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 Title : Prolonged Retention of d-Tubocurarine in Rats
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Introduction. A recent study of the pharmacokinetics of d-tubocurarine (dTc) in man revealed that on the average only 45% of a single intravenous dose of dTc is excreted in 24 hrs.¹ The pharmacokinetic data suggested the localization of dTc in a very slow (deep) compartment and excretion over a period of days to weeks. This study was undertaken to determine the long-term tissue distribution of dTc after a single intravenous injection in rats.

Method. Six adult male rats (400-475 g) were anesthetized with pentobarbital, 40 mg/kg, ip. The animals were intubated under direct vision with a #16 intracath catheter. The catheter was attached to a ventilator. At this point dTc (0.3 mg/kg) was injected into the rat's tail vein. When the effect of dTc had worn off, the animals were extubated and returned to their cages. Small volumes of blood (0.3 - 0.5 ml) were obtained daily from the tail vein to determine plasma dTc levels using radioimmunoassay. One week following the injection of dTc, the animals were killed. Liver, kidney, spleen, heart, lung, brain, diaphragm and femoral muscle together with blood were collected to be analyzed for dTc. For tissue extraction, 1 gm of tissue was homogenized in 2 ml of phosphate buffer, pH 9.5. dTc in the homogenate was extracted with 10 ml of 1-2 dichloroethane. Dichloroethane was removed to a separate tube and the extraction procedure was repeated twice with 10 ml of dichloroethane. Dichloroethane, which contained dTc, was pooled and the solvent evaporated. One ml of 0.01 N HCl was then added to redissolve the dTc. Using this material, radioimmunoassay for dTc was carried out. Using internal standards, we have determined that this method extracts 80% of dTc present in tissue. The concentration of dTc/gm of tissue was adjusted for total weight of tissue, and percent of the drug remaining in the animal one week after injection was calculated. In calculating the total values, individual organs were weighed. Muscle was estimated to be 50% of total body weight and the plasma volume was estimated to be 40 ml/kg.

Results. dTc can be detected in the plasma and tissues of rats one week after a single dose of 0.3 mg/kg of the drug. The mean plasma values of dTc were 16 ng/ml at 24 hrs, 6 ng/ml at 48 hrs, and then remained close to 4 ng/ml up to at least 7 days (Fig 1). The mean concentrations of dTc in tissues are shown in Table 1. The total percent of the drug remaining in these tissues one week after injection ranged from 0.5 to 4.0% with the mean 0.76%.

Discussion. Chagas was the first to suggest that the unaccounted-for dTc is taken up by tissues and released very slowly, based on the examination of tissues of dogs 5 hrs after the injection of metocurine.² He also advanced evidence that the binding sites are mucopolysaccharides which form a large percentage of connective tissue and ground substance. Our findings in rats that on a weight basis at one week, the liver, spleen and lung have among the highest concentrations of dTc resemble those findings of Chagas in the dog at 5 hrs. Chagas did not report on the kidney. Here

we found much higher values of dTc than in any other tissue. This is not surprising since dTc was detected in the plasma of all animals, and the kidney is the prime organ of excretion for dTc.

Plasma dTc concentration fell to 4 ng/ml by day 3 and remained at this level through day 7. This suggests that the dTc that initially enters tissues is slowly released back into the circulation (sink effect) to be excreted primarily by the kidney. The fact that dTc can be detected in the plasma and tissues of rats one week after a single injection of the drug is further evidence that dTc is stored in certain tissues in the body and that this storage and slow release can account for all of the drug administered.

Table 1. Tissue dTc Concentration in Rats

Tissue	dTc in Tissue Mean Values (ng/g tissue wet-wt.)
Plasma	4.0
Kidney	48.5
Liver	14.9
Spleen	19.7
Heart	3.0
Lung	12.2
Brain	4.2
Diaphragm	6.7
Muscle	4.7

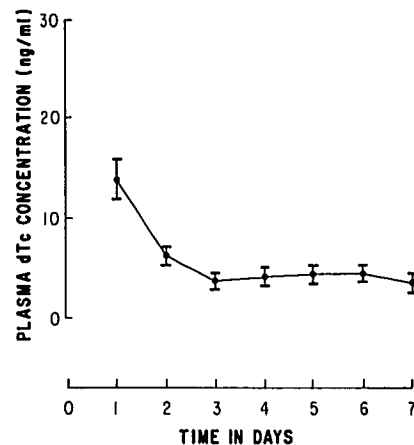


Fig 1. Plasma concentrations of dTc (mean ± SE) following a single iv injection, 0.3 mg/kg.

References

1. Matteo RS, Nishitaten K, Pua EK, Spector S: Anesthesiology 52:335-338, 1980
2. Chagas C: Ciba Foundation Study Group No 12, 1962, pp 2-10