Toxicity and Tissue Distribution of Aminophylline in Neonatal and Adult Mice and Rats

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Summary

The LD₅₀ of aminophylline in adult mice differed from young mice and rats of both ages, in which the values were remarkably similar (Table 1). With the exception of fat, which had lower concentrations in all groups, tissue concentrations after a 4 mg/kg rectal dose of aminophylline in the 10-day-old animals ranged from 10–20 µg/g as compared to 4–10 µg/g in the adults (Table 2). To evaluate preferential distribution, an analysis of tissue to blood concentration ratios was made. All ratios approximated 1.0 except fat which had a ratio of 0.1–0.6. The neonates of each species had significantly higher tissue to blood ratios for brain, heart, small intestine, skeletal muscle, and fat ($P < 0.05$). Brain to plasma and brain to blood ratios were very similar ranging from 0.4–1.3 in adults versus 0.8–1.7 in neonates. Considera-

tion of the time course indicated a trend toward unit, with the overall ratio in both groups being 1.15 at 2 hr. However, at all earlier time periods the ratios were clearly higher in neonates. Administration of aminophylline intraperitoneally to produce serum concentrations of theophylline far in excess of the 10–20 µg/ml considered to be safe and effective in clinical use did not decrease bilirubin levels in young or old Gunn rats (Fig. 1).

Speculation

The similarities of LD₅₀ and tissue distribution between neonatal and adult rodents suggests that studies of metabolism may prove to be most important in explaining differences in serum elimination times noted in prematures treated with aminophylline.

INTRODUCTION

The treatment of apnea of prematurity with aminophylline (2,14,27,32) introduces questions regarding the pharmacology of the drug in immature patients. Previous measurements of elimination (serum half-lives for the beta-phase) have established differences among individuals and between age groups. While mean half-lives for adults and children average 4.5 hours (22) and 3.5 hours (18), respectively, recent measurements in premature infants average ca. 24 hours (1,7,23).

Alterations in distribution secondary to immaturity may also contribute to age-related differences in toxicity. Such a change may be extremely important for a drug such as theophylline, which is known to exhibit significant central nervous system (CNS) toxicity in clinical use. For example, irritability and seizures have been reported in children and adults associated with high doses and elevated serum levels (25,29,35).

Despite widespread use of aminophylline for over forty years (19), developmental studies in animals are lacking. Acute toxicity has been determined in mice and rabbits (8,13,20,31), tissue distribution studied in rabbits (26) and blood-CSF ratios reported for the dog (30). None of these studies involved immature animals.

The purpose of this investigation was to study the acute toxicity and tissue distribution of aminophylline, emphasizing the blood:brain ratio in young and older rodents. Since the rectal route of administration has been employed clinically (2,14), it was included in the acute toxicity experiments and used for all groups in the distribution experiments. For adequate retention of the rectal solution, the young animals had to be at least ten days old. Mice and rats were used since brain myelination is delayed in these animals and resembles the preterm human (10,33). As hyperbilirubinemia is common in premature infants, an *in vivo* assessment of the ability of theophylline to displace bilirubin from serum binding sites was studied in young and old jaundiced Gunn rats (9).

MATERIALS

Aminophylline Injection, U.S.P. (25 mg/ml) was a gift from Abbott Laboratories, North Chicago, Ill. ^3H -theophylline (18.4 Ci/mmol) was purchased from Amersham/Searle Corp., Arlington Heights, Ill. Soluene-350, PPO (2,5-diphenyloxazole), Dimethyl POPOP (1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene), bis MSB (p-bis-(0-methylstyryl)-benzene), and Triton X-100 were purchased from Packard Instrument Co., Downer's Grove, Ill. Toluene, isopropyl alcohol, and hydrogen peroxide (30%) were reagent grade and were purchased from Fisher Scientific. The Gunn rats were from the colony maintained at the University of Vermont. Random bred Swiss mice and Sprague-Dawley rats were purchased from Canadian Breeders Labs., St. Constant, Quebec.

