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Theophylline-induced mesenteric periarteritis in F344/N rats

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Abstract The toxicity and carcinogenic potential of theophylline (an alkaloid bronchodilator drug) was investigated in male and female F344/N rats in 16-day, 14-week, and 2-year gavage and feeding studies. In 16-day studies, rats were fed diets containing 0, 500, 1000, 2000, 4000, and 8000 ppm of theophylline or given 0, 12.5 (twice daily), 25 (once daily), 50 (once daily), 50 (twice daily), 100 (once daily), 200 (once daily), 200 (twice daily), and 400 (once daily) mg theophylline/kg body weight in corn oil by gavage. In 14-week studies, rats were fed diets containing 0, 1000, 2000, and 4000 ppm theophylline or given 0, 37.5, 75, and 150 mg/kg body weight theophylline in corn oil by gavage. In 2-year gavage studies, rats were given 0, 7.5, 25, and 75 mg/kg body weight in corn oil. In 16-day gavage studies, treatment-related periarteritis occurred in arteries of the pancreas and adjacent to the mesenteric lymph nodes of early death male and female rats given 400 mg/kg once daily. In the 14-week studies, treatment-related periarteritis occurred at similar sites and in male rats exposed to 75 and 150 mg/kg, and in all exposed female rats (gavage studies), in females exposed to 1000 ppm, and in both sexes exposed to 2000 and 4000 ppm (feeding studies). In the 2-year study, chronic periarteritis was significantly increased only in the males receiving 75 mg/kg of theophylline. The adventitia, media and intima of medium- and large-sized mesenteric arteries were involved. Similar to other vasodilator chemicals, the pathogenesis of theophylline-induced vascular lesions may be a consequence of hemodynamic changes induced in the vascular wall.

Key words Theophylline · Alkaloid · Pharmaceutical · Bronchodilator · Arteritis

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Introduction

Theophylline (1,3-dimethylxanthine) is an alkaloid found in cocoa (*Theobroma cacao*) and tea (*Thea sinensis*) and is structurally related to caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine). Theophylline is used as a pharmaceutical agent having the following effects: it stimulates the heart and central nervous system, relaxes bronchial and vascular smooth muscle, and causes diuresis. The main pharmaceutical uses are as a bronchodilator in obstructive airway diseases, such as bronchial asthma, and for myocardial stimulation. The therapeutic doses of theophylline range from 3 to 6 mg/kg, yielding a serum level of 10 to 20 μg/ml (Kodama et al. 1980). Theophylline is an inhibitor of tumor necrosis factor (TNF-α; Semmler et al. 1993) and therefore has therapeutic use in the treatment of chronic obstructive pulmonary disease, a disease in which TNF-α has been shown to play a pathogenic role (Semmler et al. 1993; Di Francia et al. 1994).

Theophylline was nominated by the National Cancer Institute in 1980 for toxicologic and carcinogenicity testing by the National Toxicology Program as part of a class study on alkaloid compounds. Theophylline is a methylxanthine structurally similar to purines and purine bases (Cornish and Christman 1957; Grygiel and Birkett 1980) and was selected for study as a representative of the purine structural subclass, particularly because of its relationship to purines, such as caffeine, 1-methyl-3-hydroxyguanine, and 3-hydroxy-1-methylxanthine. The last-mentioned two compounds have been shown to induce sarcomas in rats when injected subcutaneously (Clayson and Garner 1976). The nomination was also based upon its widespread use in humans as a pharmaceutical agent, possible genotoxicity in vitro, and the lack of information on the potential toxicity and/or carcinogenicity under conditions of chronic oral usage.

Sixteen-day, 14-week and 2-year studies of theophylline were conducted in male and female F344/N rats. The oral route of administration was selected

because it is the primary route of human exposure to this pharmaceutical agent. Male and female F344 rats were given theophylline (>99% pure) in feed or by gavage in corn oil for 16 days or 14 weeks, or by gavage in corn oil for 2 years. Dosed feed was initially selected as the route of administration for the 16-day acute toxicity studies because theophylline is only moderately soluble in water. Additional 16-day studies were conducted using corn oil gavage as the route of administration in order to mimic human therapeutic use. Gavage studies also compared the toxicity of equivalent doses of theophylline given once versus twice daily; the latter split-dosing regimen more closely simulates human therapeutic exposure to theophylline (normally four times per day). Based on the results of the 16-day studies, 14-week studies were designed and conducted using two oral routes of administration, dosed feed and corn oil gavage given once daily. Two-year (chronic) toxicity/carcinogenicity studies were conducted using the gavage route of exposure.

