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Chronic Theophylline Administration Has No Apparent Effect on Theophylline Concentrations Required to Produce Seizures in Rats[1,2] (42324)

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Abstract. Theophylline, the widely used antiasthmatic drug, can cause life-threatening, generalized seizures when administered in excessive doses. The plasma concentrations of theophylline associated with these seizures vary widely among patients, thereby complicating efforts to prevent seizures by timely initiation of appropriate treatment. Some investigators suspect that chronic administration increases the neurotoxicity of theophylline but others have suggested the opposite. We have studied this problem in an animal model of theophylline-induced seizures. Osmotic pumps containing theophylline solution or drug-free solvent (for the surgical control group) were implanted in adult female Lewis rats, yielding almost constant serum theophylline concentrations of about 14 mg/liter for 7 days in the treated group. On the seventh day, theophylline was administered by much more rapid iv infusion to the two groups of animals and to one nonimplanted (nonsurgical) control group until onset of maximal seizures. There were no statistically significant differences between the three groups with respect to the concentrations of theophylline in serum, serum water, brain, and cerebrospinal fluid at onset of seizures. The concentrations of theophylline metabolites were either very low or undetectable. Under the experimental conditions, preexposure of rats for 7 days to theophylline in the human therapeutic concentration range had no apparent effect on the acute neurotoxicity of the drug.

Theophylline (1,3-dimethylxanthine) is a bronchodilating agent widely used in the treatment of reversible, obstructive airway disease (1). It produces adverse effects on the cardiovascular and central nervous systems when plasma concentrations exceed the upper limit of the usual therapeutic concentration range, about 20 mg/liter (2). A particularly serious manifestation of theophylline overdose is generalized convulsions which can occur without preliminary warning signs and often cause permanent neurologic damage or death (3). Some patients have had seizures at plasma theophylline concentrations between 20 and 30 mg/liter (4, 5) while others have not experienced convulsion despite plasma theophylline concentrations above 100 mg/liter and as high as 330 mg/liter (6, 7). The reasons for the pronounced interindividual differences in the theophylline concentrations associated with seizures are not readily apparent but must be determined so that appropriate measures can be instituted in a timely manner to minimize or prevent theophylline-induced neurotoxicity.

One of the factors that may affect the neurotoxic response to theophylline is prior exposure to the drug. Sahney *et al.* (8) and Ellis (9) have suggested that supertherapeutic plasma theophylline concentrations maintained for some time may cause greater neurotoxicity, with poorer clinical outcome, than even higher concentrations produced by acute ingestion of an overdose. Similarly, Park *et al.* (10) believe that the development of seizures is determined not only by the plasma theophylline concentration but also by the duration of elevated concentrations. On the other hand, Jacobs *et al.* (11) have noted toxicity occurring at lower concentrations in patients who had received theophylline for the first time. It is unlikely that these uncertainties and apparently conflicting views can be resolved solely by clinical observation of the very diverse patients who experience theophylline-induced convulsions and other neurotoxic reactions. We have therefore developed an animal model of theophylline-induced seizures which per-

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mits an assessment of the effects of various pathophysiologic and other variables on the drug concentration versus convulsive response relationship (12). This model has been used to investigate the effect of prior exposure to theophylline on the concentrations of this drug that cause seizures.

Materials and Methods. Adult female Lewis rats (Charles River Breeding Laboratories, Wilmington, Mass.), maintained on Charles River Rat–Mouse–Hamster Formula, were used in this investigation. One group of these animals had osmotic pumps filled with theophylline solution implanted, another group received osmotic pumps filled only with solvent, and a third group did not have pumps implanted.

Alzet osmotic pumps (Alza Corp., Palo Alto, Calif.), Model 2ML2, designed to deliver 5 μl of solution per hour for 14 days, were filled either with an aqueous solution of aminophylline equivalent to theophylline, 75 mg/ml, dissolved with the aid of sodium hydroxide to yield a pH of 9 to 10, or with drug-free solvent. The solutions were passed through a bacteria-retentive membrane filter (0.22-μm pore size) before use. Preliminary tests showed that the drug was stable in the osmotic pump for at least 7 days. On Day 0, the filled pumps were primed by immersion in sterile normal saline solution at 37°C for 4 hr. One pump per animal was then inserted sc below the neck of lightly ether-anesthetized rats, with the opening directed caudally. The surgical incision was then closed with silk sutures and the animals were returned to individual, wire mesh bottom, metal cages and maintained in a temperature- and light-controlled facility.

