Br. J. Pharmac. (1972), **45**, 385–390.

Sotalol for the protection of turkeys from the development of β-aminopropionitrile-induced aortic ruptures

CHARLES F. SIMPSON

Department of Veterinary Science, University of Florida, Gainesville, Florida 32601

Summary

1. The influence of feeding 2 levels of sotalol on the incidence of β-aminopropionitrile (BAPN)-induced aortic ruptures of immature turkeys was determined.

2. Four of 22 turkeys fed 0·12% sotalol and 0·07% BAPN died of aortic ruptures, but 6 of 21 turkeys fed only BAPN died of the syndrome.

3. Blood pressure, heart rate, aortic tensile strength, and aortic structure as seen by light- and electron-microscope were similar in turkeys fed BAPN alone or both BAPN and 0·12% sotalol concurrently.

4. In a second experiment, 13 of 24 turkeys fed 0·7% BAPN alone died of aortic ruptures, but only 2 of 24 turkeys fed BAPN and 0·2% sotalol concurrently died of the disease.

5. Aortic tensile strength was lower, heart rate was faster, alterations of aortic elastic fibres as seen by light- and electron-microscope were more severe, and aortic salt soluble collagen with a higher amino acid content was increased in turkeys fed only BAPN, as compared to turkeys fed both BAPN and 0·2% sotalol.

Introduction

Aortic ruptures, resulting from the development of dissecting aneurysms, occur in 40–50% of turkeys fed β-aminopropionitrile (BAPN). Reserpine prevents BAPN-induced aortic ruptures of turkeys (Simpson, Kling, Robbins & Harms, 1968a), and propranolol both prevents (Simpson, Kling & Palmer, 1968b) and is effective in the treatment (Simpson, Kling & Palmer, 1970) of aortic rhexis induced by BAPN. Fragmentation and swollen elastic fibres in all tunicae of the abdominal aorta of the turkey fed BAPN are the lesions of the disease observed by light- and electron-microscopy (Simpson, Pritchard, Harms & Sautter, 1962). Since similar ultrastructural alterations of elastic fibres have been described in the aorta of man with a spontaneous aneurysm (Simpson & Harms, 1969), and since aortic aneurysms in the human and BAPN-fed turkey respond favourably to the same therapy, reserpine (Wheat, Palmer, Bartley & Seelman, 1965) and propranolol (Palmer & Wheat, 1967), it has been suggested that the BAPN-fed turkey could be utilized as an experimental animal for the evaluation of drugs potentially valuable for the treatment of aortic dissecting aneurysms in man.

In the present study, sotalol [MJ 1999; 4-(2-isopropylamino-1-hydroxyethyl) methane sulphonanilide-HCl] (Mead Johnson, Evansville, Indiana, U.S.A.), a β-adrenoceptor blocking agent, and a toxic level of BAPN were fed concurrently to turkeys to determine if this drug would protect turkeys from the development of BAPN-induced aortic ruptures.

Methods

Broad-breasted white male poults were raised by conventional methods and fed a 22% protein ration until 4 weeks of age. At this time, the immature turkeys were randomized into groups and their diet was changed to one containing 16% protein with various supplements.

In the first experiment four variations of the 16% protein diet were fed: (1) no supplementation; (2) 0·07% BAPN fumarate; (3) 0·07% BAPN plus 0·12% sotalol; (4) 0·12% sotalol. Each diet was fed to 6 groups of poults, each group contained 4 turkeys.

In the second experiment four variations of the 16% protein diet were fed to 6 pens of 4 turkey poults: (1) no supplementation; (2) 0·07% BAPN fumarate; (3) BAPN plus 0·2% sotalol; (4) 0·2% sotalol.

The experiments were terminated when the turkeys were 9 weeks of age.

