil *et al*., 2001). The stock solution consisted of 5 mg/ml ephedrine and 6 mg/ml caffeine. Controls received 0.5% methyl cellulose alone. Animals were dosed once daily at approximately 1:00 p.m. for 1–3 days at 5 ml/kg body weight to deliver a total daily dose of 25 mg/kg ephedrine and 30 mg/kg caffeine. Animals were observed hourly after dosing, during the evening, and again in the morning for clinical signs of toxicity. The numbers of animals included in each group were derived from a series of three studies (Table 2).

Pathology procedures. All animals were sacrificed with pentobarbital, with the exception of one 14-week-old animal receiving ephedrine-caffeine, which was sacrificed with CO_2, and three 14-week-old animals receiving ephedrine/caffeine, which died in distress. At necropsy, all organs and tissues were examined for grossly visible lesions. The heart was fixed in 10% neutral buffered formalin, processed, and trimmed into two longitudinal halves; both sides were embedded in paraffin, sectioned to a thickness of 4–6 µm, and stained with hematoxylin and eosin (H&E) for microscopic examination. The pathological evaluation was conducted in a blinded manner, with participants having no knowledge of the control and treated groups. A semiquantitative grading scheme was used to evaluate the extent of the cardiac necrosis or inflammation in the heart section as follows: minimal (grade 1) involved 1–10% of the section; mild (grade 2), 11–40%; moderate (grade 3), 41–80%; and marked (grade 4), 81–100%.

Barbeito-Lopez trichrome stain (BLTS). This histochemical staining method was applied for routine diagnosis of myocardial degeneration or necrosis (Milei and Bolomo, 1983; Milei and Storino, 1986).

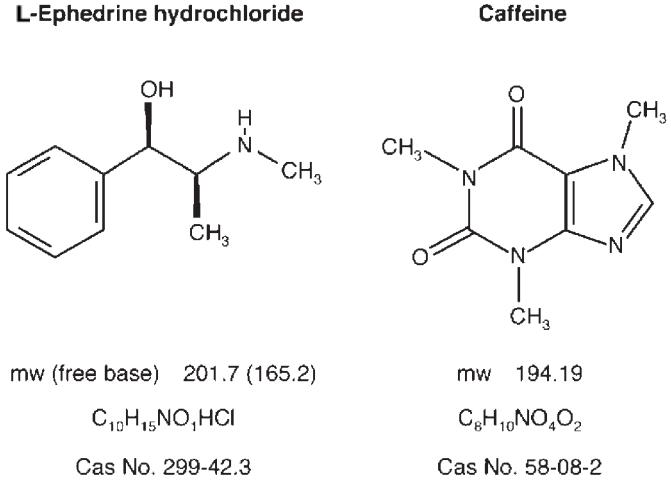

FIG. 1. Chemical structure of ephedrine and caffeine.

TABLE 2
Incidence and Severity of Heart Lesions in Male Rats*

					Heart histopathology				
Dosed group	Number of animals	Age of animals (Wks)	Time of sacrifice	Mean body weight. on day 1 (gram ± SE)	Hemorrhage, multifocal	Myofiber degeneration (hyalinization, vacuolation, and with loss of striations), multifocal	Myofiber necrosis (apoptosis, loss of myofibers), multifocal	Macrophage infiltration, multifocal	Mononuclear-cell infiltration, multifocal
Control	3	7 weeks	Sacrificed after 1–3 doses	136 ± 8	0/3[a]	0/3	0/3	0/3	3/3 (1)[b]
Ephedrine + caffeine	5	7 weeks	Sacrificed after 2–3 doses	150.9 ± 8	1/5 (0–1)	5/5 (1–2)	3/5 (0–1)	5/5 (1–2)	5/5 (1–2)
Control	2	14 weeks	Sacrificed after 3 doses	280.9 ± 8	0/2	0/2	0/2	1/2 (0–1)	2/2 (1–2)
Ephedrine + caffeine	5	14 weeks	Died acutely 4–5 h after 1 dose	303.6 ± 10	5/5 (2)	5/5 (2)	5/5 (2)	5/5 (2)	4/5 (0–2)
Ephedrine + caffeine	2	14 weeks	Sacrificed after 2–3 doses	313.8 ± 2	1/2 (1)	2/2 (2)	2/2 (1)	2/2 (2)	2/2 (1–2)

*Controls and those administered ephedrine (25 mg/kg) + caffeine (30 mg/kg).
[a]Incidence of lesion.
[b]Range of severity grade.