Blood samples (~0.3 ml) were obtained from the tail artery of each rat (all three groups) on Days 2 and 4. On Day 6, all rats had a cannula implanted in the right external jugular vein (13) under light ether anesthesia and another blood sample was obtained at that time. On Day 7, the animals were weighed, a blood sample was obtained via the cannula for assay of theophylline and metabolites and for determination of hematocrit, and an iv infusion of theophylline (100 mg/ml, prepared by dissolving aminophylline in normal saline with the aid of sodium hydroxide) at a rate of 1.03 mg/min was started. The infusion was stopped at the onset of maximal seizures (evidenced by tonic flexion of the forelimbs and, usually, tonic extension of the hindlimbs) and samples of cerebrospinal fluid, blood (for serum), and brain were obtained promptly. During the infusion, the rats were placed on isothermal pads to maintain their normal body temperature. These experiments were performed on two consecutive days, with half of each group being tested each day, in random order.

The concentrations of theophylline and its metabolites in serum, brain, and cerebrospinal fluid were determined by high-performance liquid chromatography after selective extraction (12). The detection limit for all compounds was between 1 and 2 mg/liter. The serum protein binding of theophylline was determined by equilibrium dialysis at 37°C (12). The osmotic pumps were removed from the animals at the end of the experiment and were weighed. Each of the pumps filled with theophylline solution was placed for 24 hr in 25 ml normal saline at 37°C and the concentration of drug in the solution was then determined to establish the pumping rate of the individual units. This rate divided by the steady-state serum concentration yielded the serum clearance of theophylline.

Results. Continuous administration of theophylline by means of an implanted osmotic pump produced essentially constant serum theophylline concentrations between Day 2 and Day 7 (Table I). The slightly lower mean value and the larger coefficient of variation on Day 2 were due to an unusually low concentration, 5.7 mg/liter, in one animal on that day only. The theophylline delivery rate of the pumps, 9.89 ± 0.44 mg/24 hr (mean ± SD, n = 12), was very reproducible. The calculated serum total clearance of the drug was essen-

TABLE I. SERUM CONCENTRATIONS AND SERUM CLEARANCE OF THEOPHYLLINE IN RATS DURING PRETREATMENT PERIOD[a,b]

Day	Serum concentration (mg/liter)	Serum clearance (ml/min/kg)
2	12.9 ± 3.2	
4	14.6 ± 2.4	
6	14.0 ± 2.0	
7	14.2 ± 1.4	2.63 ± 0.29
2 to 7		2.69 ± 0.36

[a] Results are reported as means ± SD, n = 12.

[b] The directly determined theophylline delivery rate of the osmotic pumps was 9.89 ± 0.44 mg/24 hr on Day 7, after their removal from the rats.

tially identical when based on the concentrations of theophylline on Day 7 only or on Days 2, 4, 6, and 7 combined (Table I). The theophylline metabolite 1,3-dimethyluric acid was detected in all serum samples, but in concentrations below the limit of quantitation (<1 mg/liter). The metabolite 1-methyluric acid was found in concentrations between 3 and 5 mg/liter only on Day 2 in 3 of the 12 theophylline-treated rats; the metabolites 1-methylxanthine and 3-methylxanthine were not detected in any of the serum samples. The rats receiving the slow, subcutaneous infusion of theophylline for 7 days acted and appeared healthy and normal during the entire period.

The results of the rapid iv theophylline infusion experiments are summarized in Table II. The hematocrit values and serum protein concentrations of animals with osmotic pumps were slightly lower, on the average, than those of animals who did not have these surgically implanted devices. Serum protein binding of theophylline was significantly lower in rats with implanted pumps than in rats that did not undergo surgery and pump implantation. The body weight of animals in all three groups was similar. There were no statistically significant differences between the three groups with respect to the total infused dose, the serum concentration (both free and total drug), the brain concentration, and the cerebrospinal fluid concentration of theophylline at onset of maximal seizures (Table II).

The theophylline metabolites 1-methyluric acid, 1,3-dimethyluric acid, and 3-methylxanthine, but not 1-methylxanthine and caffeine, were found in serum, brain, or both at the end of the rapid infusion of theophylline, i.e., at onset of seizures (Table III). None of the metabolites were detected in cerebrospinal fluid.

Discussion. The 7-day sc infusion of theophylline administered to one group of rats was designed to maintain total drug concentrations in serum in the generally accepted human therapeutic concentration range of 10 to 20 mg/liter (1, 2). This was accomplished in that concentrations of about 14 mg/liter were obtained. Corresponding concentrations of unbound drug were about 10 mg/liter, compared to the human therapeutic free drug concentration range of 4 to 8 mg/liter [based on 60% serum protein binding of theophylline in man (14)]. Thus, the concentrations of what is presumably the pharmacologically active moiety, i.e., free drug, were slightly above the human therapeutic range. Since theophylline has a biologic half-life of only about 70 min in rats at the concentrations used in this study (15), steady-state concentrations can be expected to have been attained within about 5 hr after osmotic pump implantation unless there occurred time-dependent changes in the

