Cardiovascular Effects after Inhalation of Large Doses of Albuterol Dry Powder in Rats, Monkeys, and Dogs: A Species Comparison

Janet M. Petruska,*1 J. Gregory Beattie,† Bruce O. Stuart,* Sudhakar Pai,* Karen M. Walters,* Christopher M. Banks,† George W. Lulham,† and Elmer J. Mirro*

*Drug Safety and Metabolism, Schering-Plough Research Institute, P.O. Box 32, 144 Route 94, Lafayette, New Jersey 07848; and †ClinTrials BioResearch Ltd, Montreal, Quebec, Canada

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Cardiovascular Effects after Inhalation of Large Doses of Albuterol Dry Powder in Rats, Monkeys, and Dogs: A Species Comparison

Albuterol is a quickly acting β2-adrenergic agonist bronchodilator widely used by asthmatics. Because recent case–control studies have suggested a relationship between the increase in mortality of asthmatics over the past decade and the use of β2-adrenergic agonists in the control of asthma, concern has developed regarding the potential cardiotoxicity of β2-specific adrenergic agonists, including albuterol. The aim of this investigation was to assess the potential for cardiotoxicity of inhaled albuterol dry powder in rats, monkeys, and dogs. All species were exposed to an aerosol of albuterol 1 h per day, 7 days per week, for at least 2 weeks. Control groups were exposed to filtered conditioned air and handled in the same manner as the albuterol-exposed animals. Plasma concentrations of albuterol confirmed systemic exposure. The daily inhaled dose received by the animals was calculated based on measured respiratory minute volumes, published respiratory tract deposition data, as well as HPLC-determined particle size distribution data and aerosolized albuterol concentrations. Multiples of the maximum daily clinical dose (presentation of 15 μg/kg in a 70-kg human) were approximately 0.25-2500-fold in the rat, 9-100-fold in the monkey, and 0.5-50-fold in the dog. No findings attributed to albuterol were observed in the monkey. Tachycardia and transient hypokalemia occurred in rats at multiples of 1.5 times or greater of the maximum clinical dose. Absolute and relative heart weights increased in rats receiving multiples of 47 times or greater of the maximum human dose. In the absence of histopathologic findings, the increases in rat heart weights were considered a physiologic hypertrophic response to tachycardia. In dogs tachycardia and transient hypokalemia occurred at all doses tested. Slight to mild fibrosis in the papillary muscles of the left ventricle of the heart occurred in dogs at multiples ≈19 times the clinical dose. The cardiovascular effects observed were consistent with the known pharmacologic action of β2-adrenergic agonists. Due to the lack of toxicologically relevant findings in rats and monkeys and the wide safety margin in dogs, the findings in this study do not suggest a cardiotoxicity risk in the human population after repeated exposures to clinical doses of albuterol currently used in the treatment of asthma. © 1997 Society of Toxicology.

For almost 30 years, albuterol has been used as a prophylactic and rescue medication for the relief of bronchospasm associated with reversible obstructive airway disease (Anonymous, 1971). Albuterol possesses greater than a 500-fold activity toward β2-adrenergic receptors in comparison to β1-adrenergic receptors (Jack, 1991). Because β2-adrenergic receptors predominate in the smooth muscle of the bronchi and activation of these receptors results in bronchodilation (Barnes, 1995), albuterol is well-suited for its indication. Albuterol also is rapidly acting, potent, efficacious, and has a long history of safe use (Price and Clissold, 1989).

Concern has developed in the past decade regarding the safety of β2-adrenergic bronchodilators, including albuterol, given their widespread use and the increase in asthma mortality (Sears and Taylor, 1994). Recent case–control studies have suggested a link between the clinical use of albuterol and the increase in asthma mortality (Spitzer et al., 1992; Coughlin et al., 1995). In addition, reports describing inhalation or ingestion of doses of albuterol exceeding the maximum clinical daily dose and the subsequent tachycardia, hypokalemia, and potential for eliciting arrhythmias have caused concern (Leitch et al., 1976; Lewis et al., 1993; Wong et al., 1990). The objective of this investigation was to evaluate the potential for cardiotoxicity of inhaled large doses of albuterol in rats, monkeys, and dogs. The animals were exposed to albuterol dry powder using well-characterized exposure systems. Measurements of plasma concentrations of albuterol and serum potassium, evaluation of electrocardiographic data, and evaluation of heart tissue for organ weight and histopathologic changes were performed.