Caspase-3. Immunostaining for the localization of cleaved caspase-3 protein expression was performed, using a polyclonal antibody (Cell Signaling Technology, Beverly, MA) to detect apoptosis. Sections were first deparaffinized in xylene and hydrated through a series of ethanols to 1× Automation Buffer (1× AB) (Biomeda, Foster City, CA). Endogenous peroxidase was blocked using 3% H_2O_2 for 15 min at room temperature. Antigen unmasking was accomplished by heating sections in 200 ml of citrate buffer (pH 6.0) in Decloaker™ (both, Biocare Medical, Walnut Creek, CA). Following depressurization, slides were allowed to cool for 10 min before the reaction was stopped in running distilled water. Prior to the application of the primary antibody, a protein block (Dako, Carpinteria, CA) and an avidin-biotin block (Vector Laboratories, Burlingame, CA) were applied. The primary antibody, rabbit anti-cleaved caspase-3, was then applied for 1 h at room temperature at a dilution of 1:50. Nonimmune rabbit IgG (Jackson Immunoresearch Labs, West Grove, PA) was used as the negative control at equivalent conditions in place of the primary antibody. Localization of the primary antibody was detected using the LSAB + Kit (Dako, Carpinteria, CA). The antibody complex was visualized with a diaminobenzidine (Liquid DAB™, Dako) reaction occurring for 6 min in the dark. Finally, slides were rinsed in running tap water, counterstained with Harris Hematoxylin (Harelco, Gibbstown, NJ), dehydrated through a series of ethanols to xylene, and coverslipped with Permount (Fisher Scientific, Norcross, GA). The positive control tissue for the stain was necrotic acinar pancreatic tissue from a rat.

Anti-phospho-H2A.X. Immunostaining of hyperphosphorylated H2A.X provided another indicator of apoptosis (Talasz *et al*., 2002). A commercially available polyclonal antibody for H2A.X (Upstate Signaling Solutions, Waltham, MA) was used at a dilution of 1:250. The positive control tissue for the stain was testis. The procedure for staining of H2A.X was the same as that for caspase-3.

RESULTS

Clinical Observations

Five of the seven 14-week-old ephedrine-and-caffeine-treated rats died or were sacrificed *in extremis* 4–5 h after one dose; the remaining 2 ephedrine-and-caffeine-treated 14-week-old rats were sacrificed 4 h after the second or third dose of ephedrine-caffeine (Table 2). No clinical signs of toxicity were observed in 7-week-old or 14-week-old control rats or in the five 7-week-old rats receiving ephedrine-caffeine.

Histopathological Findings

The incidences of the changes observed in the hearts are presented in Table 2. In the hearts of animals found dead or sacrificed in distress 4–5 h after the first dosing of ephedrine and caffeine (i.e., 5/7 of the 14-week-old treated rats), changes were observed chiefly in the interventricular septum and, to a lesser extent, the left and right ventricular walls. Massive interstitial hemorrhage occurred at the subendocardial myocardium of the left ventricle and interventricular septa (Figs. 2A and 2B). The hemorrhage was associated with degeneration of the surrounding myofibers that appeared hyalinyzed, vacuolated, and with loss of striations. In some of these cells the nuclei were pyknotic or had disappeared. The incidence and severity of hemorrhage in the hearts of 7-week-old ephedrine-and-caffeine treated rats were lower than in 14-week-old ephedrine-and-caffeine-treated rats, and none of the 7-week-old treated rats died acutely after the first dose of ephedrine-caffeine. The remainder of the pathological changes seen in the 7-week-old animals were, however, comparable in nature and severity to those seen in the 14-week-old animals.