In the present report, we describe the treatment-related vascular lesions (periarteritis) observed in male and female F344/N rats given theophylline orally for 16 days, 14 weeks and 2 years. The lesions are discussed in relation to their pathogenesis and anatomical location. Details of the 14-week studies have been already published (Collins et al. 1988). However, for completeness, the 14-week pathological data is also included in the present paper. Additional data are included in the National Toxicology Program (NTP) Technical Report no. 473 (1998).

Materials and methods

Study design

Sixteen-day feeding and gavage studies were conducted to determine target organ toxicity and the dose levels to be used in the 14-week studies. For the feeding studies, five rats/sex per group were fed diets containing 0, 500, 1000, 2000, 4000, and 8000 ppm theophylline for 16 days. For the gavage studies, five rats/sex per group were given theophylline in 100% pure Mazola corn oil at doses of 0, 12.5 (twice daily), 25 (once daily), 50 (once daily), 50 (twice daily), 100 (once daily), 200 (once daily), 200 (twice daily), and 400 (once daily) mg theophylline/kg body weight, 5 days per week. Gavage volumes were 5 ml/kg body weight. For both the dosed feed and the gavage studies, animals were observed twice daily for clinical signs of toxicity. In the feeding studies, selected tissues were evaluated histologically. In the gavage studies, complete histopathological examination was performed on all gross lesions and all tissues of the control, and groups given 200 (once daily), 200 (twice daily), and 400 (once daily) mg/kg body weight.

Fourteen-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to theophylline and to determine the appropriate doses to be used in the 2-year studies. In the feeding studies, ten rats/sex per group were fed diets containing 0, 1000, 2000 and 4000 ppm theophylline for 14 weeks. In the gavage study, ten rats/sex per group were given theophylline in 100% pure Mazola corn oil at doses of 0, 37.5, 75, and 150 mg/kg body weight, 5 days per week. The animals were observed twice daily for clinical signs of toxicity. Body weights were recorded weekly and at the end of the studies. Complete histopathological examination was performed on all 0 ppm and 4000 ppm animals in the feeding study and on the controls and 150 mg/kg rats in the gavage study. Additionally, the mesenteric tissue was examined grossly for evidence of arterial lesions. In the 2-year studies, groups of 50 male and 50 female F344/N rats were given 0 7.5, 25, and 75 mg theophylline/kg body weight in corn oil by gavage, 5 days per week for 2 years. Complete gross necropsy and histopathological examinations were performed on rats from all groups. The mesenteric tissue surrounding the pancreas and mesenteric lymph nodes were examined grossly for evidence of arterial lesions.

Chemicals

Theophylline was obtained from Henley and Company (New York, N.Y.) in one lot, which was used for all studies. The purity of the compound was determined by elemental analyses, Karl Fischer water analysis, nonaqueous titration, thin-layer chromatography, and high-performance liquid chromatography (HPLC). The overall purity was determined to be >99% and with no impurities. Dose formulations were prepared twice during the 16-day studies and weekly during the 14-week studies and every 2 weeks during the 2-year study by mixing theophylline with feed or corn oil to give the required concentrations. Homogeneity and stability studies of the dose formulations were performed using HPLC and ultraviolet/visible spectroscopy. Homogeneity was confirmed for both the feed and gavage formulations.

Stability of the feed formulations was confirmed for at least 21 days at −20 °C when protected from air and light, and for at least 7 days at room temperature when exposed to air and light. Stability of the gavage formulations was confirmed for at least 21 days at 5 °C and at room temperature when stored in sealed vessels protected from light, and for at least 3 h when exposed to air and light. Periodic analyses of the dose formulations of theophylline were conducted using HPLC and ultraviolet/visible spectroscopy (16-day and 14-week studies) or visible spectroscopy (2-year studies) once during the 16-day study and three times during the 14-week study. All dose formulations used during the 16-day, 14-week, and 2-year studies were within 10% of the target concentration.