METHODS

Acute Toxicity. Groups of 6 - 40 ten-day-old, and adult mice (30 - 40 days old), and ten-day-old and adult rats (250 - 350 g), were given four to six graded doses of aminophylline ranging from 150 to 300 mg/kg i.p. (6-12 ul/g). Six groups of ten-day-old mice were similarly dosed via the rectal route. Expulsion of the large volume of the solution required to deliver lethal doses precluded a similar study in adults. The number of dead animals was counted at two hours.

Tissue Distribution. Each of the four species-age groups was given 4 mg/kg per rectum aminophylline solution (4 mg/ml with ^3H -theophylline 14 uCi/ml, 1 ul/g) via a Hamilton syringe, and decapitated at 15, 30, 60, and 120 minutes. Tissues were dissected, a sample of blood, brain, lung, heart, liver, kidney, colon, small intestine, skeletal muscle, and subcutaneous fat was weighed, placed in glass counting vial with 1 ml/100 mg tissue wt of Soluene-350/isopropanol (1:1) and solubilized at 40 - 50°C overnight. Bleaching of colored samples, such as heart, liver and kidney, was accomplished by the addition of 0.2 ml of 30% hydrogen peroxide/ml of Soluene-isopropanol solution and incubation for 12 - 24 hours at 40 - 50°C. Ten ml of a "scintillation cocktail" (5 g PPO, 0.5 g bis-MSB/L of toluene) were added and the samples stored in the dark at 25°C for more than 48 hours to minimize chemiluminescence. Radioactivity was determined in a Packard Tri-Carb Liquid Scintillation Counter (Model 2018) and samples recounted after addition of an internal standard (^3H -theophylline). The efficiency of counting was used to calculate the activity of the sample in dpm/ml, and this was converted to ug theophylline/mg tissue wet weight by determination of the specific activity of the solution administered. While this method did not differentiate theophylline from such possible metabolites as 1-methylxanthine and 3-methylxanthine which would retain the ^3H in the C-8 position, Lohmann et al. (17) found no metabolism of theophylline in the brain, heart, lung, kidney, spleen, intestine, or adrenals of the rat. Moreover, the major liver metabolites were the oxidized products, 1-methyluric acid and 1,3-dimethyluric acid, which would not be included in our determination of radioactivity. In addition, minor metabolites which would retain the radio-label, such as 3-methylxanthine, would not be expected to accumulate appreciably in the short time of the experiment (two hours).

Blood-Brain Distribution. Ten-day-old and adult mice were given a sublethal dose of aminophylline (50 mg/kg) per rectum (25 mg/ml with ^3H -theophylline 140 uCi/ml, 2 ul/g), and groups of ten were decapitated at 15, 30, 60, and 120 minutes, the brains dissected, weighed, solubilized, and radioactivity determined. Blood was collected in heparinized test tubes. Radioactivity in aliquots of plasma was determined in a "scintillation cocktail" (16.5 g PPO, 0.3 g Dimethyl POPOP, 1 L Triton X-100, and 2 L toluene). Efficiency of counting was determined by internal standardization and the concentration of theophylline was calculated as described above.

Bilirubin Displacement. Aminophylline was administered i.p. to one-month-old (50 mg/kg) and adult (100 mg/kg) homozygous Gunn rats. These animals suffer a genetically determined nonhemolytic unconjugated hyperbilirubinemia (11). Blood samples were taken from the tail vein at 0, 30, 60, 120, 180 minutes, and assayed for theophylline by radioimmunoassay (23) and for bilirubin by the method of White, et al. (34). As a positive control sulfisoxazole (100 mg/kg i.p.), was administered to two adult Gunn rats.

Statistical Procedures. The acute toxicity data were analyzed by the method of Miller and Tainter (21). The percentage of deaths was converted to probits and plotted versus the logarithm of the dose. The LD_{50} and its standard error were determined graphically. The slopes and intercepts for the linear regression lines were compared using the technique of Snedecor and Cochran (28), except that differences were analyzed for all five lines instead of their two line model.