The protocol was identical in each experiment except that shortly before completion of the trials, indirect systolic blood pressure was determined by use of an inflatable cuff applied to the leg (Simpson & Harms, 1965) in the first experiment, and direct blood pressure measurement was recorded from the carotid artery after cannulation under local anaesthesia in the second experiment. Blood pressure measurements were recorded from 6 turkeys per treatment regimen in each experiment. During the course of recording blood pressure readings, control and sotalol-fed turkeys in both experiments were injected intravenously with isoproterenol (5 $\mu g/kg$ body weight) to ascertain the efficacy of β-adrenoceptor blockade provided by the 2 levels of sotalol.

Five abdominal aortae from each treatment group in the second experiment were homogenized in 12 volumes of cold buffered 1 M NaCl (O'Dell, Bird, Ruggles & Savage, 1966), and the soluble portion (collagen) was precipitated by the addition of NaCl to 20% (w/v) (Bornstein & Piez, 1966). The resultant precipitate was dried and weighed. Ten mg of this material was hydrolyzed in 6 N glass-distilled HCl at 109° C for 72 h for determination of amino acid content with a Technicon analyser (Piez & Morris, 1960).

Hearts and posterior aortae from at least 6 turkeys per dietary regimen were examined histologically. Sections were stained with hematoxylin and eosin stain and orcein–Van Gieson stain for demonstration of elastic fibres. Sections from the same part of the posterior aorta of 4 birds on each regimen were also examined by electron microscopy. The sections were fixed in 3·5% glutaraldehyde, post-fixed in 1% OsO_4 and embedded in Araldite. Thin sections on grids were stained with uranyl acetate and lead citrate (Reynolds, 1963) prior to examination with a Philips EM200 electron microscope. The tensile strength of a 2·9 mm length of aorta located immediately cephalad to the sciatic arteries (location of aortic rhexis in BAPN toxicity) was determined by the use of a motor driven device which exerted a constant tension on the ring, and also recorded the stress applied (Simpson *et al.*, 1968b). The mean thickness of the aortic wall was calculated by measuring the width at four 90° intervals around the circumference of the artery, and tensile strengths were calculated in g/mm^2.

Results

In the first experiment no turkeys that were fed the unsupplemented diet or the 0·12% sotalol diet died of aortic ruptures. However, 6 of 21 poults fed 0·07% BAPN died of aortic rhexis, and 4 of 22 poults fed BAPN and sotalol died of the disease (Table 1). Turkeys that died of causes other than aortic rupture were not included in per cent mortality from aortic rhexis.

Blood pressures of turkeys in all treatment groups in the first experiment were similar, but heart rate was lowest in birds fed only sotalol (Table 1). Injection of control turkeys with isoproterenol resulted in a reduction of blood pressure of 51 mmHg, and injection of isoproterenol to poults fed only BAPN caused a lowering of blood pressure of about the same magnitude. There was partial β-adrenoceptor blockade in turkeys fed sotalol or sotalol and BAPN, as indicated by an average depression of systolic blood pressure of 6 and 16 mmHg respectively, following injections of isoproterenol. Aortic tensile strengths were similar in turkeys fed control or sotalol-supplemented diets, and in turkeys fed a BAPN diet or a BAPN-sotalol-supplemented diet. However, these values were more than twice as high in the groups fed control or sotalol supplemented diets than in the 2 dietary regimens containing BAPN.

The histology of the abdominal aortae of turkeys fed control or sotalol-supplemented diets was characterized by areas of intimal thickening of various sizes and shapes. Elastic fibres in such plaques were continuous and stained intensely with orcein–Van Gieson stain. Elastic fibres in the aortic tunicae media of these poults were also continuous.

The histology of the aortae of birds fed BAPN or BAPN and 0·12% sotalol was similar. Elastic fibres were fragmented in the area of intimal hyperplasia, and large segments of the internal elastic membrane were missing. As a result, the aortic wall was irregularly thickened. In addition, elastic fibres in the tunica media were swollen, fragmented or eliminated.