METHODS

Inhalation and exposure system. Seven-week-old Sprague–Dawley rats (Crl:CD (SD)BR/viral antibody-free, Charles River, Canada), young
adult cynomolgus monkeys (Charles River, TX) and 6- to 7-month-old beagle dogs (HRP, MI) were exposed to aerosols of micronized albuterol dry powder (99.9% purity), 1 h/day, for 7 days/week, for approximately 2 weeks. In all studies, a control group consisting of animals exposed to filtered conditioned air only was included and was handled in the same manner as the albuterol-exposed animals, with the exception of exposure to drug.

For all studies, the aerosol of albuterol dry powder was generated using a fluid energy air mill (Trost Mill, Gem T Research model, Garlock, Inc., Newton, PA) (Cheng et al., 1985). Different concentrations of aerosolized albuterol were obtained by controlling the rate at which the mill was supplied the albuterol dry powder. For each daily exposure, concentrations of albuterol were continually estimated during exposures using a real-time aerosol monitor (RAM-S, MIE Inc., MA) and confirmed by HPLC analyses of filter samples collected quarter hourly at the animal’s breathing point during the exposure period. In addition, once weekly, particle size analyses were performed using an Andersen 1ACFM cascade impactor (Andersen Instruments, Atlanta, GA) situated at the animal’s breathing point. The effective cutoff diameters (ECD50) for each stage were 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.7, and 0.4 µm. The mean mass aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated as well as mean MMADs and GSDs. The mean particle size distribution data were used for the calculations of total achieved dose levels of albuterol.

Rats, positioned in restraint cones, were exposed using “flow-through” nose-only inhalation chambers (ADG Developments, England). Monkeys were positioned on slings with only their heads exposed in a stainless steel chamber to the appropriate aerosols. Dogs were positioned on slings and were exposed using masks fitted to the mouth and nose. For dog exposures, the aerosol was delivered from a central chamber through tubing to which the masks were attached. The care and use of all animals were conducted in accordance with the regulations of the USDA Animal Welfare Act (relevant sections of 9 CFR, Parts 2 and 3) and the Canadian Council on Animal Care.

For all studies, animals were acclimated to the exposure systems prior to initiation of exposures. The airflow was maintained so as to provide more than adequate oxygen to all animals during each day’s exposure. Prior to initiation of exposures, at intervals during the dosing period and after completion of dosing, filter samples were obtained to determine the uniformity of aerosol concentrations within each dose group and the uniformity of particle size distributions within each dose group and/or study. Analyses confirmed that aerosol concentrations were uniform within each dose group within a study and that particle size distributions were uniform within each dose group and among all groups within each study. Animals were placed onto the exposure systems only after the target aerosol concentrations were reached, and were rotated to a different position for the following day’s exposure. All aerosol exhausted from the exposure systems was purified using a 5-µm coarse filter and an absolute filter (99.97% efficient at 0.3 µm) before expelling the air from the building.

**HPLC analyses of filters collected from the exposure system.** Filters were extracted using a solution of dodecyl sulfate, glacial acetic acid, methanol, and distilled water. The albuterol extracted from the filters was separated by reverse phase HPLC using a Bondapak phenyl column (Waters Co., MA) and a mobile phase consisting of dodecyl sulfate, glacial acetic acid, methanol, and distilled water. An internal standard was included in each separation and the peak area ratios of albuterol to the internal standard were calculated. Albuterol was detected at 280 nm and quantitated using a standard curve.

**Respiratory minute volume measurements.** Respiratory minute volume (RMV) measurements were obtained prior to initiation of exposure for four rats/sex/group and on all monkeys and dogs. All RMV measurements were obtained using the Buxco Electronics LS-20 system (Sharon, CT). The same rats also had RMV measurements during the exposure period during Week 1 or 2. Monkeys and dogs had RMV measurements during Week 1 or 2 within 2 h following the exposure period.