Care and maintenance of animals

Male and female F344/N rats were obtained from the Frederick Cancer Research Center, Frederick, Md. (16-day studies), Taconic Farms, Germantown, N.Y. (14-week studies), and Simonsen Laboratories, Gilroy, Calif (2-year studies). On receipt the rats were approximately 4 weeks of age. After an 11–12 day quarantine period for health screening, the animals were identified and randomly assigned to control or exposure groups and were approximately 6 weeks old at the start of the study. The animals were housed five males and five females per cage in solid bottom polycarbonate cages (Lab Products, Maywood, Ill.) suspended on stainless steel racks. HEPA-filtered room air underwent a minimum of ten air changes/h. Animal rooms were maintained at 20–25 °C with 35–70% relative humidity and were on a 12-h (6:00 a.m. to 6:00 p.m.) photoperiod cycle. Feed (NIH-07 open formula mash rodent feed for dose feed studies from Zeigler Bros., Gardners, Pa.; pellets for the gavage studies) and water were available ad libitum. All animals were observed twice daily for clinical signs of toxicity, and moribund animals were necropsied. The health status of the animals was monitored by serological analysis of serum samples from sentinel rats placed in the study rooms. Sentinel rats remained negative for *Mycoplasma pulmonis*, *M. arthritidis*, Sendai, pneumonia virus of mice, and rat coronavirus/sialodacryoadenitis virus for the duration of the study.

All studies were conducted at Southern Research Institute (Birmingham, Ala.) in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC, Rockville, Md.). The experimental protocols were approved by the Laboratories' Institutional Animal Use and Care Committee. All animal treatments were conducted in accordance with the

Principles of Laboratory Animal Care (NIH Publication no. 85–23, revised 1985). Animal use and care were in accordance with the United States Public Health Service policy on humane care and use of laboratory animals and the Guide for the Care and Use of Laboratory Animals (National Research Council 1995).

Histopathology

The animals were killed by CO_2 asphyxiation. Tissues were fixed in 10% neutral phosphate-buffered formalin and embedded in paraffin. The paraffin blocks were sectioned at a thickness of 5 μm and stained with hematoxylin and eosin (H&E) for histopathological evaluation. All tissues were evaluated histologically.

Statistical analysis

Arterial lesions were evaluated by logistic regression methods (Dinse and Lagakos 1983; Dinse and Haseman 1986) in the 2-year study and by Cochran-Armitage trend tests and Fisher's exact tests (Armitage 1971) in the 16-day and 14-week studies. Differing statistical methodologies were used because it was unnecessary to adjust for survival differences in the shorter duration studies.

Results

Mortality or clinical signs considered to be related to the vascular lesions were not noted in any of the described studies. In addition, no gross lesions were noted in the abdominal vasculature in any of these studies. Theophylline-related pathology was diagnosed only by histopathology studies.

Sixteen-day studies

In the gavage studies, acute to subacute periarteritis of minimal to moderate severity was observed in medium-sized mesenteric arteries adjacent to the mesenteric lymph nodes of four early death animals (two male and two female rats given 400 mg/kg once daily) and adjacent to the pancreas of one of the two males (Table 1). The cause of death was attributed to theophylline-induced respiratory distress and not to the arterial lesions. Arterial lesions were segmental or circumferential and consisted of medial hemorrhage and fibrinoid necrosis. The adventitia contained a mixed inflammatory cell infiltrate consisting of neutrophils, macrophages, and proliferating fibroblasts (Figs. 1 and 2). Arterial lesions were not evident in the feeding studies.

Fourteen-week studies

Arterial lesions noted in both 14-week studies were more advanced and chronic in nature. In the gavage studies, there was a slight dose-dependent increase in the incidence of periarteritis of the medium-sized arteries adjacent to the mesenteric lymph nodes of male and female rats (Table 1). For male rats, this dose-related increase was statistically significant ($P = 0.021$). The lesions were focal or circumferential and were characterized by

Table 1 Incidences of periarteritis in F344/N rats exposed to theophylline for 16 days, 14 weeks or 2 years