The tissue concentrations and tissue:blood ratios were analyzed by Analysis of Variance using a factorial design. For each tissue the contributions to the variance from species, age, and time differences as well as the appropriate interactions were calculated.

RESULTS

Acute Toxicity. Deaths of the ten-day-old animals of both species occurred within one hour of dosing. Untreated animals of this age were able to withstand at least four hours separation from their mothers without apparent ill effects. Deaths in the adult mice occurred within two hours of dosing, with no additional deaths occurring during a five day post-treatment observation period. In contrast, occasional additional deaths of adult rats occurred 12 - 24 hours post-treatment. For this reason calculation of the LD_{50} was based on deaths within the first two hours post-treatment.

Cause of death appeared to be central in origin, manifested by protrusion and biting of the tongue, tonic-clonic seizures, opisthotonus, rigidity, and cessation of respiration. Late deaths of adult rats appeared to have another basis, since they were flaccid and had no evidence of tongue-biting.

The LD_{50} of aminophylline in adult mice differed from young mice and rats of both ages, in which the values were remarkably similar (Table I). Analysis of the regression line (probits vs. log-dose) of adult mice revealed that both its slope and intercept differed significantly ($P < .05$) from those of the other four groups combined. Notably, the LD_{50} in ten-day old mice was virtually the same when aminophylline was given rectally as when given i.p. (Table I).

Tissue Distribution. Following administration of equivalent doses, based on body weight, concentrations of theophylline in each tissue were significantly greater in the 10-day-olds of both species than in the adults ($P < 0.001$). With the exception of fat, which had lower concentrations in all groups, tissue concentrations after a 4 mg/kg rectal dose of aminophylline in the 10-day-old animals ranged from 10 to 20 ug/g as compared to four to 10 ug/g in the adults (Table II). While no statistically significant differences were found between time periods in any of the tissues, levels in the colon tended to be higher at 15 and 30 minutes than they were at one and two hours following rectal administration.

Species differences were found only in the heart and kidney. In both tissues, adult rats had significantly higher levels than did adult mice ($P < 0.05$).

To evaluate preferential distribution, an analysis of tissue:blood concentration ratios was made. All ratios approximated one, except fat which had a ratio of 0.1 - 0.6. The neonates of each species had significantly higher tissue:blood ratios for brain, heart, small intestine, skeletal muscle, and fat ($P < 0.05$). The only tissue exhibiting a time dependency was fat where the ratios in at the first two time periods were significantly greater than in the last two in all groups except adult rats ($P < 0.01$).

Species differences were found in three tissues. In adults brain:blood ratios were higher in mice, whereas the reverse was found for the kidney: blood ratios ($P < 0.05$). In the 10-day-olds, ratios for small intestine: blood were higher in mice ($P < 0.05$).

Blood-Brain Distribution. To further delineate the relative permeability of the blood-brain barrier of the neonate vs that of the adult, the dose administered per rectum was increased from 4 to 50 mg/kg. This 12.5-fold increase in dose resulted in plasma concentrations that were, in adults, only three- to four-fold greater than found in the previous experiment. In neonates only a two- to three-fold increase was found one and two-hours post-treatment (Table III). Similarly, brain concentrations were elevated two- to eight-fold in adults, but only two-fold in neonates. Thus brain:plasma and brain:blood ratios were very similar ranging from 0.4 - 1.3 in adults vs. 0.8 - 1.7 in neonates. Consideration of the time course indicated a trend toward unity, with the overall ratio in both groups being 1.15 at two hours. However, at all earlier time periods the ratios were clearly higher in neonates.

Displacement of Bilirubin. Administration of aminophylline i.p. to produce serum concentrations of theophylline far in excess of the 10 - 20 mg/ml considered to be safe and effective in clinical use did not decrease serum bilirubin levels in young or old Gunn rats (Figure 1). As expected, sulfisoxazole in equivalent dosage (100 mg/kg, i.p.) produced decreases in serum bilirubin to 70% of control values at 30 and 60 minutes post-administration.