The aortae of turkeys fed the control diet or the 0·12% sotalol diet were similar ultrastructurally. Adjacent smooth muscle cells in the tunica media were strongly bound by elastic fibres and bundles of collagenous fibres, and the extracellular spaces were almost completely filled with these connective tissue elements. Elastic fibres had smooth surfaces.

It was observed by electron microscopy that adjacent smooth muscle cells were not closely bound, and that elastic fibres in the tunicae media of the aortae of turkeys fed 0·07% BAPN or BAPN and 0·12% sotalol were swollen and had knobby blebs on their surfaces. Such alterations of elastic fibres usually caused

TABLE 1. *Incidence of aortic ruptures, aortic tensile strength, blood pressure, heart rate, and efficacy of β-adrenoceptor blockade among turkeys fed BAPN and* 0·12 *per cent sotalol* (*experiment* 1)

Treatment	(No. died/ total)	Aortic tensile§ strength (g/mm²)	Heart rate§ (beats/min)	Systolic blood pressure§ Before isoproterenol	After isoproterenol
None	(0/23*)	149·9^{a}	300^{a}	175^{a}	124^{a}
0·07% BAPN	(6/21*)	61·5^{b}	292^{a}	185^{a}	127^{a}
0·12% Sotalol	(0/24)	142·2^{a}	282^{a}	168^{a}	162†^{b}
BAPN and sotalol	(4/22*)	65·2^{b}	296^{a}	176^{a}	160‡^{b}

* Turkeys that died of causes other than aortic rupture not included in total. † β-Adrenoceptor blockade: 5/6 turkeys. ‡ β-Adrenoceptor blockade: 4/6 turkeys. § Six turkeys per treatment group. Numerals with different superscripts are significantly different ($P<0.05$) according to Duncan's Multiple Range Test.

dilatation of the extracellular spaces and compression atrophy of the surrounding smooth muscle cells.

In the second experiment turkeys fed an unsupplemented diet or a diet supplemented with 0·2% sotalol did not die of aortic ruptures. However, 13 of 24 poults fed 0·07% BAPN, and 2 of 24 turkeys fed both BAPN and 0·2% sotalol died of aortic ruptures (Table 2).

Blood pressures were not remarkably different among turkeys fed the 4 different dietary regimens; however, heart rates were significantly lower among poults fed 0·2% sotalol alone or BAPN and sotalol, when compared to heart rates of turkeys fed an unsupplemented diet or only 0·07% BAPN (Table 2). There was β-adrenoceptor blockade, as judged from lack of response to isoproterenol, among 5 of 6 turkeys fed sotalol alone. Aortic tensile strength was greatest in turkeys fed control or sotalol-supplemented diets, intermediate in poults fed BAPN and sotalol and lowest in turkeys fed only BAPN.

The least amount of soluble collagen precipitated by 20% NaCl (mg/100 mg wet weight of aorta) was in the aortae of turkeys fed a control diet or a diet containing 0·2% sotalol, and the greatest amount of precipitated soluble collagen was in the aortae of turkeys fed BAPN alone (Table 3).

It was found, from the analysis of the precipitated, dried and hydrolyzed soluble collagen, that amino acids were highest in the aortae of turkeys fed BAPN, lowest in the aortae of poults fed a control diet and intermediate in the aortae of turkeys fed both BAPN and sotalol (Table 3).

The histology of the aortae of poults fed the control diet and the 0·2% sotalol diet was similar, and resembled the histology described for the aortae of turkeys fed similar diets in the first experiment. The aortae of birds fed BAPN alone also had a histological appearance similar to those fed BAPN in the first experiment. The aortic intima of turkeys fed BAPN and 0·2% sotalol contained mildly swollen and fragmented elastic fibres. There were only small breaks of the internal

TABLE 2. *Incidence of aortic ruptures, aortic tensile strength, heart rate, blood pressure, and efficacy of β-adrenoceptor blockade among turkeys fed BAPN and* 0·2 *per cent sotalol* (*experiment* 2)