Other treatment-related changes in the heart consisted of a multifocal, generalized loss of myofibers associated with macrophage infiltration and the presence of hyperbasophilic fragments of nuclei (Figs. 2C and 2D). The macrophages appeared to be digesting lysed fibers and fragments of nuclear chromatin. The fragmented nuclei were markedly positive for anti-phospho-H2A.X, a histone variant that becomes phosphorylated during apoptosis (Figs. 3A and 3B) (Talasz *et al*., 2002). Caspase-3 staining revealed in all animals dying acutely within 4–5 hours after the first dosing the presence of multifocal, intracytoplasmic, positive myofibers, seen frequently in the interventricular septa (Figs. 3C and 3D). Infrequently these positively stained myofibers demonstrated morphological degenerative alterations only, indicating the usefulness of this method in detecting an activated apoptotic process at an early stage. Application of the histochemical BLTS for diagnosis of myocardial degeneration and/or necrosis indicated the presence of generalized patchy yellow myofibers, consistent with cytoplasmic homogenization and loss of striation (Fig. 3E). Normal-appearing fibers in control and treated animals stained green to light blue (Fig. 3F).

In the ephedrine-and-caffeine-treated 7- and 14-week-old rats, the foci of myocardial degeneration and necrosis were relatively more extensive after the two or three doses, especially in subepicardial regions, and were associated with an infiltration of mixed inflammatory cells (Figs. 2E and 2F).

DISCUSSION

The FDA previously reported that ephedra-containing products can alter heart rate and blood pressure but, in issuing the 2004 ban on ephedra-containing products, had little or no information on pathologic consequences in the heart resulting from exposure to ephedra-containing herbal medicines (United States Food and Drug Administration, 2004). This investigation focused, therefore, on identifying pathologic changes in the heart following exposure to ephedrine and caffeine, components of the herbal medicines. Our study demonstrated that ephedrine administered in combination with caffeine caused severe acute hemorrhage, myocardial necrosis, and inflammation in male F344 rats after one to three daily exposures. This ephedrine-and-caffeine treatment caused rapid death of five of seven of the 14-week-old male rats and heart toxicity without death in the remaining two 14-week-old-rats and the 7-week-old rats.

Different studies in the literature suggest that older rats, as well as older humans, have a diminished ability to respond to ischemia compared to younger individuals (Isoyama and Nitta-Komatsubara, 2002). This difference may result from