Concentration	16-day gavage[a]			14-week gavage[a]				14-week feed[a]				2-year gavage[a]			
	200 once daily	200 twice dialy	400	0	37.5	75	150	0	1000	2000	4000	0	7.5	25	75
Male	0/5[b]	0/5	2/5	1/10	1/10	2/10	5/10	0/10	0/10	2/10	3/10	2/50	2/50	3/50	15/50**
Significance of trend				$P = 0.021$				$P = 0.016$				$P < 0.01$			
Female	0/5	0/5	2/5	0/10	2/10	2/10	3/10	0/10	1/10	1/10	5/10*	1/50	0/50	2/50	0/50
Significance of trend				$P = 0.068$				$P < 0.01$				$P > 0.50$			

*$P < 0.05$ vs control
**$P < 0.01$ vs control
[a] Dosages in gavage studies are in mg/kg/day; dosages in feed studies are in ppm
[b] Number observed with lesion/number of animals examined

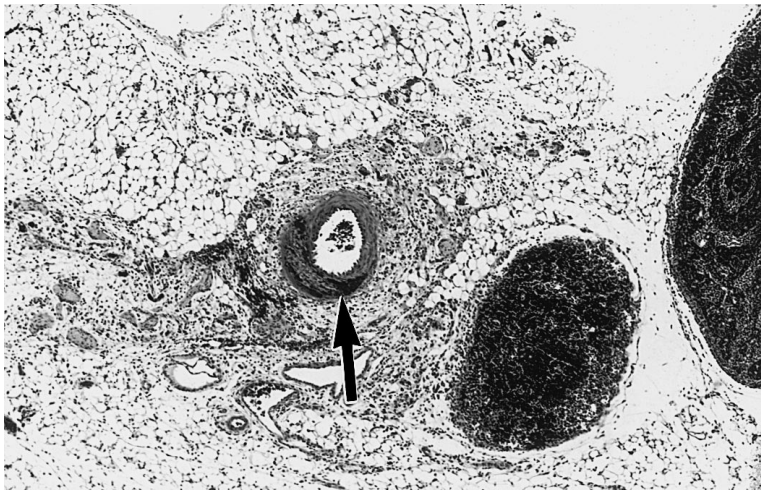

Fig. 1 Hemorrhage and necrosis (*arrow*) within the media of a mesenteric artery of a male F/344N rat given 400 mg theophylline/kg body weight by gavage for 16 days. Hematoxylin and eosin (H&E), ×13.2

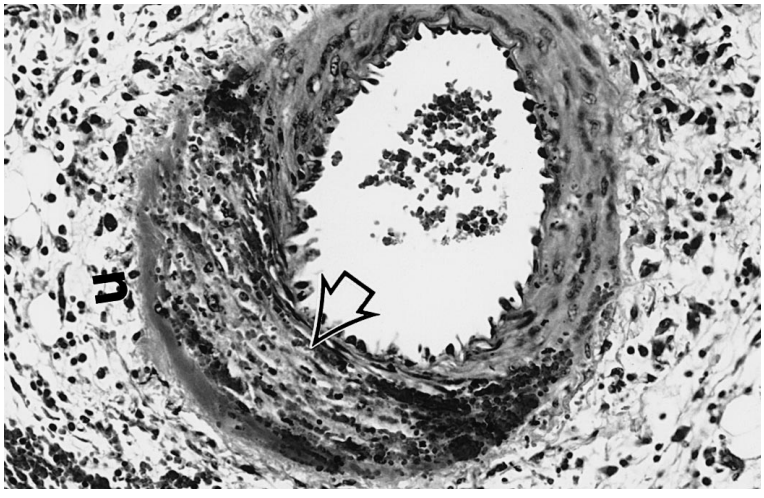

Fig. 2 Higher magnification of fig. 1. Note medial necrosis (*open arrow*) and the mixed inflammatory infiltrates within the media and adventitia. H&E, ×66

infiltrates of mononuclear and polymorphonuclear leukocytes in the media and adventitia. The adventitia was often expanded by connective tissue, which contained small-diameter endothelial-lined spaces. In the feeding studies, a significant dose-related increased incidence of periarteritis of medium-sized mesenteric arteries adjacent to the pancreas and/or mesenteric lymph nodes was observed in males and females (Table 1). Arterial lesions were similar to those observed in the gavage studies.

