Acute Clenbuterol Induces Hypotension, Atrioventricular Block and Cardiac Asystole in the Rabbit

Yan Ke · Li-Lan Fu · Xia-Fei Hong · Run Dong · Tian-Ming Xu · Jing-Fei Guo · Yan Liu · Ji-Min Cao

© Springer Science+Business Media, LLC 2012

Abstract Clenbuterol is a long-lasting β-adrenoceptor (β-AR) agonist and was once medicated as a bronchial dilatator, and is also used by body-building enthusiasts and athletes and in livestock breeding because of its anabolic effect on skeletal muscles and ability to promote lipolysis. Though prohibited from pharmacological uses, clenbuterol intoxication cases are frequently reported, and most of the cardiac symptoms are tachyarrhythmia. Here, we reported a different cardiovascular toxic response to clenbuterol. Using a rabbit model, we tested the dose–response pattern of the cardiovascular system to intravenous administration of clenbuterol. Routine arterial blood pressure (BP) and surface electrocardiogram (ECG) were monitored. We observed that clenbuterol at a lower dose (0.4 mg/kg, \( n = 3 \)) did not significantly affect the ECG, but decreased the mean BP roughly by 15–18 mmHg. At a medial dose (3.6 mg/kg, \( n = 3 \)), clenbuterol induced significant hypotension (mean BP dropped by about 30 mmHg), first-degree atrioventricular (AV) block and intermittent ectopic activities with a relatively slow rate. The hypotension and arrhythmia recovered slowly, and animals did not die. Higher-dose clenbuterol (10 mg/kg, \( n = 6 \)) induced severe hypotension, second-degree AV block (Mobitz type II), 2:1 ventricular capture and progressive prolongations of P–R intervals and QRS duration, and the animals soon died of cardiac asystole. Different from other reports, we had not observed lethal tachyarrhythmia in all experiments except for the slight heart rate acceleration during the recovery stage of medial clenbuterol dosage. These results indicate that acute intravenous administration of clenbuterol has serious, dose-dependent cardiovascular toxicities and is even life threatening.

Keywords Clenbuterol · Beta-adrenoceptor · Blood pressure · Electrocardiogram · Arrhythmia · AV block · Sudden cardiac death

Introduction

Clenbuterol can relax bronchial smooth muscle and increase the size of skeletal muscle cells via agonizing β2-adrenoceptor (β2-AR) and stimulate lipolysis via the β3-adrenoceptor (β3-AR) in adipocytes [1]. Because of these pharmacological effects, clenbuterol was once used as a bronchial dilatator in treating chronic obstructive pulmonary disease (COPD) and asthma [2]. However, increasing evidences showed the toxicity of this agent to the cardiovascular system. FDA (U.S. Food and Drug Administration) has banned the use of clenbuterol as a therapeutic drug. In recent decades, clenbuterol has also been using in livestock breeding in China as well as in some other countries, in that clenbuterol was illicitly added to the feeds of cattle, pig or other livestock in order to decrease the fat content and increase the proportion of lean meat for a good sale. Outbreaks of clenbuterol intoxication by consuming contaminated meat or liver occurred in some areas of the world [3]. Human case reports of clenbuterol

Y. Ke · L.-L. Fu · X.-F. Hong · R. Dong · T.-M. Xu · J.-F. Guo
Department of Medicine, Peking Union Medical College, Chinese Academy of Medical Sciences, 5 Dong Dan San Tiao, Beijing 100005, China

Y. Liu · J.-M. Cao (✉)
Department of Physiology and Pathophysiology, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences, School of Basic Medicine Peking Union Medical College, 5 Dong Dan San Tiao, Beijing 100005, China

e-mail: caojimin@126.com

Published online: 19 September 2012
intoxication also appear occasionally in that bodybuilders or athletes consume clenbuterol for a purpose of increasing their muscle bulk and strength [4, 5]. Clenbuterol has become a routinely checked doping in the Olympic Games [6]. In addition, a series of case reports raise the awareness that heroin might be contaminated by clenbuterol [7–12], suggesting a role of clenbuterol in drug abuse.