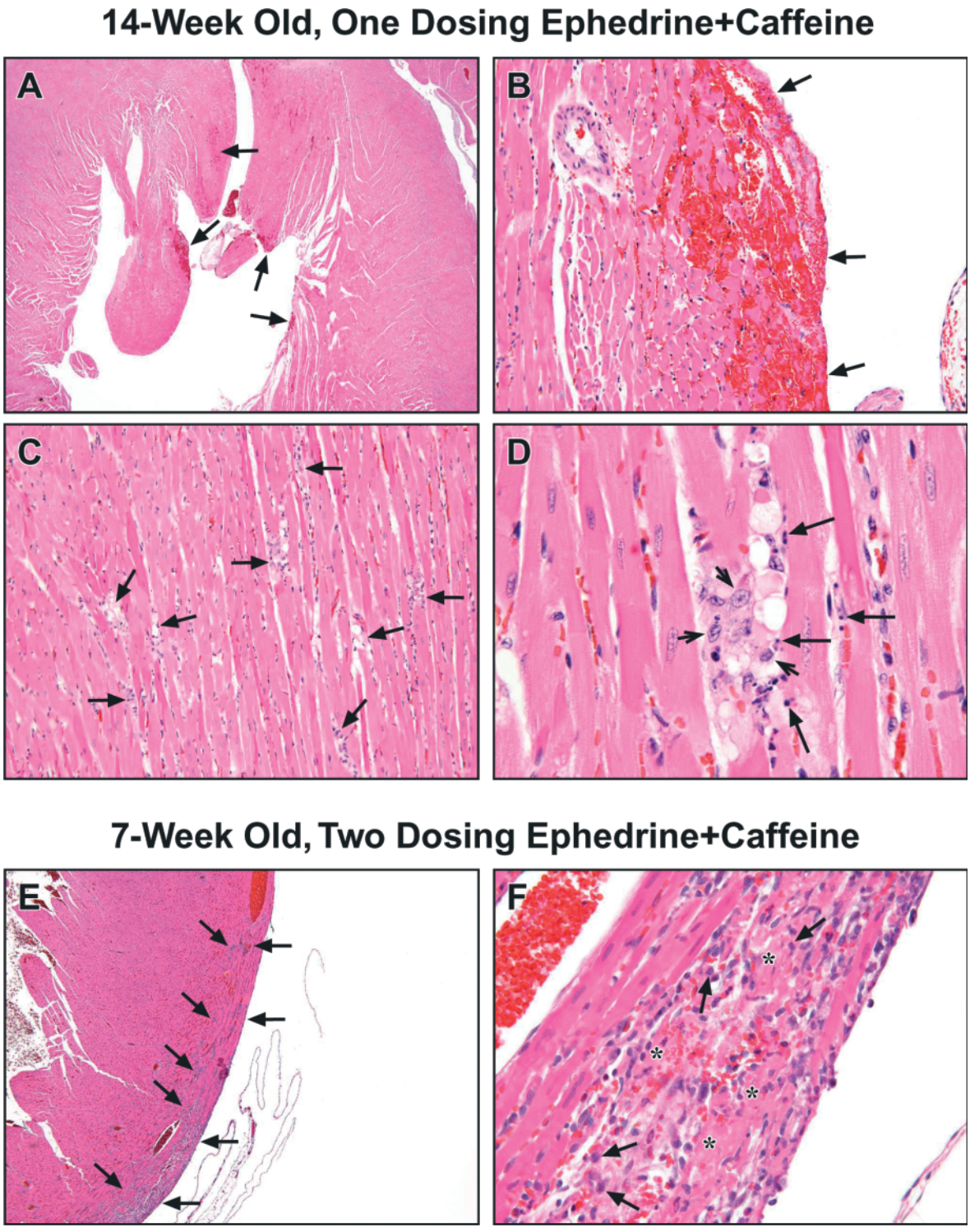

FIG. 2. 14-week-old rats administered one dose of 25 mg/kg ephedrine and 30 mg/kg caffeine and sacrificed *in extremis* ~4–5 h after dosing. H&E-stained sections showing grade-2 (mild) cardiac damage. (A) Hemorrhage (arrows) in subendocardial myocardium of left atrium and interventricular septum; ×2.5. (B) Higher magnification of (A); interstitial hemorrhage and myofiber degeneration (hypereosinophilia, loss of striation) are apparent; ×25. (C) Multifocal myofiber necrosis (arrows) in myocardium of interventricular septum; ×20. (D) Higher magnification of (C); necrotizing myofibers are being digested by infiltrating macrophages (small arrows) and fragmenting nuclei (apoptotic bodies, long arrows) of necrotic myofibers are apparent; ×80. (E) and (F) show heart damage in a 7-week-old rat after two doses of ephedrine (25 mg/kg) and caffeine (30 mg/kg) and sacrificed 4 h after the second dose. (E) Extensive subepicardial area (arrows) of myofiber necrosis and inflammation in left ventricular wall; ×5. (F) Higher magnification of (E); myofibers undergoing necrosis (asterisks), accompanied by macrophages (arrows) and inflammatory cell infiltration; ×50.