Treatment	(No. died/ total)	Aortic tensile strength (g/mm^2)	Heart rate (beats/min)	Blood pressure Before isoproterenol	Blood pressure After isoproterenol
None	(0/24)	119·0^a	316^a	178^a/137^a	132^a/78^a
0·07% BAPN	(13/24)	53·7^c	292^a	189^a/145^a	—
0·2% Sotalol	(0/24)	121·0^a	246^b	169^a/127^a	150^{*b}/112^{*b}
BAPN and sotalol	(2/24)	74·4^b	251^b	172^a/131^a	—

* β-adrenoceptor blockade: 5/6 turkeys. Numerals with different superscripts are significantly different ($P<0·05$) according to Duncan's Multiple Range Test.

TABLE 3. *Relationship of diet to quantity and hydroxyproline content of aortic soluble collagen precipitated by* 20% *NaCl* (*experiment* 2)

Diet	Soluble collagen precipitated[a]	Hydroxyproline[b]
Control	2·20	0·56
0·2% Sotalol	1·56	—
0·07% BAPN	6·75	1·90
BAPN and sotalol	4·17	1·02

[a] mg/100 mg wet weight aorta. [b] mg/g precipitated collagen. Average of 2 aliquots.

elastic membrane; elastic fibres in the tunica media were slightly swollen and a few of the fibres were fragmented.

The ultrastructure of the aortae of turkeys fed only BAPN or 0·2% sotalol alone in experiment 2 was similar to that described for turkeys fed BAPN alone or only 0·12% sotalol in the first experiment. However, it was observed that elastic fibres were not as severely altered in the aortae of poults fed BAPN and 0·2% sotalol, as in the aortae of poults fed only BAPN.

Discussion

Propranolol at a level of 0·04% is effective in the treatment of BAPN-induced ruptures of turkeys and also prevents the development of the disease in turkeys when fed at a level of 0·03% (Simpson, Kling & Palmer, 1970). This β-adrenoceptor blocking agent is a potent local anaesthetic.

Sotalol, at the highest level used in this study (0·2%), lowered the incidence of BAPN-induced aortic ruptures of turkeys. Sotalol is a β-adrenoceptor blocking agent but it does not have local anaesthetic and intrinsic sympathomimetic actions or a quinidine-like effect.

Propranolol administered orally to animals is 5 to 10 times more active than sotalol in blocking the cardiac actions of isoproterenol, but in man it has been noted that propranolol has only twice as much activity as sotalol (Kofi Ekue, Lowe & Shanks, 1970). In the present trial in turkeys, the highest level of sotalol fed (0·2%) was 5 times as great as the level of propranolol (0·04%) found to be an effective treatment for BAPN-induced aortic ruptures (Simpson *et al.*, 1970).

The mechanisms of protection of turkeys from BAPN-induced aortic ruptures by sotalol are not readily apparent. In the second experiment, in which mortality from BAPN-induced aortic ruptures was reduced from 40·6 to 6·25% by the feeding of 0·2% sotalol, there was bradycardia and increased aortic tensile strength as a result of sotalol treatment, but no significant depression of blood pressure. Since sotalol, unlike propranolol, is reported not to have a quinidine-like effect, it is possible that reduction of heart rate by sotalol was an important factor in the protection of turkeys from BAPN-induced aortic ruptures by this agent. Even though sotalol is not a local anaesthetic, it is possible that the drug does have a direct or indirect influence on the aortic wall. This possibility exists because ultrastructural changes of aortic elastic fibres were less severe in turkeys fed BAPN and 0·2% sotalol, as compared to elastic fibres of turkeys fed only BAPN, and, in addition, aortic tensile strength was greatest in turkeys receiving the former treatment regimen. Moreover, even though there was more salt soluble collagen in aortae of turkeys fed BAPN and sotalol than in aortae of turkeys fed a control diet, less soluble collagen was present in the aortae of poults fed both BAPN and sotalol than in the aortae of poults fed only BAPN and the concentration of all amino acids was highest in precipitated collagen from aortae of poults fed only BAPN. It has been shown previously that the lathyritic aorta contains more soluble collagen that the control aorta (Savage, Bird, Reynolds & O'Dell, 1966; Tanzer & Gross, 1964).