Two-year studies

There was no evidence of carcinogenic activity related to theophylline administration. The incidence of periarteritis of the mesenteric arteries was significantly increased in male rats given 75 mg/kg (Table 1). The lesions were more advanced and of a more chronic nature than those of the 14-week studies and most frequently involved the medium to large arteries associated with the pancreas and, less often, with the mesenteric lymph nodes (Fig. 3). In general, the lesions consistently involved the adventitia and, in more severe lesions, the media and intima. Perivascular fibrosis with infiltrates of small macrophages mixed with low numbers of lymphocytes and degenerate cellular debris expanded the adventitia. Within the media, smooth muscle cells were disorganized and occasionally contained cytoplasmic vacuoles. Severely affected arteries had variable adventitial and medial thickening, intense mononuclear cell and neutrophilic infiltrates, fibrinoid necrosis with small foci of mineralization, and focal intimal and/or medial hemorrhage. Hemosiderin was noted in the cytoplasm of some macrophages. An increase in the incidence of arterial lesions was not observed in female rats.

Discussion

Theophylline is widely prescribed in the treatment of obstructive airway diseases but has a low therapeutic index (Minton and Henry 1996): there is a narrow range between therapeutic doses and doses giving unacceptable toxicity. The rate of administration is a major factor in human fatalities. Deaths have resulted from as

Fig. 3 Periarteritis in pancreatic arteries of a male F344/N rat given 75 mg theophylline/kg body weight by gavage for 2 years. Note the thickened tortuous arterial walls. H&E, ×5

little as 500 mg theophylline (aminophylline) given as a rapid intravenous bolus, and it is recommended that the drug is injected over a 20- to 40-min period (Gilman et al. 1990). The bolus effect was observed in the present studies. In the 16-day dosed feed studies, all rats that received 8000 ppm theophylline (equivalent to 1000 mg/kg) survived, whereas all rats that received once-daily doses of 400 mg/kg or twice-daily doses of 200 mg/kg by gavage died. In dosed feeding, the animals were never exposed to theophylline at $\geq$200 mg/kg at any given time. The highest dose (75 mg/kg) given to male and female rats in the 2-year studies did not result in significant differences in mortality compared to controls.

In the present studies, periarteritis was noted in rats given theophylline for 16 days by gavage, for 14 weeks in feed or by gavage, or for 2 years by gavage. The incidences generally followed a dose-related trend, and in exposed rats in the 14-week feeding study and dosed males in the 2-year gavage study, were significantly different from those of the respective control groups. Arterial lesions were not observed in B6C3F1 mice tested under similar experimental and exposure conditions (NTP Technical Report 1998).

With the exception of exposure to certain vasoactive compounds including angiotensin, norepinephrine, dopamine agonists, and xanthine compounds, the vascular system is not a common site for chemically induced lesions in the rat (Mitsumori 1990). The incidence of spontaneous polyarteritis (synonyms: polyarteritis nodosa, periarteritis nodosa, chronic arteritis, necrotizing arteritis, necrotizing vasculitis) varies among different rat strains but is relatively uncommon in the F344 rat (Mitsumori 1990; Carlton and Engelhardt 1991). Incidences of 1.8% (male) and 0.9% (female) have been reported in F344 rats. The pancreatic, mesenteric, and spermatic arteries are most commonly affected (Mitsumori 1990). The cause of spontaneous polyarteritis in rats is unknown, but morphologically, the lesions resemble the immune-mediated arteritis observed in other species. That lesion is associated with deposition of antigen-antibody complexes in the arterial walls resulting in degeneration and necrosis of the vessel wall (Mitsumori 1990).

The morphology and distribution of the arterial lesions noted in the present studies are very similar to that occuring spontaneously in F344 rats (Mitsumori 1990; Carlton and Engelhardt 1991). Lesions similar to spontaneous polyarteritis were noted in the mesenteric vessels of Sprague-Dawley rats given caffeine in feed for up to 117 weeks (Johansson 1981); in the small- and medium-sized arteries of the pancreas, lymph node, kidney, and stomach of rats given LY-195115 (an experimental inotropic agent) in feed for 3 months (Sandusky and Means 1987); in the large mesenteric arteries of rats given fenoldopam mesylate (a post-synaptic DA_1 dopaminergic vasodilator) intravenously for 24 h (Kerns et al 1989); in the small and large arteries of the mesentery, cerebrum, heart, and kidney of rats given dopamine intravenously for 24 h (Kerns et al. 1989); in rats given a variety of structurally unrelated vasodilators (Mitsumori 1990; Carlton and Engelhardt 1991; Kerns et al. 1991); and in male Wistar rats given one of four phosphodiesterase III (PDE III) inhibitors as a single subcutaneous dose (Joseph et al. 1996). The lesions induced by PDE III inhibitors given to rats were similar to lesions induced by PDE III inhibitors given to dogs, except for the site of predilection (Boor et al. 1995). In rats, the lesions occurred in the mesentery rather than coronary vasculature.