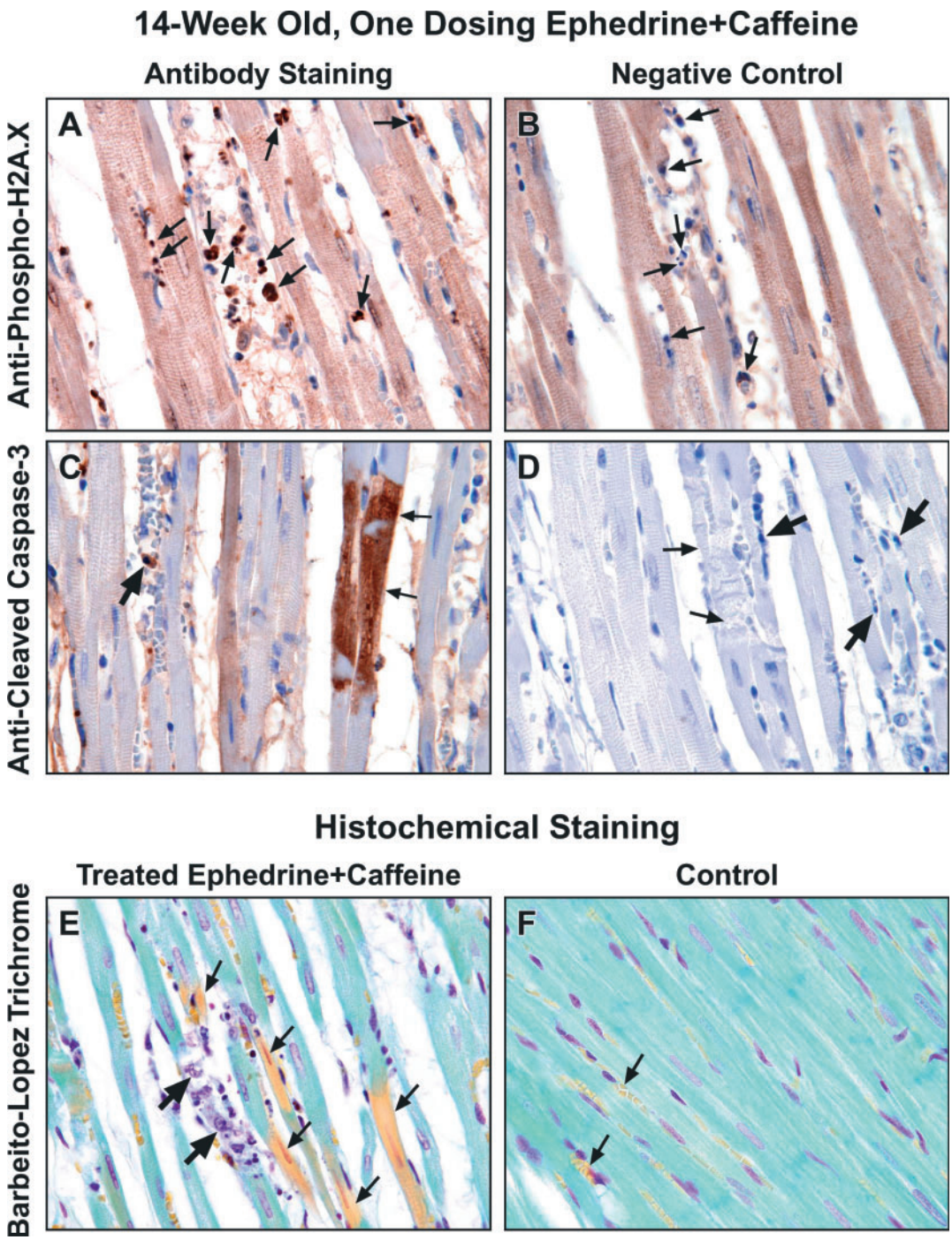

FIG. 3. Heart damage in 14-week-old rats administered one dose of 25 mg/kg of ephedrine and 30 mg/kg of caffeine, and sacrificed *in extremis* approximately 4–5 h after dosing. Sections demonstrating immunohistochemical stainings. (A) Interventricular septum stained by anti-phospho-H2A.X, a histone variant that becomes phosphorylated during apoptosis; note strongly stained apoptotic bodies (arrows); ×50. (B) Demonstration of the negative control of the same section shown in (A); nonimmune rabbit IgG was used at equivalent conditions in place of the primary antibody. Note the unstained apoptotic bodies (arrows); ×50. (C) Cleaved caspase-3 staining for apoptosis reveals the presence of intracytoplasmic positive myofibers (thin arrows) located in the interventricular septum; thick arrow indicates apoptotic body; ×50. (D) Negative control of the same heart demonstrated in (E); specific antibody for caspase-3 was not added. Thin arrows indicate degenerating myofibers; thick arrows indicate apoptotic bodies; ×50. (E) Interventricular septum stained by the Barbebeitto-Lopex trichrome histochemical method, which detects myocardial degeneration and necrosis; degenerating and necrotic myofibers are stained yellow (thin arrows), while unaffected fibers are stained green to light blue; note the macrophages within the necrotizing myofiber (thick arrows); ×50. (F) Interventricular septum of 14-week-old control rat stained by Barbebeitto-Lopex trichrome histochemical method; note that the normal fibers are stained green to light blue, while collections of erythrocytes are stained yellow (thin arrows); ×50.

an age-related diminished capacity to adapt to increased hemodynamic overload and ischemia and combat heart disease (Goyns *et al*., 1998). Identifying the critical genes involved in the response to ischemia is an area of future exploration.

Reports of possible toxicity in humans induced by herbal supplements containing both 3–7% ephedrine and guarana-derived caffeine have indicated an increased risk of stroke, myocardial infarction, and sudden death (Samenuk *et al*., 2002). In one of the human sudden-death cases, histopathological examination of the heart revealed changes comparable to those seen in this study, consisting of multifocal and confluent myocardial necrosis with the occurrence of scarring in one to two weeks. Interpretative analysis indicated that myocardial necrosis