**Calculation of achieved dose levels.** Total achieved dose levels of albuterol were calculated according to

\[
\text{total achieved dose} = \text{RMV} \times \text{albuterol} \times 60 \times \text{deposition factor} / \text{mean body weight of animal} \ \text{kg}.
\]

For the dog and monkey studies, the individual mean RMV was used in the equation. For the rat studies, the mean RMV for each sex in each dose group was used. The overall mean albuterol concentrations for each dose group were used in the equation. Sixty minutes was the daily duration of dosing for all studies. Individual mean body weights over the course of the dosing period also were utilized.

The deposition factor was calculated for each species based upon published respiratory tract deposition data. Total or regional deposition curves were constructed for each species encompassing the range of ECD50s of the stages in the Andersen 1ACFM cascade impactor. For the rat, the regional deposition curves of the Fischer 344 rat following inhalation of monodisperse aerosols were used (Raabe et al., 1988). The doses calculated for each region in the rat were summed to give a total achieved dose of albuterol. A curve of total respiratory tract deposition in cynomolgus and rhesus monkeys was used to determine the deposition factor for the monkey exposures (Palm et al., 1956). Because the monkey deposition data were similar to those found in the human and because no data were available for particles with an aerodynamic size greater than 5.0 µm for monkeys, the deposition factor for particles greater than 5.0 µm was assumed to be 100%, as is approximated for the human (Palm et al., 1956). For the dog studies, a total deposition curve was constructed from particle size diameters ranging from 0.02 to 2.45 µm using the deposition data of Kanapilly et al. (1982), Wolff et al. (1981, 1982), and Cuddihy et al. (1973). Due to the lack of suitable published canine particle size deposition data for aerodynamic sizes greater than 2.45 µm, human oral inhalation data (Phalen et al., 1991) was used to extend the deposition curve prepared from the canine data over the range of aerodynamic particle sizes separated by the Andersen cascade impactor.

The mean relative quantities of albuterol aerosol collected at each stage of the cascade impactor during the weekly particle size analyses were used in the calculation of achieved doses. The mean quantity of aerosol collected at each stage of the impactor was multiplied by the deposition factor interpolated from the curves of deposition data for each species. In order that the proportion of particles of aerodynamic size below 0.4 µm not be excluded from the calculation of the total deposition factor and because the Andersen cascade impactor could not separate particles of aerodynamic sizes between 0 and 0.4 µm, the fraction of particles collected below the final stage of the cascade impactor were added to the fraction of particles collected on the last stage of the impactor (ECD50 of 0.4 µm). The products of the quantity of albuterol at each stage (and below for the last stage of the cascade impactor) and of the deposition fraction at each impactor stage were summed to give a deposition factor. This deposition factor was used in the equation to determine the dose for each animal. Mean total achieved dose levels were calculated for each sex in each dose group.

**Cardiovascular studies.** Prior to initiation of the daily exposure and at intervals during and postexposure (Exposure Days 1–2, 13–15), electrocardiograms (EKGs) were obtained for 3–9 animals/sex/group. Electrocardiographic recordings were made using limb leads I, II, and III for the rats; limb leads I, II, III, aVR, aVL, and aVF and chest leads V1, V2, and V3 for the monkeys; and limb leads I, II, III, aVR, aVL, and aVF and chest leads V2, V3, and V4 for the dogs. All EKGs were obtained while the animals were positioned in the restrainers used for the exposures. The Cambridge Model MC 6400 analog instrument (Cambridge, England) and the Schiller Model CS 612 digitized instrument (Schiller-Reomed AG, Switzerland) were used for recording the EKGs. Heart rate values were obtained from the EKGs and the waveforms were qualitatively evaluated.
Determination of plasma albuterol concentrations. Prior to exposures and at intervals up to and including 24 h following exposures, heparinized blood samples were collected from rats (while under ether-induced anesthesia from the abdominal aorta), monkeys (via femoral venipuncture), and dogs (via jugular venipuncture). The plasma was separated by centrifugation, frozen at approximately -80°C, and later assayed for the presence of albuterol using a HPLC method with a limit of quantitation of 2.0 ng/ml. Briefly, the method involved adsorption onto a C-8 solid-phase cartridge, followed by an extensive washing procedure. Albuterol and the internal standard were eluted with methanolic ammonium acetate, the eluate was evaporated, and an aliquot of the reconstituted extract was analyzed by HPLC with fluorometric detection. The assay was validated for rat, monkey, and dog plasma. In addition, for each study, plasma collected from control animals (not exposed in the exposure system to control air or albuterol) served as assay blanks.

