

Cardioactive Properties of *d*-Tubocurarine with and without Preservatives

Elizabeth G. Dowdy, M.D., Ph.D.,* William C. Holland, M.D.,†
Ikuo Yamanaka, M.D.,‡ Keiji Kaya, M.D., Ph.D.‡

The authors previously reported that *d*-tubocurarine (dTe) had a negative inotropic effect on isolated perfused rabbit hearts; the effect was concentration-dependent and related to myocardial calcium exchange. This paper reports results of a concentration-response study of the effects of crystalline dTe, various commercial preparations of dTe, and their preservatives on the contractile amplitude of stimulated rabbit atrial strips. Crystalline dTe produced a slight positive inotropic effect which was significantly different from control, while the commercial preparation, Tubarine (used in previous studies), produced depression with 4.3×10^{-5} to 5.4×10^{-4} [M]. This depression closely paralleled that produced by antibacterial preservative, *p*-chloro-*m*-cresol, while the antioxidant preservative, K metabisulfite, produced minor depression. Other commercial preparations containing antibacterial preservatives also produced depression similar to the depression produced by their antibacterial preservatives. The commercial preparations dispensed without antibacterial preservatives produced relatively little depression. The authors conclude that their previous results were due to the preservative, *p*-chloro-*m*-cresol, and not to dTe. (Key words: *d*-Tubocurarine; Inotropic effect; Preservatives.)

SINCE THE EARLY PART of the twentieth century, investigators have described cardioactive properties of curare.¹⁻³ With its introduction into clinical use, it became evident that hypotension often occurred promptly after rapid in-

travenous administration of large doses.⁴ Histamine release,⁵⁻¹⁰ ganglionic blockade,^{9, 11-14} and a direct negative inotropic effect on the myocardium^{1-3, 15-20} have been suggested as pharmacologic actions of *d*-tubocurarine (dTe) which may contribute to decreases in arterial blood pressure.

During open-chest operations in acutely ill patients, we found visible changes in contractile force immediately after intravenous administration of dTe. We subsequently demonstrated that in the isolated perfused rabbit heart dTe had a negative inotropic effect which was concentration-dependent and related to decreased calcium exchange in the myocardium.^{18, 19} Iwatsuki *et al.*, using an *in vivo* preparation in dogs and a strain-gauge technique, demonstrated a negative inotropic effect of dTe and attributed this to the direct effect of histamine on the myocardium.²⁰ Trendelenburg found that histamine had a direct positive inotropic effect on the myocardium of the cat, rabbit and guinea pig which was not blocked by antihistamine.²¹ Others have found that dTe produced a positive inotropic effect under certain conditions.^{15, 22}

The cardiovascular effects found in early studies could have been the result of impurities, since techniques of isolating the pure alkaloid dTe had not yet been developed, however, disagreement about cardiac effects continues to exist even after the development of isolation techniques. In personal communications, European investigators have informed us that they have not observed a cardiac depressant effect of dTe like that we described, while those in Japan have found this phenomenon. This inconsistency could be explained by the use of pharmaceutical preparations which differ in the preservatives added to dTe. The purpose of this study was to determine if the reduction in myocardial contractility produced by dTe might be due to the preservatives added by the drug manufacturers.

* Professor of Anesthesiology; Associate Professor of Pharmacology; Director, Anesthesia Research Division, Department of Anesthesiology, University of Alabama School of Medicine, Birmingham, Alabama 35233.

† Professor and Chairman of Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi 39216.

‡ Anesthesia Research Division, Department of Anesthesiology, University of Alabama School of Medicine, Birmingham, Alabama 35233.