was not specific and could have been due to primary myocytic toxicity or occurred secondarily to ischemia. Furthermore, the presence of hemorrhage and cellular necrosis was consistent with the effects of factors that collectively could have been responsible for vascular damage, vasoconstriction of small arterial vessels, and myocytic toxicity. These pathological features are reminiscent of experimental and clinical manifestations of adrenergic and sympathomimetic agents (Samenuk *et al*., 2002).

The myocardial changes seen in our rats after 1 to 3 days of ephedrine/caffeine exposure consisted of acute interstitial hemorrhage, myocytic necrosis, apoptosis, and early inflammatory cell infiltration. Caspase-3 staining revealed positivity in multiple myofibers in the animals dying within 4–5 h of exposure. Caspase-3, which belongs to a family of highly homologous endopeptidases, plays a crucial role in executing apoptosis and has been found to be a reliable marker of apoptosis in other models of chemically induced cardiotoxicity (Li *et al*., 2002). Using an *in vitro* model of heart ischemia, investigators suggested that, within 1 h, heart ischemia can cause apoptosis mediated by release of cytochrome c from mitochondria and subsequent activation of caspase-3-like proteases (Borutaite *et al*., 2001; Naka *et al*., 2003).

The immunostaining for the DNA-phosphorylated histone H2A.X proved to be reliable in detecting apoptotic cells in which H&E staining had revealed nuclear fragments. Histones are highly conserved proteins that serve as the structural scaffold for the organization of nuclear DNA into chromatin; histone modifications, such as phosphorylation, may affect chromatin function during both the cell cycle and apoptosis (Talasz *et al*., 2002). Hyperphosphorylation of H2A.X is a necessary step in apoptotic pathways, occurring during the induction of apoptosis concomitantly with the appearance of the condensation of chromatin, but before nucleosomal fragmentation (Rogakou *et al*., 2000). The H2A.X histone becomes specifically phosphorylated at serine 13 within seconds after the induction of DNA double-strand breaks. The formation of H2A.X during apoptosis is a downstream consequence of caspase activation (Rogakou *et al*., 2000). In our study, the caspase-3 staining identified early necrotizing myofibers, while the anti-H2A.X staining was effective in confirming the fragmentation of nuclear chromatin.

