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BRIEF COMMUNICATION

Theophylline Neurotoxicity Is Unaffected by Glycerol-Induced Renal Failure

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RAMZAN, I. *Theophylline neurotoxicity is unaffected by glycerol-induced renal failure.* PHARMACOL BIOCHEM BEHAV 37(3) 583–585, 1990.—Glycerol acute renal failure (ARF) was examined to see if it alters theophylline (Th) neurotoxicity in rats. Concentrations of Th in serum, cerebrospinal fluid and in brain at seizure onset were similar in control and ARF rats infused with Th. Thus, glycerol ARF fails to alter Th neurotoxicity, an effect similar to that noted previously with uranyl nitrate but not with ureter ligation.

Theophylline neurotoxicity Glycerol renal failure Seizures

THEOPHYLLINE (Th) neurotoxicity is increased in rats with acute renal failure (ARF) induced with bilateral ligation of the ureters (UL) but not in rats with renal damage induced chemically with intravenous (IV) uranyl nitrate (UN) treatment (11). Reason(s) for this difference in toxic response to Th between the two models of renal dysfunction is not known but may be related to the greater severity of the surgical model compared to UN treatment (11) or to the fact that these two models represent two different categories of ARF namely ARF caused by tubular obstruction (ureter ligation) and nephrotoxic ARF caused by UN treatment (12). In any case, the pronounced increase in sensitivity to Th in the surgically induced renal failure model is consistent with clinical findings. In a retrospective study of 58 consecutive patients, seizures occurred in six out of 22 patients with impaired renal function and in only 2 out of 36 patients with normal renal function, a five-fold increase in incidence in ARF (1). The aim of the present study was therefore to examine the effect of another model of ARF, that produced with intramuscular (IM) glycerol, on Th neurotoxicity in rats. Glycerol ARF was chosen since it yet again represents another category of renal failure and is haemodynamically mediated. It resembles the 'crush syndrome' in humans with IM glycerol producing myohaemoglobinuria which leads to renal ischaemia (12,13). Specifically, a model of theophylline-induced seizures in rats (10) was used to examine if glycerol ARF alters Th concentrations in serum, cerebrospinal fluid (CSF) and brain at onset of maximal seizures.

METHOD

The experimental animals were twenty male Sprague-Dawley rats maintained in a temperature/humidity- and light-controlled vivarium on a 0600–1800 hr lighting schedule for at least one week before experimentation. They were deprived of drinking water for 24 hr but allowed food ad lib, following which they were anaesthetized briefly (10 min) with ether and a silicone rubber cannula was placed in the right external jugular vein. An IM injection of 50% v/v glycerol in sterile saline, 10 ml/kg b.wt., was then administered, under ether anaesthesia, in divided doses to two sites in each of the hind limbs as described previously (2,13). The drinking water was immediately restored. Control (or sham injected) rats were similarly dehydrated but injected with sterile saline only (10 ml/kg b.wt.). It has been reported that severe azotemia only develops when a 24-hr period of dehydration is used prior to glycerol treatment; half of nondehydrated animals fail to become azotemic (13). Both groups of rats were studied 48 hr later in a random fashion.

On the study day, Th solution (100 mg/ml base as aminophylline) was infused into unanaesthetized (and unrestrained) rats at 0.051 ml/min via the IV cannula until onset of a maximal seizure as evidenced by forelimb flexion and tonic hindlimb extension. The animals were then immediately (and briefly) anaesthetized with ether and samples of CSF (by cisterna magna puncture), blood (for arterial serum from abdominal aorta) and brain (after decapitation) were obtained in that order and assayed for total (protein bound and unbound) theophylline using liquid chromatography (10). Theophylline-free (unbound) fraction in serum was determined by equilibrium dialysis (10); this allowed the calculation of serum-free concentration as serum total theophylline multiplied by the free (unbound) fraction (10). Serum concentrations of creatinine and urea nitrogen were determined using commercially available kits for human samples (11); creatinine by a modified Jaffè reaction involving a picrate complex (6) and urea