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TABLE 1. Some Commercial Preparations of dTc and Their Preservatives

	Pharmaceutical House	dTc (mg/ml)	Antibacterial Preservative (mg/ml)	Antioxidant Preservative (mg/ml)
Tubarine	Burroughs Wellcome & Co., Inc., U. S. A.	3	p-chloro-m-cresol 1	K metabisulfite 1
Tubocurarine chloride injection, U.S.P.	E. R. Squibb and Sons, Inc., U. S. A.	3	Benzyl alcohol 9	Na bisulfite 1
Tubocurarine chloride injection	Abbott Laboratories, U. S. A.	15	—	Na metabisulfite 1
Amelizol	Yoshitomi Pharmaceutical Industries, Ltd., Osaka, Japan	3	Chlorobutanol 5	—
Curarin-IIAF	Ethicon, Ltd., Hamburg, Germany	3	—	—

Methods and Materials

Pure crystalline *d*-tubocurarine chloride was obtained from Burroughs Wellcome and Co., U. S. A. The commercial preparations of dTc investigated are listed in table 1, along with the concentrations dispensed and the concentrations of the preservatives.

Rabbits (2-3 kg) were stunned by a blow on the head and the left atria were isolated. Atrial strips were bathed in a physiologic salt solution of the following composition (mM/l): NaCl 120, KCl 5.63, CaCl₂ 2.16, MgCl₂ 2.1, NaH₂PO₄ 1.03, NaHCO₃ 25, dextrose 11.1, sucrose 13.14. The solution was aerated vigorously with 95 per cent O₂-5 per cent CO₂ and maintained at pH 7.35-7.45 and a constant temperature of 30 C. The atria were attached to platinum-iridium electrodes and stimulated at a constant rate of 100/min with square-wave pulses (6 v, 5 msec duration). Two grams of tension were applied to the preparation and changes in isometric tension recorded by means of a force-displacement transducer (Grass FT-03) and recording polygraph (Grass Model 7). Each preparation was allowed to equilibrate for two hours prior to study. The reduction of contractile tension was expressed as percentage change from the original tension and plotted as a function of the negative logarithm of the concentration of dTc.

A concentration-response study of pure crystalline dTc and the commercial preparations of dTc listed in table 1 was done. A concentration-response study of each preservative listed

in table 1 except sodium metabisulfite was also done. Sodium metabisulfite was omitted because commercial sodium bisulfite is known to exist primarily as sodium metabisulfite and their actions are similar. The concentrations of preservatives studied were those found in the commercial preparations with concentrations of dTc listed in table 2. Therefore, the points plotted on the graphs and the data listed in table 2 are the percentage changes in contractile tension produced by the concentrations of the preservatives as they exist commercially with the listed concentrations of dTc. The time intervals between additions of drugs were 10 min in all preparations. Control studies were performed by adding small amounts of physiologic media to the bath instead of equal volumes containing the test drug.

Statistical comparisons were made by an analysis of variance with nonorthogonal single-degree-of-freedom comparisons of control vs. each test drug.

Results

The data describing the effects of crystalline dTc, the commercial preparations, and the preservatives are listed in table 2 and plotted in figures 1 and 2.

Crystalline dTc exerted a small positive inotropic effect, and from pD 4.37 to 3.27 values were significantly different from controls ($P = 0.0001$). Tubarine exerted a marked negative inotropic effect that was significant in all concentrations from pD 4.37 to 3.27 ($P = 0.0001$).

TABLE 2. Percentage Changes of Contractile Tension of Rabbit Left Atria Produced by dTc and Its Preservatives*