The BLTS detected myocardial degeneration in the rat as early as 4–5 h after ephedrine/caffeine exposure. In contrast, in the H&E-stained heart sections from the ephedrine/caffeine-treated rats, the early degenerating myofibers could not be easily identified, especially when they exhibited only loss of striation. In the BLTS-stained heart sections, degenerating myofibers were identified with a yellow color, in contrast to the green-blue coloration of the undamaged myofibers. Milei and Storino (1986) have also used BLTS to demonstrate heart toxicity in the rat following exposure to isoproterenol sulfate. In these studies, isoproterenol induced coagulative necrosis in rat hearts 30 min after myocardial ischemia, and BLT stained the cytoplasm of these necrotic cells a patchy pale-yellow color. In the coagulative necrosis that had become established after 24 h, the cytoplasm appeared golden yellow (Milei and Storino, 1986).

In our ephedrine/caffeine rat studies, the combination of caspase-3, H2A.X, and the BLTS staining methods was shown to be particularly effective in locating early degenerating myofibers. We recommend, therefore, that, in evaluating the cause of sudden death where cardiac damage is suspected, this triple-combination staining be added to the routine H&E staining.

Although the myocardium can be damaged by various agents or factors, such as anoxia, ischemia, infectious agents, and physical and chemical agents, the pattern of response is relatively limited (Greaves, 2000). In ephedrine/caffeine-treated rats, myofiber degeneration, apoptosis, and necrosis reflected a typical range of acute damage, while the infiltration of macrophages represented the process of elimination of dying cells. If the animal did not die due to extensive heart failure, this stage could be followed in a few days by further scarring by fibrosis. The myocardial necrosis, morphologically and histochemically consistent with apoptosis, was frequently associated with acute inflammation. Inflammation has been documented in the case of myocardial ischemia (Vinten-Johansen, 2004); however, experimental studies provide strong but somewhat conflicting evidence that neutrophils are involved in the myocardial response leading to lethal injury upon reperfusion. Whether the accumulation of neutrophils within an ischemic-reperfused area represents a response to injury or is an active process contributing to injury of the myocardial cells is not clear.

We hypothesize that, in our study, many of the 14-week-old rats exposed to a single dose of ephedrine and caffeine developed severe heart insufficiency, leading to sudden death. In contrast, when ephedrine alone was given to rats or mice for 13 weeks at doses up to 120 mg/kg/day, or for 104 weeks at doses up to 18 mg/kg/day (National Toxicology Program, 1986), heart toxicity was not observed. Even when mice were administered ephedrine alone at doses up to 600 mg ephedrine/kg/day for up to seven days, acute heart toxicity and death did not occur (Minematsu *et al*., 1991). In our studies, administration of caffeine alone to rats did not cause acute heart toxicity and death. Other studies do show that long-term administration of caffeine alone may cause some cardiac toxicity (Johannsson, 1981); however, the exposure to the combination of ephedrine

and caffeine causes acute heart toxicity. The animals were dosed in the afternoon when the stomach would have been relatively empty, and this dosing on an empty stomach may have facilitated uptake of ephedrine and caffeine (Yuan, 1993).

In humans to whom ephedrine alone (Cohn, 1965; Franciosa and Cohn, 1979) or caffeine alone was administered (Vlachopoulos *et al*., 2002), acute heart toxicity and death did not occur. In humans, however, consumption of products containing ephedrine and caffeine may cause acute heart toxicity and death (Samenuk *et al*., 2002). The combined effects of caffeine, which can antagonize the adenosine receptors resulting in increased blood pressure (Nurminen *et al*., 1999), and ephedrine, which is an α- and β-adrenergic agonist (Nurminen *et al*., 1999), work together to cause the acute and sometimes fatal heart toxicity. Our study design can serve as a model to elucidate further these combined mechanisms that function in this type of cardiotoxicity and can be used to develop preventive strategies.

The heart damage observed in this rat study is suggested to be caused by ischemia, followed by myofiber necrosis. Combined ephedrine and caffeine exposure probably induced intense diffuse vasoconstriction of the coronary arterial system, decreasing myocardial blood supply, leading to ischemia and apoptotic death of the cardiomyocytes. These findings support the recommendations of the FDA (United States Food and Drug Administration 2004) to ban the over-the-counter sale of dietary supplements containing ephedra.

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