The clinical manifestations, such as tachycardia, hypokalemia and headache, have been observed in a series of patients who have a detectable level of clenbuterol in blood [13]. Supraventricular tachycardia and atrial fibrillation attributable to acute use of clenbuterol has been reported [1]. In an in vitro atrial model of guinea pig, clenbuterol induced heart rate increase by acting chiefly on β2-AR [14], a phenomenon which is in consistent with that in clenbuterol-intoxicated patients [1]. In these reports, tachyarrhythmia appears the most relevant cardiac symptoms after clenbuterol intake. Clenbuterol was used as inhaled drug for asthma and COPD, whereas it is generally administered orally in athletes. To our knowledge, AV conduction abnormalities and sudden cardiac death after clenbuterol consuming are seldom shown in case reports and/or experimental studies.

However, some other studies provide clues which correlate β2-AR activation and AV conduction disturbance. For example, stimulation of the β2-AR with autoantibodies directed against the second extracellular loop of the β2-AR induced AV block in the mouse heart in vitro, and clenbuterol exerted similar effect as β2-AR autoantibodies on AV conduction in the same model [15]. The present study aimed to assess the acute toxicities of clenbuterol on hemodynamics and cardiac electrophysiology in a rabbit model in vivo, especially focusing on how and at what a dosage regime clenbuterol would induce sudden cardiac death.

Materials and Methods

Animal and Reagent

Adult male New Zealand white rabbits (2.0–2.5 kg) were used in the experiment. Before the experiment, each animal was housed in cages equipped with wire-net bottoms and had free access to water and regular chow for at least 7 days. The experimental protocol was approved by the Life Ethics Committee of Peking Union Medical College and in compliance with the U.S. National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication 85–23).

Clenbuterol hydrochloride was purchased from Dr. Ehrenstorfer (Augsburg, Germany) and was then dissolved in saline to reach a final concentration of 2 mg/ml for use.

Blood Pressure Monitoring

The animals were anesthetized with bolus intravenous infusion of urethane solution (25 % v/v, 4 ml/kg). Then, the left carotid artery was cannulated, and arterial blood pressure (BP) was monitored by a pressure transducer connected to the catheter and the BL-420E data acquisition system (Taimeng Software Co. Ltd., Chengdu, China). Femoral vein was also cannulated for drug infusion. Each catheter was filled with heparin (0.5 % v/v) before cannulation to prevent coagulation.

Recording of Electrocardiograms

Surface electrocardiogram (ECG) was recorded to monitor the heart rate and rhythm. The ECG leads were connected to the subcutaneous tissues of the left and right arm and left inguinal region. ECG signals were also recorded by the BL-420E data acquisition system. The readout of ECG was equivalent to human ECG lead I. ECG signals, such as heart rate (HR), P–P intervals, P–R intervals, duration of QRS complex and arrhythmias, were routinely inspected and analyzed.

Statistical Analysis

Statistics were performed mainly for identifying the variation in P–R intervals before and after clenbuterol administration. Using the Kolmogorov–Smirnov test of SPSS 17.0.0 software, we first verified that the baseline P–R intervals and the P–R intervals after clenbuterol infusion were subjected to normal (parametric) distribution (P < 0.05). Then, we calculated the mean ± 1.96 (standard deviation/sqrt(n)) (95 % confidential interval) of these P–R intervals using Prism 5.01 software.

Results

The baseline BP was 60–75 (diastolic)/78–115 (systolic) mmHg in the tested rabbits under anesthesia. To determine the dose–response relationship, three dosages of clenbuterol (0.4, 3.6 and 10 mg/kg) were tested. Clenbuterol was infused into the left femoral vein within 10 s. Intravenous administration of clenbuterol at a lower dose (0.4 mg/kg, n = 3) decreased the mean BP roughly by 15–18 mmHg (Fig. 1A), but did not induce any ECG change (Fig. 1B). The BP recovered to the baseline level about 2 min after the end of clenbuterol infusion. Clenbuterol infusion at a medial dose (3.6 mg/kg, n = 3) induced significant hypotension, and the mean BP dropped roughly by 28–32 mmHg (Fig. 2A). Clenbuterol at this dosage obviously interfered with the cardiac pacemaker and conduction.
system, as shown by a disrupted pattern of ECG (Fig. 2B). The first-degree AV block (P–R interval prolongation) and intermittent ectopic activities with relatively a slow rate were occasionally observed (Fig. 2B-f). After recovery from the disrupted ECG pattern (about 75 s after clenbuterol infusion), the heart rate became stable and was
slightly accelerated compared with the baseline (from baseline 260 ± 3 to 309 ± 4 bpm) (Fig. 2B), but not likely turning to sinus rhythm at this period (Fig. 2B-g). Hypotension and arrhythmia recovered to normal roughly 2 h after clenbuterol infusion, and animals did not die at this dosage. Rabbits received larger-dose (10 mg/kg, \( n = 6 \)) clenbuterol rapidly developed severe hypotension (mean BP dropped to <30 mmHg within 15 s) (Fig. 3A).