Number of Experiments	Concentration of d-Tubocurarine									
	$4.31 \times 10^{-7} M$ (pD = 3.37)†	$2.10 \times 10^{-6} M$ (pD = 5.67)†	$1.20 \times 10^{-5} M$ (pD = 4.89)†	$4.27 \times 10^{-4} M$ (pD = 3.37)†	$8.40 \times 10^{-4} M$ (pD = 4.07)†	$1.61 \times 10^{-3} M$ (pD = 3.81)†	$3.48 \times 10^{-3} M$ (pD = 3.61)†	$3.78 \times 10^{-3} M$ (pD = 3.42)†	$6.30 \times 10^{-3} M$ (pD = 3.27)†	
Control	0.1 ± 0.2†	0.1 ± 0.7	+0.1 ± 0.8	0.4 ± 0.8	0.6 ± 0.6	1.5 ± 0.6	1.8 ± 0.6	1.7 ± 0.8	2.1 ± 0.8	
Pure crystalline dTc	0.1 ± 0.0	+0.1 ± 0.2	+0.3 ± 0.3	+0.7 ± 0.6	+1.1 ± 0.6	+1.6 ± 0.7	+0.9 ± 0.6	+1.0 ± 0.7	+0.7 ± 0.7	
Tubarine (B. W. U. S. A.)	1.2 ± 0.4	2.0 ± 0.8	1.4 ± 1.5	7.7 ± 1.6	17.2 ± 1.3	30.6 ± 1.2	62.3 ± 2.1	83.0 ± 1.1	93.5 ± 1.3	
p-chloro- m-cresol§	0.0 ± 0.3	2.0 ± 0.6	4.0 ± 0.8	10.7 ± 1.1	26.5 ± 2.3	49.4 ± 2.1	75.7 ± 1.9	91.5 ± 1.8	98.7 ± 0.9	
K meta-bisulfite§	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.8 ± 0.4	1.5 ± 0.6	2.8 ± 0.9	4.0 ± 1.1	7.2 ± 1.2	9.0 ± 1.3	
Amelized (Japan) Chlorobutanol§	0.7 ± 0.3 +0.4 ± 0.4	2.0 ± 0.6 1.1 ± 0.9	1.4 ± 0.4 1.3 ± 0.7	1.9 ± 0.5 4.1 ± 0.7	5.7 ± 0.8 12.3 ± 1.4	14.3 ± 1.7 25.1 ± 2.0	24.8 ± 2.0 44.1 ± 2.4	44.6 ± 2.9 67.9 ± 2.5	63.3 ± 3.1 86.4 ± 2.6	
dTc Cl injection U.S.P., Squibb (U. S. A.)	+0.8 ± 0.3	0.1 ± 0.8	2.0 ± 1.5	12.8 ± 1.7	23.0 ± 2.3	39.4 ± 3.2	56.6 ± 4.0	81.5 ± 5.1	90.1 ± 0.9	
Benzyl alcohol§ Na bisulfite§	0.7 ± 0.3 0.1 ± 0.2	1.3 ± 0.4 0.5 ± 0.4	5.8 ± 1.1 1.1 ± 0.6	16.1 ± 1.9 2.1 ± 0.8	27.6 ± 2.8 2.7 ± 1.0	43.0 ± 4.1 3.5 ± 1.1	59.5 ± 5.6 4.3 ± 1.0	84.5 ± 5.1 5.8 ± 1.3	90.2 ± 0.5 7.9 ± 1.1	
dTc Cl injection Abbott (U. S. A.)	0.0 ± 0.0	1.2 ± 1	1.5 ± 0.8	1.2 ± 0.9	1.7 ± 1.0	2.1 ± 1.0	2.5 ± 1.0	2.8 ± 1.0	3.3 ± 0.7	
Curarin-HAP (Germany)	+0.1 ± 0.1	0.2 ± 0.2	0.4 ± 0.4	0.3 ± 0.5	0.9 ± 0.5	1.0 ± 0.6	2.5 ± 0.7	4.3 ± 0.8	5.8 ± 0.8	

* Mean values (designated increases in contractile amplitude; all others are percentage decreases).

† Mean values (designated increases in contractile amplitude; all others are percentage decreases).

‡ Mean and SD.

§ The concentrations of the preservatives used are those found in the commercial preparations with concentrations of dTc equivalent to those listed.