Determination of serum potassium concentrations. On Days 1 and 14 or 15 for rats and dogs and on Day 13 for monkeys, serum was collected at the same intervals used for collection of plasma for determination of albuterol concentrations and analyzed for potassium using a Hitachi-717 analyzer (Mito, Japan).

Histopathology and organ weight assessment of heart tissue. Following completion of the dosing (during Week 3) or a 4-week postdose recovery period, the animals were fasted overnight and weighed. They were then anesthetized, exsanguinated, and subjected to a complete necropsy. The hearts were dissected and weighed; relative heart weights were calculated by dividing the absolute heart weight by the terminal body weight of the animal. The hearts were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. Deeper sections of the heart (specifically sections containing the left ventricular papillary muscle) of monkeys and dogs also were stained.

Achieved Aerosol Concentrations and Dose Levels of Albuterol, Plasma Albuterol Concentrations, and Particle Size Distributions

All animals survived the dosing regimen. A summary of mean albuterol aerosol concentrations, achieved doses of albuterol, C_max plasma concentrations following a single 1-h exposure and particle size distribution data is presented in Table 1. Mean achieved albuterol aerosol concentrations were within 20% of target aerosol concentrations. The coefficient of variation for achieved albuterol aerosol concentrations varied no more than 20% for all studies. Mean MMADs ranged from 1.0 to 2.1 µm for all species and mean GSDs were no greater than 2.1. Within each exposure system for each species, the MMADs increased slightly as the albuterol aerosol concentration increased. This is commonly seen in dry powder exposure systems, as agglomeration may occur as the aerosol concentration is increased. For each species, the slight differences in MMADs did not result in a significant difference in total percentage deposition of albuterol among the dose groups.

Rats were exposed to target albuterol aerosol concentrations ranging from 0.25 to 1000 µg/L. The mean total doses the rats received ranged from 5.1 µg/kg daily to approxi-

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**TABLE 1**

Summary of Mean Aerosol Concentrations, Achieved Dose Levels, C_max Plasma Albuterol Concentrations, and Particle Size Analyses

<table>
<thead>
<tr>
<th>Species</th>
<th>Target albuterol aerosol conc. (µg/L)</th>
<th>Achieved albuterol aerosol conc. (µg/L)</th>
<th>Achieved dose level (µg/kg/day, mean ± SD)</th>
<th>Day 1 C_max* (ng/ml)</th>
<th>MMAD* (µm) ± GSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>0.25</td>
<td>0.233</td>
<td>5.1 ± 0.35</td>
<td>5.8 ± 0.37</td>
<td>1.3 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.2</td>
<td>12 ± 0.7</td>
<td>22 ± 1.7</td>
<td>13.4 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.5</td>
<td>84 ± 4.7</td>
<td>73 ± 3.8</td>
<td>11.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>11.6</td>
<td>178 ± 12.5</td>
<td>245 ± 18.1</td>
<td>30.7 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.233</td>
<td>5.1 ± 0.35</td>
<td>5.8 ± 0.37</td>
<td>1.3 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.2</td>
<td>12 ± 0.7</td>
<td>22 ± 1.7</td>
<td>13.4 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
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<td>73 ± 3.8</td>
<td>11.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>11.6</td>
<td>178 ± 12.5</td>
<td>245 ± 18.1</td>
<td>30.7 ± 3.0</td>
</tr>
<tr>
<td>Monkeys</td>
<td>4</td>
<td>4.2</td>
<td>132 ± 10.3</td>
<td>141 ± 24.7</td>
<td>81.2 ± 25.6</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>12.9</td>
<td>419 ± 50.8</td>
<td>495 ± 85.5</td>
<td>112.9 ± 14.3</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>36.5</td>
<td>1,542 ± 218</td>
<td>1,138 ± 306</td>
<td>987 ± 62.6</td>
</tr>
<tr>
<td>Dogs</td>
<td>0.5</td>
<td>0.53</td>
<td>15 ± 10.9</td>
<td>9 ± 2.2</td>
<td>7.17 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4.3</td>
<td>82 ± 24.3</td>
<td>66 ± 22</td>
<td>5.85 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>16.6</td>
<td>352 ± 176</td>
<td>280 ± 88.3</td>
<td>35.9 ± 3.5</td>
</tr>
</tbody>
</table>

* C_max is the maximal plasma albuterol concentration. This occurred immediately following exposure.