DISCUSSION

The acute toxicity of aminophylline, expressed as the LD_{50} , from the present study in ten-day-old mice and in both ten-day-old and adult rats correlated well with those reported for adult rabbits (31), rats (4), and cats (8). The data, viewed collectively, suggest an LD_{50} of ca. 175 mg/kg (i.p.). In contrast, a higher LD_{50} (ca. 270 mg/kg) was found in adult mice in our study and in previous reports (6,12,13,19). Thus the data indicate that one must not assume that an LD_{50} will be lower for neonates than for adults, or merely make extrapolations from one species to another to estimate relative doses, since inconsistencies can be found even between species as closely related as mice and rats.

Excessive stimulation of the CNS by aminophylline appeared to be the cause of death in the rodents we studied, consistent with observations in higher mammalian species such as cats and dogs (13). Likewise, excessive CNS stimulation, manifested by hyperirritability and seizures, has been reported in man following aminophylline overdosage (29,35).

The clear differences in blood-brain distribution between ten-day-old and adult animals, while of interest, do not explain the age and species differences in LD_{50} . The results do confirm that the so-called "blood-brain barrier" is less developed in immature mice and rats. Combining mean values at both doses and at all time periods yielded brain:blood ratios for neonatal of 1.2 vs 0.7 for adult mice and values of 0.7 vs 0.35 for immature and adult rats. While this observation is compatible with and might be used to explain the age-related differences in LD_{50} in mice, such reasoning cannot be applied universally, because rats did not exhibit an age-related difference in LD_{50} .

Tissue concentrations of theophylline were consistently and significantly higher in ten-day-old animals of both species, but tissue:blood ratios approximated unity. This observation is important, having been demonstrated before only in two adult rabbits (26), for it provides an experimental basis for the use of serum and saliva theophylline concentrations for monitoring

therapeutic and toxic effects. Calculated tissue compartment theophylline concentrations from a two-compartment pharmacokinetic model differ, and must be interpreted cautiously. Paalzw (24) found the tissue compartment theophylline concentrations to be 2.3 times the central compartment concentrations in rats, whereas Levy and Koysooko (16) found the tissue compartment values to be only about 20% of the central values in humans. However, the central (plasma) theophylline concentrations correlate well with the pharmacologic effects (16,24). The conclusions of Paalzw and of Levy and Koysooko, as well as other studies in children (15,18) and the data of the present study all support the use of a one-compartment pharmacokinetic model.

The known binding of theophylline by serum proteins generates concern over bilirubin displacement. Aranda et al. (1) have shown by an ultracentrifugation method that 35% (at a serum concentration of 17 ug/ml) of theophylline was protein bound in the cord plasma of neonates versus 56% in adult plasma. In their study, theophylline (15 ug/ml) did not reduce bilirubin binding as determined by the Sephadex G-25 column technique. Similarly Brodersen (3), using the peroxidase method, found theophylline to be a very weak bilirubin displacer, causing a 5% displacement at 10 ug/ml. Our *in vivo* studies in Gunn rats agree with these *in vitro* findings and support the view that bilirubin displacement is unlikely to present problems in the treatment of neonatal apnea with theophylline.

CONCLUSION

Our results in animals support the general clinical experience regarding the safety of the use of aminophylline in neonates. Ten-day-old rodents were not more sensitive than adults to its lethal effects. Although the LD₅₀ for newborn mice was less than that for adult mice, it was consistent with that found for adults of several other species, indicating that the adult mouse is unusually resistant to this drug. The uniform distribution of theophylline in body tissues supports the utility of serum theophylline levels in monitoring therapeutic and toxic effects and the analysis of such data by a one-compartment pharmacokinetic model. The relatively greater permeability of the neonatal blood-brain barrier necessitates careful observation and avoidance of excessive serum levels to avoid CNS toxicity. Since the site of action may be central respiratory chemoreceptors (5), relatively low serum concentrations may be sufficient for a therapeutic effect. Thus, while at present there are no data available as to optimum serum levels in premature, such levels may prove to be different from the 10 - 20 ug/ml generally accepted as therapeutic and safe for treatment of bronchial asthma in children and adults.