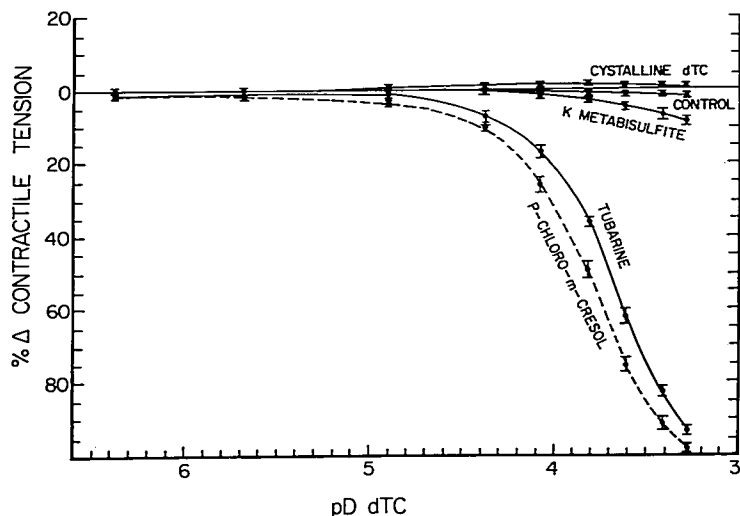


FIG. 1. Concentration-responses of contractile tension of stimulated left atrial strips of rabbits to crystalline dTc and to Tubarine and its preservatives, p-chloro-m-cresol and potassium metabisulfite. pD = negative log of molar concentration of dTc. The concentrations of the preservatives are those found with the concentrations of dTc in Tubarine, as listed.

This depression was almost identical to the depression produced by the antibacterial preservative, p-chloro-m-cresol, alone. The antioxidant, potassium, metabisulfite, had a less depressant effect than p-chloro-m-cresol or Tubarine at all concentrations; however, both preservatives were significantly depressant at pD 4.37 to 3.27 ($P = 0.0001$).

Amelizol also significantly depressed contractile amplitude, and the depression was similar to that produced by the antibacterial preservative, chlorobutanol, alone. Squibb's *d*-Tubocurarine Chloride Injection, U.S.P., and its preservatives, benzyl alcohol and sodium bisulfite, all significantly depressed contractile amplitude. The depression produced by benzyl alcohol was similar to that produced by the commercial preparation, while sodium bisulfite produced only slight depression.

The greatest depression produced by Abbott's *d*-Tubocurarine Chloride Injection was 3 per cent and that produced by Curarin-HAF, 5 per cent, both being significantly different from control at pD 4.07 to 3.27 ($P = 0.0001$).

Discussion

Crystalline dTc had a slight positive inotropic effect which was significant. This could be the result of release of some bioamine, such as histamine, which is known to be liberated by dTc and known to have a positive inotropic effect in the rabbit.

The depressant effect of Tubarine was very similar to that of its antibacterial preservative, p-chloro-m-cresol, while the antioxidant, potassium metabisulfite, produced less than 5 per cent change. This suggests that the myocardial depression produced by Tubarine, which we used in our previous studies,^{18, 19} was not due to dTc, but to the preservative, p-chloro-m-cresol. The depressant effect of Squibb's *d*-Tubocurarine Chloride Injection, U.S.P., was similar to that of its antibacterial preservative, benzyl alcohol, while the depression produced by equivalent sodium bisulfite was slight, although significant. The depression produced by Amelizol was similar to that produced by its preservative, chlorobutanol.