At the same time, second-degree AV block (Mobitz type II) (Fig. 3B-e), progressive prolongations of P–R intervals and QRS duration and 2:1 ventricular capture (Fig. 3B-f) were observed, and the animals soon died of cardiac asystole (Fig. 3B). No lethal tachyarrhythmia (ventricular tachycardia or fibrillation) was observed at this dosage.

**Discussion**

The present study aimed to evaluate the cardiovascular toxicity and lethality of clenbuterol when administrated intravenously. The results indicate that the toxicity of clenbuterol is serious and even life threatening when acutely administrated at a larger dose. This kind of acute toxicity usually cannot occur merely by consuming clenbuterol-contaminated animal products or oral clenbuterol intake at a medicated dosage, but may encounter by malpractice, accident or poisoning. The toxicities for chronic and minimal intake of this agent, such as oral intake of clenbuterol tablet or syrup, inhaling of aerosol or daily eating of clenbuterol-contaminated animal tissues (meat or liver), are not well investigated except for some case reports \([1, 3, 4]\). It should be noted that the elimination half-life of clenbuterol is pretty long (25–39 h) \([12]\), daily intake of this drug might lead to accumulation in the body, and therefore, the deleterious and potentially lethal effects of clenbuterol might be underestimated.

The toxicity of clenbuterol depends on several factors including the dosage, the lasting time of medication and the route of administration. It is difficult to determine the dose range of clenbuterol which can cause acute toxicity in humans, because most of the acute clenbuterol intoxication cases could not provide their exact intake dose except for one case, in that a total dose of 108.75 \( \mu \)g clenbuterol syrup was orally consumed and caused supraventricular tachycardia and atrial fibrillation \([1]\). However, information about the blood and/or urine levels of clenbuterol of whose...
with clenbuterol intoxication (often combined with other drugs) is available in the Emergency Department or post-mortem case reports [3, 12]. The recommended clenbuterol dose for people with asthma was 20–40 mg orally twice daily, or 20 mg by inhalation given at 8-h intervals [1]. For the illicit users such as bodybuilders, no standardized clenbuterol dosing regimens was recommended except for the anecdotal experiences [1], and the commonly used doses are as high as 200 mg orally taken 1–3 times daily over 6- to 12-week cycles [1]. This high dosage places users at a high risk of intoxication if also compared with the doses used in the present study.

The present study observed that acute clenbuterol administration significantly affected the cardiac electrophysiology, as shown by AV block and also potential ventricular conduction block, ectopic arrhythmia and cardiac asystole. Supposing a doctor comes across a patient with acute clenbuterol poisoning, cardiac conduction block and sudden death should be early warned in his mind. Or, should a patient presenting with AV block be admitted, clenbuterol could be on the list of differential diagnosis. We also noticed that the clenbuterol-induced AV conduction disturbance is highly dose-dependent. Lower dose of clenbuterol did not induce obvious AV conduction abnormalities. Medial dose began to interfere with conduction and lead to first-degree AV block and ectopic rhythms, while larger dose caused second-degree AV block and prolongation of QRS duration which is suggestive of ventricular conduction disturbance. This is significant because it indicates that the clinical manifestation of clenbuterol intoxication could vary from case to case, due to the dosage effect. This study gives the first in vivo evidence that acute clenbuterol poisoning could lead to AV block and cardiac asystole.