TABLE II. EFFECT OF CHRONIC THEOPHYLLINE ADMINISTRATION ON THEOPHYLLINE CONCENTRATIONS CAUSING MAXIMAL SEIZURES IN RATS[a]

	Theophylline-pretreated	Surgical controls	Nonsurgical controls
No. of animals	12	7	7
Body weight (g)[b]	186 ± 12	175 ± 20	172 ± 26
Hematocrit (%)[c]	37 ± 3	39 ± 1	41 ± 2
Onset time of seizures (min)[d]	46 ± 2	46 ± 2	46 ± 2
Total infused dose (mg/kg)[d]	255 ± 20	276 ± 28	280 ± 35
Serum concentration of total drug (mg/liter)[d]	369 ± 20	372 ± 23	384 ± 16
Serum concentration of free drug (mg/liter)[d]	273 ± 17	267 ± 8	255 ± 12
Brain concentration (mg/kg)[d]	250 ± 24	236 ± 6	229 ± 12
CSF concentration (mg/liter)[d]	251 ± 36	269 ± 34	257 ± 25
Serum free fraction (%)[e]	74.0 ± 1.6	72.1 ± 3.3	65.9 ± 1.8
Serum protein concentration (%)[e]	6.50 ± 0.29	6.71 ± 0.25	7.62 ± 0.36

[a] Results are reported as means ± SD.

[b] Total body weight minus the weight of the implanted osmotic pump in the case of the treated animals and the surgical controls.

[c] Significant difference between groups by analysis of variance, $P < 0.001$. Nonsurgical controls differ significantly from treated rats, $P < 0.001$.

[d] No statistically significant difference between the three groups of animals.

[e] Significant difference between groups by analysis of variance, $P < 0.001$. Nonsurgical controls differed significantly from each of the other two groups, $P < 0.001$.

TABLE III. CONCENTRATIONS OF THEOPHYLLINE METABOLITES IN SERUM AND BRAIN OF RATS AT ONSET OF MAXIMAL SEIZURES[a]

Metabolite	Theophylline-pretreated	Surgical controls	Nonsurgical controls
1-Methyluric acid			
Serum	6.0 ± 1.2	3.5 ± 1.0	5.4 ± 1.7
Brain	4.3 ± 3.3	2.9 ± 2.8	5.9 ± 3.5
1,3-Dimethyluric acid			
Serum	7.6 ± 2.8	7.0 ± 3.6	13.7 ± 1.9
Brain	D[b]	D	D
3-Methylxanthine			
Serum	ND[c]	ND	ND
Brain	9.0 ± 5.0	4.0 ± 2.5	3.7 ± 2.4

[a] Results are reported in units of mg/liter for serum and mg/kg for brain as means ± SD, n = as in Table II. No metabolites were detected in the CSF. No 1-methylxanthine was detected in serum and brain.
[b] Detected, but concentrations too low to quantify.
[c] Not detected.

drug's pharmacokinetics on the first day. Theophylline has been reported to have an inductive effect on its own metabolism in rats (16) but there is no evidence of induction in the serum concentration profile from Day 2 to Day 7. However, enzyme induction may have occurred before Day 2.

The decreased serum protein binding of theophylline in the rats with surgically implanted osmotic pumps (containing either drug solution or solvent only) is probably due to the occurrence of hypoalbuminemia. The decreased serum protein concentration and lower hematocrit in the rats with osmotic pumps indicates moderate blood loss and/or hemodilution, perhaps referable to the surgery. The pumping rate of about 5 μl/hr was too low to produce hemodilution. None of the rats bled overtly from the incision or from any other site, lost weight, or looked ill.

The concentrations of theophylline in serum, brain, and cerebrospinal fluid associated with onset of maximal seizures were not significantly different between theophylline-pretreated rats, surgical control animals with implanted pumps containing only solvent, and the nonsurgical control group without osmotic pumps. The concentrations of theophylline metabolites at onset of seizures were negligible compared to the concentration of the parent drug and far below concentrations obtained when the metabolites were infused as such at a high rate without any apparent pharmacologic effect (12). Thus, the neurotoxicity of theophylline appears to be due to the drug itself. Under the conditions of this investigation, chronic administration of theophylline had no apparent effect on the concentrations that produced seizures. Of particular relevance in this respect are the theophylline concentrations in cerebrospinal fluid, which reflect free drug concentrations at the site of action (12). The relatively small interindividual variation of these concentrations under the standardized and controlled conditions of the animal study compared to the very large interindividual variation of neurotoxic theophylline concentrations in the much more heterogenous human patient population is striking. It makes the animal model used in this investigation particularly suitable for the assessment of potential risk factors for theophylline neurotoxicity.

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