**Note.** Achieved albuterol aerosol concentrations were determined daily. Achieved doses of albuterol were calculated for 10 rats/sex/group, 3 or 6 monkeys/sex/group, and 3 or 6 dogs/sex/group. C_max plasma concentrations following a single 1-h exposure to albuterol dry powder were obtained from 1 to 5 rats/sex/group, 3 or 6 monkeys/sex/group, and 6 or 9 dogs/sex/group. Particle size analyses were performed weekly.

* MMAD is the mass median aerodynamic diameter.

* GSD is the geometric standard deviation.

* BLD is below the limit of detection of the plasma albuterol assay.
INHALED ALBUTEROL IN RAT, MONKEY, AND DOG

FIG. 1. Individual male Cmax plasma albuterol concentrations in rats (○), monkeys (▲), and dogs (■) following a single 1-h exposure to albuterol dry powder aerosol. The achieved albuterol aerosol concentrations are shown in Table 1. N = 4-5 males/group for rats, N = 3 or 6 males/group for monkeys, and N = 6 or 9 males/group for dogs.

approximately 38 mg/kg/day. The maximum daily clinical dose of marketed albuterol is approximately 15 µg/kg in a 70-kg human, assuming that the total presented dose of 1080 µg administered via oral inhalation from a metered dose inhaler (Physician’s Desk Reference, 1996) is inhaled and deposited in the body via the airways and gastrointestinal tract. Therefore, the total achieved dose levels in rats corresponded to multiples of the maximum human dose ranging from approximately 0.25 to 2500 times.

Target aerosol concentrations for monkeys ranged from 4 to 36 µg/L albuterol. The mean total achieved doses in monkeys ranged from 132 µg/kg/day to approximately 1.5 mg/kg/day, corresponding to multiples of the maximum human dose ranging from approximately 9- to 100-fold.

Dogs were exposed to target albuterol aerosol concentrations ranging from 0.5 to 64 µg/L. The mean total achieved doses the dogs received ranged from approximately 9 µg/kg/day to 1.4 mg/kg/day. The achieved dose levels in dogs corresponded to approximately 0.5 to 90 times the maximum daily clinical dose.

Albuterol was not detected in the plasma of rats exposed to a target aerosol concentration of 0.25 µg/L albuterol and of dogs exposed to a target aerosol concentration of 0.5 µg/L albuterol. For all species, Tmax occurred immediately following exposure to albuterol. Mean and individual Cmax values (Fig. 1) increased in a dose-related manner with respect to achieved albuterol aerosol concentration and achieved dose. Additionally, across all species, Cmax plasma concentrations were comparable at similar achieved albuterol aerosol concentrations. Plasma samples collected following up to 3 months of consecutive daily exposures to albuterol aerosol revealed that albuterol did not accumulate after repeated dosing (data not shown).

Electrocardiography

There were no sex-dependent or day-related differences in heart rates in any species; therefore heart rates for males after one or two exposures only are shown (Fig. 2). Due to the marked variation in and high values of resting monkey heart rates prior to exposure to albuterol, heart rate differences following exposure to albuterol in monkeys could not be discerned. In rats during and immediately following exposure, tachycardia was evident at target albuterol aerosol concentrations of ≥1 µg/L after 1 and 15 consecutive days of exposure. Tachycardia occurred at all doses in dogs after 2 and 13 days of exposure. Heart rates in rats and dogs returned to preexposure values at 6 h following exposure to albuterol.

There were no albuterol-related EKG waveform findings in rats and monkeys (Table 2). However, dogs exposed to target albuterol aerosol concentrations of 0.5, 1.6, and 64 µg/L exhibited ventricular extrasystole and/or reversal of the polarity of T waves in chest leads rV2 and V10.