It is also meaningful to compare the acute and chronic arrhythmogenic effects of clenbuterol. In those with chronic oral intake of clenbuterol, tachyarrhythmia is the most prominent cardiac manifestation [1, 3, 4, 16], while in the acute administration of this agent at larger dose, AV block and even cardiac asystole predominate, no lethal tachyarrhythmia was observed (the present study). The following signaling pathways may underlie the beat-to-beat conduction failures in rabbit hearts after high clenbuterol administration. (1) Because β2-AR couples to Gi protein, activation of β2-AR by its agonists such as clenbuterol leads to release of Gβγ dimmer from the β2-AR–Gi complex. The released Gβγ dimmer can directly activate the G protein-regulated potassium channels (GKIR) especially the inwardly rectifying K+ (IK1) channel and hyperpolarize the cardiac cells (possibly including those in the AV node) [3], leading to a decreased excitability and conductivity, and therefore a disturbance in AV conduction. Clenbuterol at high concentrations predominantly activates the Gi protein [17], further supporting this hypothesis. (2) As the Gβγ dimmer of Gi protein inhibits β1-type Ca2+ channels [18], activation of β2-AR by clenbuterol may lead to blockade of β1-type Ca2+ channels and as a result, suppress cell depolarization in the AV node (and even potentially the ventricular myocytes) and therefore AV and/or ventricular conduction failure. We did not find supraventricular tachycardia or ventricular tachyarrhythmia in the present study, the potential mechanisms might include: (1) the maintenance of supraventricular tachycardia depends on normal AV conduction, and damage of AV conduction by clenbuterol may therefore prohibit the conduction of supraventricular pacemaking signals to the ventricle; (2) the inhibition of β1-type Ca2+ channels by clenbuterol may also occur in the ventricular ectopic pacemakers. In this case, the latent pacemaker in the ventricle may encounter difficulty in firing; (3) although not strictly confirmed by the present study, it is likely that clenbuterol may also interfere with ventricular conduction at higher dose, as evidenced by prolongation of QRS duration at a sinus rhythm (Fig. 3B-f). If this is finally proved true, then it may explain why ventricular tachyarrhythmia could not occur in the present study.

We noted in this study that the heart rate was actually accelerated after recovering from the ectopic arrhythmia at a medial clenbuterol dose, a phenomenon which is consistent with other observations [14] that clenbuterol could increase the heart rate via activating β1-AR at higher dose beside of its vasorelaxing effect via β2-AR. The β2-AR contributed less to clenbuterol-stimulated heart rate increase than β1-AR [14]. In addition, clenbuterol-induced hypotension may stimulate baroreflex and therefore increase the heart rate. We also noticed that higher clenbuterol did not accelerate the heart rate but rather induced conduction block, a possible explanation might be that higher clenbuterol predominantly affects the cardiac conduction, and therefore, its stimulating effect on heart rate could not take a leading role compared with relatively smaller clenbuterol dose.

We also observed that clenbuterol induced hypotension in a dose-dependent manner, a β2-AR-mediated vasorelaxing effect which is consistent with the human case report [9] but more serious. A very low BP induced by overdose clenbuterol actually means hypotensive shock. The sudden death induced by clenbuterol in the present study may be caused by AV conduction and/or ventricular conduction failure, hypotensive shock and acute deterioration of the pump function induced by the negative inotropic effect of clenbuterol owing to its inhibition of β1-type Ca2+ current and Ca2+ transient [17]. In addition, the effects of clenbuterol on vasculature and the heart may interact with each other during the induction of sudden death.
Apart from its toxicity at high dose, clenbuterol may exert some beneficial effect on the cardiovascular system at an appropriate dosage. Clenbuterol reduces oxidative stress and cardiac myocyte apoptosis in an experimental rat model of myocardium ischemia/reperfusion [19]. It would be worth studying similar effects of clenbuterol using markers of oxidative stress in the lung including 8-iso-prostaglandin F2α release from guinea-pig lung in vitro.

In conclusion, intravenous administration of clenbuterol induces hypotension, AV block and sudden death due to cardiac asystole in the rabbit. These findings may provide some clinical implications in treating clenbuterol poisoning. Future studies aimed at clarifying the mechanism of AV block caused by clenbuterol and the relevance for its acute toxicity in humans are warranted.

Acknowledgments This study was supported by grants of Scientific Research and Entrepreneurship for Undergraduates in Beijing City and a 973 program (2011CB503900) from the Ministry of Science and Technology of China. Ke Y, Fu LL, Hong XF and Dong R contributed equally to this work.

References