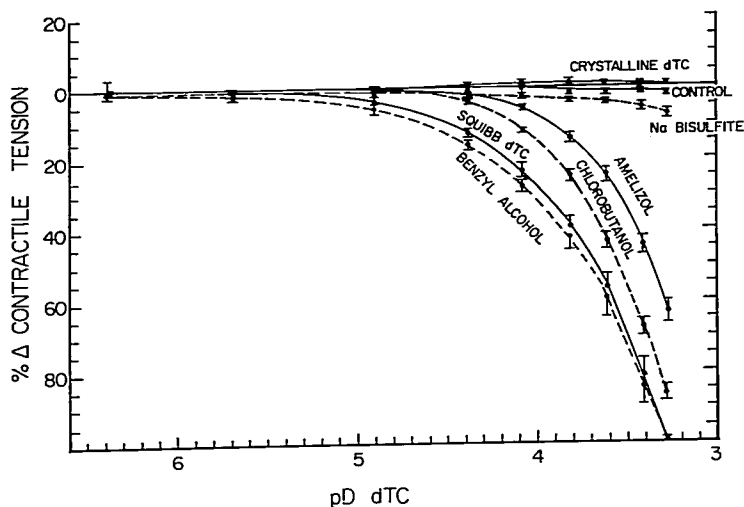


FIG. 2. Concentration-responses of stimulated left atrial strips of rabbits to crystalline dTc; Squibb, *d*-Tubocurarine Chloride Injection, U.S.P., and its preservatives, benzyl alcohol and sodium bisulfite; Amelizol and its preservative, chlorobutanol. pD = negative log of molar concentration of dTc. The concentrations of the preservatives, benzyl alcohol and sodium bisulfite, are those found with the concentrations of dTc in Squibb, *d*-Tubocurarine Chloride Injection, U.S.P., as listed. The concentrations of the preservative, chlorobutanol, are those found with the concentration of dTc in Amelizol, as listed.

Abbott's *d*-Tubocurarine Chloride Injection contains 0.1 per cent sodium metabisulfite, and Curarin-HAF (Germany) contains no preservative. Both commercial preparations produced less than 5 per cent depression, which was nevertheless significant.

It is interesting that the dose-effect curves of depression of the three antibacterial preservatives examined were always to the left of the curves of their respective commercial preparations. This could represent the summation of the positive effect obtained with crystalline dTc and the negative effect from the preservatives; however, when these data were analyzed by nonorthogonal single-degree-of-freedom contrast, there was still a considerable statistical difference, suggesting the existence of a factor not accounted for by summation. We have no explanation for this.

Preparations commonly used in the United Kingdom are Tubarine (Stabilised), dispensed as dTc, 1 per cent, with benzyl alcohol, 1.57

per cent w/v, and Tubarine (Miscible), dispensed as dTc, 1 per cent, without preservative, by Burroughs Wellcome and Co., London. These preparations were not available for this study. Tubarine (Stabilised) contains only half the amount of benzyl alcohol found with an equivalent amount of dTc in Squibb's *d*-Tubocurarine Chloride Injection, U.S.P.

Hager, 1954, described a negative inotropic effect produced by curare in the isolated perfused heart of the frog.¹⁵ The dTc preparation used in these studies was Intercostin, consisting of tubocurarine chloride pentahydrate, 0.3 per cent, and chlorobutanol, 0.5 per cent. He found this preparation caused significant depression in concentrations from 10^{-5} to 10^{-4} [M]. It can be seen from table 2 that chlorobutanol used as a preservative in this concentration range causes considerable depression. Bouyard, 1960, studied the myocardial depressant effects of dTc and also used Intercostin.^{16, 17} By using a myocardial strain-gauge

technique, Iwatsuki *et al.*, 1965, demonstrated a negative inotropic effect of dTc on the intact heart of the dog.²⁰ The dTc utilized by these investigators was Amelizol, which contains dTc, 0.3 per cent, and chlorobutanol, 0.5 per cent. Lapique and Veil, 1916, described cardiac depressant properties of curare; however, they used a sample of curare left in the laboratory by Claude Bernard,¹⁻³ which must have been approximately half a century old and probably contained many impurities.

This investigation has not identified the part of the hypotension seen after clinical administration of commercial preparations of dTc that may be the result of direct myocardial depression produced by the preservative. There is ample evidence that histamine released by dTc plays a major role in producing this hypotension.⁵⁻¹⁰ There is also evidence suggesting that ganglionic blockade is a less significant factor when dTc is given in clinical dose ranges.⁹⁻¹¹ The decreases in arterial pressure seen in cats and dogs given commercial dTc are partially, but not completely, blocked by antihistamine.^{17, 22} We believe that direct myocardial depression is insignificant in the healthy patient but may become significant in the patient with a diseased myocardium or low serum calcium. Perhaps the direct negative inotropic effect of dTc on the myocardium which has been reported since the development of efficient isolation techniques has resulted from only the preservatives in the commercial preparations used.

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