This work was supported in part by grants (H-6580 and 71AG8) from the National Heart Institute, United States Public Health Service, and the Florida Heart Association. (Florida Agricultural Experiment Stations Journal Series No. 4016.) The technical assistance of J. W. Carlisle and E. Robbins is acknowledged.

REFERENCES

BORNSTEIN, P. & PIEZ, K. A. (1966). The nature of the intramolecular cross-links in collagen. The separation and characterization of peptides from the cross-link region of rat skin collagen. *Biochem.*, **5**, 3460–3473.

KOFI EKUE, J. M., LOWE, D. C. & SHANKS, R. G. (1970). Comparison of the effects of propranolol and MJ1999 on cardiac β-adrenoceptors in man. *Br. J. Pharmac.*, **38**, 546–553.

O'DELL, B. L., BIRD, D. W., RUGGLES, D. L. & SAVAGE, J. E. (1966). Composition of aortic tissue from copper-deficient chicks. *J. Nutr.*, **88**, 9–14.

PALMER, R. F. & WHEAT, M. W. (1967). Treatment of dissecting aneurysms of the aorta. *Ann. Thoracic. Surg.*, **4**, 38–52.

PIEZ, K. A. & MORRIS, L. (1960). A modified procedure for the automatic analysis of amino acids. *Anal. Biochem.*, **1**, 187–201.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.*, **17**, 208–212.

SAVAGE, J. E., BIRD, D. W., REYNOLDS, G. & O'DELL, B. L. (1966). Comparison of copper deficiency and lathyrism in turkey poults. *J. Nutr.*, **88**, 15–25.

SIMPSON, C. F., PRITCHARD, W. R., HARMS, R. H. & SAUTTER, J. H. (1962). Electron microscopy of the cardiovascular system of the normal and beta-aminopropionitrile fed turkey. *Exp. Mol. Path.*, **1**, 321–343.

SIMPSON, C. F. & HARMS, R. H. (1965). Relationship of age and sex of turkeys to aortic ruptures induced by diethylstilbestrol. *Proc. Soc. exp. Biol. Med.*, **119**, 509–512.

SIMPSON, C. F., KLING, J. M., ROBBINS, R. C. & HARMS, R. H. (1968a). Beta-aminopropionitrile-induced aortic ruptures in turkeys: inhibition by reserpine and enhancement by monoamine oxidase inhibitors. *Toxic. Appl. Pharmacol.*, **12**, 48–59.

SIMPSON, C. F., KLING, J. M. & PALMER, R. F. (1968b). The use of propranolol for the protection of turkeys from the development of β-aminopropionitrile-induced aortic ruptures. *Angiology*, **19**, 414–418.

SIMPSON, C. F. & HARMS, R. H. (1969). Dissecting aneurysms in animals. *Adv. Vet. Sci. Compar. Med.*, **13**, 1–28.

SIMPSON, C. F., KLING, J. M. & PALMER, R. F. (1970). β-aminopropionitrile-induced dissecting aneurysms of turkeys: treatment with propranolol. *Toxic. Appl. Pharmac.*, **16**, 143–153.

TANZER, M. L. & GROSS, J. (1964). Collagen metabolism in the normal and lathyritic chick. *J. exp. Med.*, **119**, 275–289.

WHEAT, M. W., PALMER, R. F., BARTLEY, T. D. & SEELMAN, R. C. (1965). Treatment of dissecting aneurysms of the aorta without surgery. *Thoracic. Cardiovasc. Surg.*, **50**, 364–373.

(*Received December* 12, 1971)