TABLE 1

EFFECT OF GLYCEROL-INDUCED ACUTE RENAL FAILURE (ARF) ON THE ACUTE NEUROTOXICITY TO THEOPHYLLINE IN RATS

Parameters	Saline Control	Glycerol ARF
Number of animals, n	9	9
Body weight, g	258 ± 26	259 ± 15
Serum creatinine, mg/100 ml	1.1 ± 0.3	4.2 ± 1.7*
Serum urea nitrogen, mg/100 ml	15 ± 3	144 ± 55*
Infusion time, min	14 ± 2	16 ± 4
Cumulative dose, mg	71 ± 9	82 ± 21
Cumulative dose, mg/kg	275 ± 12	316 ± 89
Theophylline concentrations at onset of seizure		
Serum total, mg/l	504 ± 32	485 ± 51
Serum free, mg/l	430 ± 47	426 ± 47
CSF, mg/l	259 ± 24(7)	228 ± 41(4)
Brain, mg/kg	443 ± 41	435 ± 69
Serum free (unbound) fraction	0.852 ± 0.052	0.880 ± 0.055

Control rats received 10 ml/kg saline IM, while glycerol ARF rats received IM glycerol 10 ml/kg 48 hr earlier. On the study day, all animals received theophylline via a jugular vein cannula at 5.1 mg/min/rat until onset of a maximal seizure. Values represent means ± SD, n as shown except for CSF which are shown in parentheses. Serum-free concentration calculated as serum total concentration × the corresponding free fraction in each rat.

*Denotes values statistically different from control ($p<0.001$; Student's t-test).

nitrogen using the diacetyl monoxime method (3). Variables from the two groups of animals were compared using Student's t-test for unpaired data.

RESULTS

Two animals pretreated with IM glycerol died prior to the study day presumably due to the severity of the renal damage produced. In the remaining animals, serum concentrations of creatinine were increased three times and urea nitrogen elevated about 10-fold compared to control animals, indicative of a severe damage to the kidneys with glycerol (Table 1).

Onset times to seizure, cumulative doses and concentrations of Th at onset of seizures are summarized in Table 1. ARF rats took the same time and needed the same absolute (mg) and body weight normalized (mg/kg) dose to elicit a seizure as saline-treated controls. More importantly, serum and CNS (both CSF and whole brain) concentrations of Th at maximal seizure onset did not change with glycerol pretreatment, i.e., ARF did not increase sensitivity to Th neurotoxicity nor did it offer protection against Th-induced convulsions. In addition, glycerol ARF did not affect Th serum protein binding statistically.

DISCUSSION

Th use in patients is often associated with serious toxicity including seizures which are generally not preceded by warning signs and are associated with wide-ranging serum theophylline concentrations (14). Th is also used therapeutically in some patients with renal failure (9) and since its plasma protein binding is decreased in renal disease, these patients may show toxicity at plasma concentrations of total (free plus protein bound) Th that are normally considered to be within the therapeutic concentration range (8). ARF is also associated with increased sensitivity to the pharmacologic affect of certain CNS depressants like phenobarbitone and ethanol (4,7). Thus, there is a need to examine if ARF is a risk factor for Th neurotoxicity.

Previously, using a model of Th-induced seizures in rats (10), its neurotoxicity was examined in rats with ARF (11). Ureter ligation caused a substantial reduction in the infused dose and the serum, brain and (more importantly) the CSF concentrations of Th at seizure onset. UN treatment did not affect the drug's toxicity. The results of this present study complement the previous findings with UN in that a different mode of producing chemically induced experimental renal failure in rats, that with IM glycerol, does not affect Th neurotoxicity. It therefore appears that different chemical models of ARF which produce a less severe form of the disease at least as judged by a lower mortality (5,11) do not change sensitivity to Th toxicity. In contrast, UL which results in life-threatening renal dysfunction and a higher mortality (5,11) increases the sensitivity to Th. However, all three (UL, UN and glycerol) models for producing ARF apparently result in similar elevations in serum creatinine and urea nitrogen concentrations (2, 5, 12, 13). Thus, these (gross) biochemical indices of ARF may not adequately reflect the severity of the disease at the renal level and other more sensitive pathomorphological indices of renal damage including histological and micropuncture techniques may need to be employed to assess the damage.

The findings of this study have important implications for both animal and patient studies involving drug toxicity (and pharmacodynamics) in disease particularly ARF. Several models of each disease need to be examined in animals to rule out effects referable to a particular method of producing the disorder. In addition, in patients, the aetiology and duration of a particular disease like ARF may need to be considered when disease effects on drug toxicity (or therapeutic effect) are being documented.

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