We wish to thank Dr. Jerold F. Lucey and Mrs. Jean Hewitt for their assistance with the Gunn rat studies, Miss Ann Hughes for her technical assistance, and Mrs. Mary-Jane J. Dodds for her assistance in preparation of the manuscript.

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36. This work was supported in part by a Pharmaceutical Manufacturer's Association Foundation Medical Student Fellowship (ALN), the Ross Laboratories, and PHS Grant No. R01-HD-08708 (LFS). Presented in part at the Ross Conference on Apnea of Prematurity, November 20-21, 1975 in North Andover, Massachusetts.
37. Received for publication November 8, 1976.
38. Accepted for publication February 23, 1977.

Table I. Acute toxicity of aminophylline

Species	N	Age	Route	LD ₅₀ (mg/kg) ± SE
Mice	91 ^a	10 days	IP ^b	152 ± 16
	58	10 days	Rectal	166 ± 16
	155	Adult	IP	273 ± 5
Rats	82	10 days	IP	168 ± 8
	45	Adult	IP	188 ± 10

^aApproximately equal numbers of males and females in each group.

^bVolumes of injection = 1 - 2 ul/g.

Table II. Concentrations of theophylline (ug/g) in various tissues at 15 - 120 minutes administration of aminophylline (4 mg/kg per rectum, 1 ul/g)

Time	M I C E							
	ADULT				10 DAYS			
	15	30	60	120	15	30	60	120
Blood ^a	9.6	11.0	12.6	4.2	26.2	21.0	12.3	11.3
	2.0	12.0	5.0	4.4	1.8	13.7	7.6	9.1
	3	5	4	4	4	6	6	5
Brain	5.8	7.4	4.1	1.9	19.8	15.6	15.8	14.3
	4.1	6.0	2.7	1.5	0.9	11.8	3.8	6.5
	4	7	6	5	4	6	6	5
Lung	11.5	9.3	5.0	1.9	25.4	20.8	12.2	12.8
	7.2	10.0	3.8	0.9	1.8	16.3	8.3	8.5
	4	7	6	4	4	6	6	5
Heart	8.0	8.0	4.9	2.2	27.3	25.0	19.3	12.7
	2.4	6.8	3.6	1.1	3.6	16.5	7.8	7.1
	3	7	6	5	4	6	6	5
Liver	10.1	3.8	5.9	4.0	21.0	20.4	17.9	11.4
	6.6	1.3	4.2	3.1	1.9	12.3	4.9	5.2
	4	6	6	5	4	6	6	5
Kidney	14.6	5.2	6.0	3.0	29.9	26.6	21.7	15.1
	6.8	1.8	5.9	2.0	2.2	18.9	7.3	8.3
	4	6	4	5	4	6	6	5
Colon	15.4	4.5	7.3	3.8	24.1	25.2	21.3	10.9
	9.7	1.5	3.4	2.5	2.5	19.1	12.5	7.5
	4	5	6	5	2	6	6	5
Small Intestine	10.7	3.7	5.2	2.0	27.3	21.1	19.3	11.2
	3.9	1.3	3.2	0.9	6.4	16.7	6.4	6.4
	4	6	6	5	4	6	6	4
Skeletal Muscle	13.0	3.8	5.0	2.1	23.0	19.0	14.7	13.0
	7.5	2.1	3.0	1.1	2.2	18.9	9.9	8.7
	4	6	6	5	4	6	6	4
Fat	7.9	0.6	0.7	0.4	12.0	8.8	6.8	5.4
	9.0	0.4	0.2	0.4	3.4	9.5	3.9	3.0
	4	5	6	5	4	6	5	4

^aValues are mean, S.D., and N.