Serum Potassium

There were no sex-dependent or day-related differences in serum potassium values within each species; therefore, only male values from Day 1 are shown (Fig. 3). A minimal, transient hypokalemia occurred in rats exposed to albuterol aerosol concentrations of 1 µg/L and above after
TABLE 2
Electrocardiographic Findings

<table>
<thead>
<tr>
<th>Species</th>
<th>Target albuterol aerosol conc. (µg/L)</th>
<th>Heart rate findings</th>
<th>Waveform findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Rat</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
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<td></td>
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<td>1</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>t</td>
<td>t</td>
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<tr>
<td></td>
<td>12</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td></td>
<td>300</td>
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</tr>
<tr>
<td></td>
<td>1000</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>Monkey</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>Dog</td>
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</tr>
<tr>
<td></td>
<td>0.5</td>
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<td></td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
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<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>64</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. *N = 4/sex/group for rats, N = 3 or 6/sex/group for monkeys, and N = 6 or 9/sex/group for dogs.

a Increased relative to preexposure and concurrent control values.
b The numerator refers to the number of animals with the finding. The denominator refers to the number of animals examined.

Inhalation of albuterol aerosols at targeted concentrations up to and including 1000 and 36 µg/L in rats and monkeys, respectively, for approximately 2 weeks, resulted in no histopathologic heart lesions related to exposure to this β2-adrenergic agonist. Dogs exposed to target aerosol concentrations of 16 and 64 µg/L albuterol for 2 weeks exhibited a slight to mild, focal or multifocal cardiac fibrosis with or without a slight mineralization of the left ventricular papillary muscle (Table 3). Following a 4-week postexposure period, one of three males in the 64 µg/L albuterol group exhibited a slight focal fibrosis with slight focal mineralization of the left ventricular papillary muscle.

DISCUSSION

Inhalation of albuterol at multiples up to and including approximately 100-fold of the maximum daily clinical dose...
Albuterol-related heart tissue histopathologic findings in dogs

<table>
<thead>
<tr>
<th>Target albuterol aerosol conc. (µg/L)</th>
<th>Males</th>
<th>Recovery*</th>
<th>Females</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 3</td>
<td></td>
<td>Week 3</td>
</tr>
<tr>
<td>0.5</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>NP*</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>16</td>
<td>None</td>
<td>None</td>
<td>1/3 Slight multifocal fibrosis</td>
<td>None</td>
</tr>
<tr>
<td>64</td>
<td>2/3 Slight focal fibrosis with slight focal mineralization</td>
<td>None</td>
<td>1/3 Slight focal fibrosis with slight focal mineralization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/3 Mild multifocal fibrosis with slight focal mineralization</td>
<td>None</td>
<td>1/3 Slight focal fibrosis with slight focal mineralization</td>
<td></td>
</tr>
</tbody>
</table>

Note. N = 3 dogs/sex/group.

* Three dogs/sex/group in the 4, 16, and 64 µg/L groups were retained for a 4-week postdose period.

NP* refers to ‘not performed.”

‘The numerator refers to the number of animals with the finding. The denominator refers to the number of animals examined.

Actuated from a metered dose inhaler resulted in no remarkable findings in cynomolgus monkeys. Tachycardia and a transient hypokalemia were observed in rats at multiples of 1.5 times or greater than the maximum daily clinical dose. The tachycardia was no longer apparent at 6 h following dosing, and the transient hypokalemia was not clinically significant, as most serum potassium values were within the normal physiologic range for this species. Rats dosed with albuterol aerosol at 66 times or greater than the maximum daily clinical dose had slightly increased absolute and/or relative heart weights in comparison to filtered conditioned air control rats. This increase in heart weight also occurred in rats dosed with albuterol aerosol at 47 times the maximum daily clinical dose (data not shown). Due to the absence of albuterol-related histopathologic findings in the heart tissue of rats receiving ≤2500 times the maximum daily dose, the slight heart weight increases were considered to be due to a physiologic adaptation related to the tachycardia. Results of this study indicate that administration of exceedingly large doses of albuterol via nose-only inhalation to rats is not cardiotoxic.

Subcutaneous administration of albuterol for 3 days at doses of 0.5–50 mg/kg to Sprague–Dawley rats resulted in a slight myocardial necrosis in the apex and papillary muscle, with concomitant increases at 50 mg/kg in absolute and relative heart weights in comparison to saline controls (Magnusson and Hansson, 1973). The slight myocardial necrosis was characterized by edema, hemorrhage, and mononuclear cell infiltration in the necrotic areas. Oral administration of albuterol for 1 month at doses of 17, 50, and 150 mg/kg resulted in statistically significantly higher absolute and relative heart weights in rats (increased 13 to 27%) in comparison to saline controls (Yamada et al., 1977). Following a 1-month recovery period, heart weights of rats in all dose groups were comparable to saline controls. Orally administered albuterol resulted in edema, a slight to moderate focal myocardial necrosis in the apex and subendocardial layer of the left ventricle, and hypertrophy of muscle fibers of the left ventricle. At the end of the 1-month recovery period, the incidence and severity of heart lesions in all groups were markedly decreased. The cardiotoxicity observed after subcutaneous and oral dosing in rats occurred at doses greatly exceeding the maximum daily exposure of inhaled or orally administered albuterol (Physician’s Desk Reference, 1996).