It has been proposed that the arteriopathy induced by structurally unrelated vasodilators is the result of disturbances in critical wall tension due to relaxation of the medial smooth muscle (Carlton and Engelhardt 1991). The supposition is that vascular smooth muscle cell necrosis results from prolonged reduction in the blood pressure coupled with alteration in intramural tension, which may interfere with the diffusion of essential nutrients into the wall of affected arteries, increasing susceptibility to injury (Bugelski et al. 1989). However in other reports, infusion of rats with hydralazine or sodium nitroprusside resulted in a profound reduction in blood pressure, without the development of arterial le-

sions (Morgan et al. 1983; Bugelski et al. 1989). It has also been suggested that arterial necrosis develops due to alternating vasodilation and vasoconstriction of the arterial smooth muscles (Yuhas et al. 1985; Bugelski et al. 1989).

Male Wistar rats given four structurally dissimilar PDE III inhibitors subcutaneously, developed arterial lesions similar to those induced by fenoldopam mesylate (Joseph et al. 1996). The lesions were characterized by medial necrosis and hemorrhage, occurred with a dose-related intensity, and correlated well with the duration and degree of hypotension induced by each of the PDE III inhibitors. The authors suggested that prolonged and excessive vasodilation increases arterial compliance (blood flow) and intramural tension (shear stress) beyond critical levels, which led to increased endothelial permeability and subsequent progressive medial necrosis and hemorrhage. The molecular mechanisms for the induction of periarteritis by vasoactive agents may be related to elevated cyclic AMP (cAMP) levels due to the stimulation of dopaminergic (DA_1) or adrenergic receptors, or inhibition of PDE III (Bugelski et al. 1989; Joseph et al. 1996; Kerns et al. 1989; Yuhas et al. 1985).

In humans, drug-related vasculitis has been described clinically as hypersensitivity or allergic angitis (Mullick et al. 1979). Clinically, patients develop either localized or systemic vasculitis while medication is being taken. No single drug appeared to be associated with the development of vasculitis, and oftentimes, vasculitis developed when drug combinations were being taken. Ampicillin and penicillin, taken in combination with other drugs were implicated in 20% of the cases investigated. Clinical symptoms included skin rash, malaise, eosinophilia or unexplained fever in variable combinations. The skin, subcutaneous tissue, heart, liver, and kidney were the most commonly involved organs.

Theophylline is a non-specific phosphodiesterase inhibitor which may cause excessive vasodilatation. The mesenteric and pancreatic arteries in rats are particularly sensitive to the excessive vasodilator-pharmacological activity of theophylline, as well as caffeine (Johansson 1981) and other vasodilator drugs (Kerns et al. 1991; Boor et al. 1995). In the present studies, the incidence of the lesions was dose related, which further supports a pharmacological/toxic mode of action rather than hypersensitivity. The particular predisposition of effects in the medium-sized arteries the splanchnic vasculature (mesenteric and pancreatic vascular beds) in the rat may be related to the anatomic location of these vessels with lack of supporting tissue, and possibly, to the localization of particular receptors to the drug in the vascular wall (Greaves 1990; Joseph et al. 1996; Kerns 1996; Kerns et al. 1989; Nordborg et al. 1985). Changes in arterial compliance and/or intramural tension due to vasodilation may cause progressive damage to all layers of the arterial wall.

In the 2-year studies, theophylline was tested at doses which were approximately 3 to 12 times greater than normal human exposure (Kodama et al. 1980). In the assessment of the arteritis induced by the infusion of rats with UK-61,260, a cAMP PDE III inhibitor, it was concluded that the arterial lesions were linked to exaggerated pharmacological effects, and therefore not considered a potential risk to human patients (Hanton et al. 1995). Theophylline-related arterial lesions were noted only in male rats given the highest dose (75 mg/kg), which is approximately 12 times greater than the human therapeutic dose. Therefore, it is not considered of a potential risk to humans (Hanton et al. 1995).

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