Table II. Continued

Time	R A T S							
	ADULT				10 DAYS			
	15	30	60	120	15	30	60	120
Blood ^a	13.0 9.4 2	11.7 0.6 2	5.8 2.7 2	8.9 7.4 2	14.9 9.4 6	20.0 8.3 6	24.1 1.4 6	22.0 2.5 5
Brain	5.2 4.4 2	3.2 0.9 2	3.3 2.9 2	2.7 1.9 2	9.8 6.2 5	11.6 6.4 8	16.5 6.2 6	11.5 5.3 7
Lung	10.9 6.6 2	9.7 1.5 2	5.5 2.2 2	9.4 8.8 2	13.9 8.4 6	15.4 8.5 8	21.6 6.9 7	15.3 6.5 7
Heart	14.6 11.3 2	9.6 1.9 2	5.0 2.1 2	7.3 5.8 2	15.4 10.2 6	16.7 10.3 8	23.4 7.9 7	16.2 7.1 7
Liver	12.3 8.1 2	10.2 0.6 2	5.7 2.7 2	7.9 6.2 2	14.4 8.6 6	16.2 8.3 8	21.6 7.5 7	14.6 5.8 7
Kidney	18.1 10.3 2	15.5 0.3 2	7.7 2.4 2	19.1 19.5 2	17.6 10.4 6	19.8 10.0 8	29.3 9.7 7	20.2 8.7 7
Colon	20.4 20.7 2	8.7 0.6 2	4.7 2.0 2	7.4 5.9 2	13.5 9.0 6	36.0 17.3 7	23.7 7.3 7	20.8 9.2 7
Small Intestine	11.4 7.7 2	10.4 2.6 2	4.5 1.5 2	9.3 9.1 2	14.3 9.9 6	17.6 9.2 8	32.1 16.2 7	17.7 7.7 7
Skeletal Muscle	10.2 8.0 2	8.4 1.3 2	4.4 1.4 2	6.9 4.6 2	13.6 7.4 6	26.0 11.0 8	22.3 8.1 7	16.4 7.4 7
Fat	0.8 0.4 2	1.0 0.4 2	2.5 2.4 2	1.1 0.9 2	11.4 10.4 6	8.2 4.4 7	17.8 4.3 6	6.3 2.5 7

^aValues are mean, S.D., and N.

Table III. Comparison of blood-brain concentrations of theophylline (ug/g) following administration of low (4 mg/kg) and high (50 mg/kg) doses of aminophylline to neonatal and adult mice

	ADULT				10 d.o.			
Time (min)	15	30	60	120	15	30	60	120
	Low dose (N = 3 - 6)							
Blood	9.6 ^a 2.0	11.0 12.0	12.6 5.0	4.2 4.4	26.2 13.7	21.0 13.7	12.3 7.6	11.3 9.1
Brain	5.8 4.1	7.4 6.0	4.1 2.7	1.9 1.5	19.8 0.9	15.6 11.9	15.8 3.8	14.3 6.5
Brain Blood	0.5	0.8	0.4	1.0	0.8	0.8	1.6	1.3
	High dose (N = 10)							
Plasma	53.7 44.4	32.0 21.9	33.3 25.2	18.1 14.5	31.3 12.0	19.2 6.8	26.7 7.7	29.3 11.9
Brain	19.6 12.6	13.3 7.0	18.3 9.5	17.5 6.2	27.8 6.3	29.5 6.2	30.3 3.5	27.0 8.6
Brain Plasma	0.5	0.5	0.8	1.3	1.0	1.7	1.3	1.0

^aValues are mean and S.D. Fig. 1. Serum levels of theophylline and bilirubin (mean $\pm$ S.D.) after IP injection of aminophylline to one-month old (50 mg/kg, N = 8) and adult Gunn rats (100 mg/kg, N = 2 - 6). A positive control of two adult Gunn rats given sulfisoxazole (100 mg/kg) has been included.