Tachycardia and transient hypokalemia also were observed in dogs at all aerosol concentrations of albuterol tested. The tachycardiac effects of albuterol in dogs have been observed previously after intravenous administration of 0.5, 1, and 2 µg/kg in adult anesthetized mongrel dogs (Nayler and McInnes, 1972), after a single intravenous dose of 100 µg/kg in anesthetized dogs (Maxwell et al., 1969) and following oral or intraduodenal administration of 25 µg/kg, intratracheal administration of 400 µg per dog, and intravenous administration of 0.25, 1, and 4 µg/kg in anesthetized mongrel dogs (Minatoya, 1978).

The cardiac effects of albuterol are believed to be due to a reflex tachycardia resulting from albuterol-induced peripheral and coronary vasodilation and from a reduction in peripheral resistance. In anesthetized dogs receiving a single intravenous dose of 100 µg/kg albuterol, tachycardia occurred concomitant with an increase in cardiac output and reductions in femoral arterial pressure and total peripheral resistance (Maxwell et al., 1969). In addition, coronary blood flow was increased and coronary vascular resistance was decreased in comparison to controls. Intravenous infusion of 0.86–7.2 µg/kg albuterol in anesthetized greyhounds also resulted in increased cardiac output and reductions in femoral arterial diastolic pressure (Loh et al., 1971). Intravenous dosing of 2 µg/kg albuterol to anesthetized adult mongrel dogs resulted in tachycardia, reduced mean arterial pressure,
and increased coronary blood flow (Nayler and McInnes, 1972). In the present study, slight reductions in indirect systolic blood pressures measured over the tail occurred on occasion in addition to tachycardia (data not shown). Dogs which received ≥0.5 times the human daily dose of albuterol also exhibited T wave changes in chest leads rV₂ and V₁₀. The reversal of polarity of T waves observed is considered a secondary effect of the hypokalemia (Detweiler, 1981) and occurred simultaneously with reductions in serum potassium. Hypokalemia is believed to occur due to the stimulation of skeletal muscle Na–K–ATPase, resulting in an influx of potassium and resultant reduction in extracellular potassium (Bradberry and Vale, 1995). The changes in T waves in chest leads rV₂ and V₁₀ are relevant in a dog model, as the T waves in leads rV₂ and V₁₀ can reverse polarity due to drug-induced effects, whereas the T waves in the other leads are labile and may reverse polarity under normal conditions (Detweiler, 1981). The tachycardia, hypokalemia, and waveform changes observed were transient, and were not evident within 6–23 h following inhalation of the large doses used in this study.

Tachycardia and hypokalemia are believed to be due to
an extension of the pharmacologic action of $\beta_2$-adrenergic agents and have been observed in humans following inhalation (Rolf-Smith et al., 1984; Lipworth et al., 1988; Lipworth and McDevitt, 1989; Wong et al., 1990), intravenous administration (Leitch et al., 1976; Neville et al., 1977; Morice et al., 1986), and oral ingestion of doses of albuterol exceeding the maximum daily clinical dose (Lewis et al., 1993; Wiley et al., 1994). These studies in humans included asthmatic patients as well as healthy volunteers. The doses used in the inhalation studies in man were generally greater than the maximum daily dose and/or were given in greater frequency than recommended (Physician’s Desk Reference, 1996). The intravenous route of administration is used in asthmatics only when inhalation of a $\beta_2$-agonist fails to provide adequate bronchodilation (Nelson, 1995) and the doses used in the above referenced studies were far greater than that administered on a daily basis. The ingestion studies investigated overdoses, which occur rarely in individual patients. With all routes of administration, the tachycardia and hypokalemia were reversible. The heart rates and serum potassium values returned to normal physiologic values within no more than a few hours following withdrawal of albuterol.

Dogs dosed with 19 times or greater the maximum clinical daily dose had a slight to mild, focal or multifocal, cardiac fibrosis with or without a slight mineralization of the left ventricular papillary muscle. This finding also was observed in one dog following a 4-week postdose period. This lesion is commonly seen following the administration of other vasodilating compounds in the dog (Detweiler, 1989; Dogterom et al., 1992). The combination of waveform changes present (reversal of polarity of $T$ waves in leads $V_2$ and $V_{10}$ and ventricular extrasystoles) is consistent with the cardiac lesions observed. Dogs are believed to be uniquely sensitive to vasodilating agents (Detweiler, 1989), including $\beta_2$-adrenergic agonists, in comparison to the human population (Dogterom et al., 1992). Therefore, the heart lesions observed in the dog are believed not to be relevant to the human population after repeated clinical dosing.

In addition, the safety margin of 19-fold in the dog is probably closer to 95- to 190-fold when one takes into account that in man only approximately 10–20% of the total administered dose from a metered dose inhaler is actually deposited into the lungs and is bioavailable (Melchor et al., 1993; Newman et al., 1984). When comparing plasma concentrations among the three species tested, cardio toxicity was noted in the dog only at mean concentrations of approximately 36 ng/ml and above. These plasma concentrations are approximately 12-fold greater than that reported for humans inhaling a dose of 1200 µg from a metered dose inhaler over a 6-min period (Clark et al., 1996; Clark and Lipworth, 1996). In addition, patients who ingested overdoses of albuterol and had plasma concentrations ranging from 18 to 449 ng/ml survived without any serious cardiac arrhythmias (Lewis et al., 1993). The magnitude of exposure seen in the dog in this study is unlikely to occur in the human population except in the case of an exceedingly large overdose.

There are case-control studies which suggest that repeated exposure to clinical doses of albuterol may be responsible for the increase in asthma mortality (Spitzer et al., 1992; Coughlin et al., 1995). It is important to note that case-control studies cannot establish a cause and effect relationship (Taubes, 1995). In the Saskatchewan case control study, there was an increased risk from near death or death due to fatal asthma with the use of ≥1.4 canisters of $\beta_2$-agonist per month (Suissa et al., 1994). This corresponds to approximately 10 puffs per day, close to the maximum recommended daily dose of 12 puffs of 90 µg each for albuterol (Physician’s Desk Reference, 1996). It was then hypothesized that the severity of asthma could be linked to the increased risk for death or near death from asthma, as more severe asthmatics or those patients with poorly controlled asthma may overuse $\beta_2$-agonist inhalers (Ernst et al., 1993). However, not all appropriate indices of assessing asthma severity (National Asthma Education Program, 1991) were investigated in the later analysis (Ernst et al., 1993); therefore, one can surmise that the severity of asthma still may be a confounding variable in the analysis of the Saskatchewan study data. Another case-control study suggests that use of $\beta_2$-agonists, including albuterol, is linked to an increased risk of developing idiopathic dilated cardiomyopathy (Coughlin et al., 1995). This study employed few subjects and did not take into account the dose and frequency of $\beta_2$-agonist use. In addition, the authors stated that some of the subjects studied may not have had a history of true asthma, but rather an episode of cardiogenic bronchospasm and wheezing.

Autopsies of patients who have died from asthma fail to show cardiotoxic effects and instead reveal the hallmarks of death due to asphyxiation (Barger et al., 1988; Johnson et
al., 1984; Molfino et al., 1991; Sly, 1988). Furthermore, appropriate treatment, including the administration of albuterol, of patients with severe, potentially fatal asthma routinely resulted in improvement of symptoms and prevention of morbidity and/or mortality (Nelson, 1995). In a recent study of adequately oxygenated acute severe asthmatics administered doses up to and including 1600 μg of albuterol by inhalation using an aerosol holding chamber, cardiovascular mortality and clinically significant arrhythmias were not observed (Newhouse et al., 1996). These human data support that albuterol is not a cardiotoxic agent.

In summary, albuterol continues to be used safely as a bronchodilating agent for asthmatics. Due to the lack of toxicologically relevant findings in rats and monkeys and to the large safety margin in dogs, albuterol is not believed to be a cardiotoxic agent as used routinely for the treatment of asthma.

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