NeuroToxicology® 10:211-218, 1989

Comparison of Colchicine Toxicity on Different Dysmyelinating Mutant Models

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ABSTRACT: *The administration of colchicine to dysmyelinating mutant mice may serve as an in vivo pharmacological tool for the study of the mechanisms involved in the formation of the myelin sheath. The study of the acute toxicity of colchicine in these mutants demonstrated that male animals were much more sensitive than female animals. All of the mutants and their controls were also more resistant to colchicine than the Swiss strain usually used in toxicity studies.* © 1989 Intox Press, Inc.

Key Words: Colchicine, Intoxication, Dysmyelinating Mutants, Mice

INTRODUCTION

Inhibition of the polymerization of microtubules by colchicine makes it an ideal tool for the understanding of numerous phenomena involving transport (Périsic and Cuénod, 1972; Banks and Till, 1975). Interruption of axonal transport occurs to varying degrees and at rates which are not always identical and this may be related to slow penetration of colchicine into the myelinated fibres of the CNS (Edström *et al.*, 1979; Paulson and McClure, 1975a,b). The *in situ* application of colchicine at the level of the peripheral nervous system induces an alteration in the synthesis of the constituents of myelin (Souyri *et al.*, 1988). Myelin mutants have helped to further in understanding of the formation of myelin. The Quaking mutant has contributed to the identification of the constituents of myelin and to the clarification of part of metabolism and synthesis, as its defective genetic character induces an alteration in the assembly of myelin (Hogan and Greenfield, 1984). The Shiverer mutant is totally devoid of basic myelin protein (Baumann, 1980), while in the Trembler mutant the myelin of peripheral nerves and of the Schwann cell is altered (Bourre *et al.*, 1980). These mutants could be used to study the mechanisms of myelin assembly in more detail, by using the toxic effects of colchicine on microtubules, which are involved in transport mechanisms. Whether colchicine crosses the blood brain barrier, which it would have to in order to effect myelin constituents, has been much debated (Hunter and Klassen,1975; Stewart and Rose, 1968). Bennett *et al.* (1981) reported that 0.01% of the ip. injected dose was recovered in the central nervous system. In fact, the quantity of colchicine present in the brain represents 1:105 of the amount of tubulin. However, since the majority of this tubulin is involved in the formation of microtubules by polymerization, the ratio to free tubulin is much higher. The sub-stoichiometric action of colchicine is now generally accepted. Olmstedt and Borisy (1973) consider that a ratio of 1:25

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Accepted: May 24, 1989

TABLE 1. Equations of linear regression determined from the lethal dose curves after probit transformation with probit = f(Logdose). Level of significance of analysis of variance performed on the slope of LD curves is * ($p < 0.05$) and NS (Not Significant).

STRAINS	MALES		FEMALES	
SWISS	−9.24 + 7.66 X		0.10 + 4.51 X	
TREMBLER	−14.11 + 0.53 X	*	−9.26 + 9.96 X	NS
TREMBLER C.	−9.11 + 6.69 X		−1.62 + 5.14 X	
SHIVERER	0.52 +2.63 X	NS	−3.13 + 5.77 X	NS
SHIVERER C.	−7.45 + 7.27 X		7.24 + 10.03 X	
QUAKING	−7.66 + 7.3 X	NS	−0.95 + 4.90 X	*
QUAKING C.	−5.85 + 6.61 X		1.21 + 2.78 X	

is sufficient to inhibit polymerization of microtubules. It is therefore reasonable to consider that, despite the low percentage of colchicine crossing the blood brain barrier the quantity of colchicine present is nevertheless sufficient to exert an effect on transport systems mediated by microtubules. The objective of the present study was to compare the lethal dose of colchicine in different myelin mutants with that in normal strain. This enabled us to evaluate whether the mutation affected the sensitivity of the strain to colchicine. The second objective was to provide neurobiologists with a colchicine lethal dose profile to enable them to select a dose capable of inducing toxic effects without being lethal.

MATERIALS AND METHODS

Animals

Swiss mice were obtained from Iffa Credo (l'Arbresle, France). The Trembler mutant was on the B6-CBA strain and originate from the scottish mutation (through laboratories of Dr. Guenet, Pasteur Institute) Shiverer, on C3H-SWV, was obtained from Washington University (Dr. Bird) and the Quaking mutant belonged to the C57-B6 black strain and was obtained from Jackson Laboratories. These mutants were bred in our laboratory. Animals were compared with their normal appearing littermate except for Shiverer which were compared with a control strain (mice issued from the same cross but without expression of the mutation). Adult animals were randomized into groups of 10 mice for each injected dose. At the time of injection, they were a minimum of 60 and a maximum of 90 days old. Mice were housed in an air-conditioned animal house under standardized temperature conditions (22 ± 1° C) with a day and night cycle (12 hr - 12 hr) and were fasted on the day prior to the administration and had free access to water.

Conditions of Administration

Colchicine {base, SIGMA} dissolved in 0.9% NaCl was always administered by intraperitoneal (ip) injection between 9:00 a.m.

TABLE 2. Comparison of the LD50 and LD10 for different mice strains after colchicine intoxication, with the same levels of significance for statistical analysis as described in Table 1.

	MALES		FEMALES	
STRAINS	LD 50	LD10	LD 50	LD10
SWISS	2.98	2.21	6.42	5.49
TREMBLER	4.19	3.86	6.14	5.47
	} NS {		} * {	
TREMBLER C.	3.29	2.8	8.24	6.89
SHIVERER	4.09	3.25	5.49	3.48
	} NS {		} NS {	
SHIVERER C.	3.39	3.0	5.54	4.7
QUAKING	3.37	2.63	5.66	4.8
	} NS {		} NS {	
QUAKING C.	3.91	2.54	5.16	4.3

and 12:00 a.m. (conformity with chronopharmacology). The animals were observed for the first six hr following intoxication and regularly each day for 15 days.

Determination of the LD50, LD10 and Statistical Analysis

We used TREVAN's method (1927) modified by Miller and Tainter's correction (1944) with the probit transformation of the percentage of death using GENSTAT V (General Statistical program. NAG, Oxford, United Kingdom). For each strain an estimation of linear regression was determined using the least squares method. The LD50 and LD10 were calculated. Comparison of these data using analysis of variance (ANOVA) (Snedecor and Cochrane, 1957) led us to separate the males of the females for the subsequent statistical analysis. Then comparison of the estimated linear regression was performed using the same statistical method, first taking into account all the strains. Then analysis of variance was also used to compare the linear regression of the same strain between the mutant and its control.

RESULTS

Signs of Colchicine Intoxication Observed

The first signs of intoxication were observed between the fifth and sixth hr considerable huddling together of the animals due to hypothermia. On the following morning, the majority of animals were hypothermic (ΔT = 10°C). Marked gastrointestinal disorders occurred whose manifestations were dehydration and diarrhea. The first deaths regardless of the strain occurred before the fourth day, between the 40th and 48th hour but some deaths occurred up to the eighth day after intoxication. Deaths were exceptional after that time.

Comparison of Lethal Doses in Different Strains and Statistical Analysis

Fig. 1 and 2 present the lethal dose curves according to a log-probit representation (% mortality on the probit coordinates versus dose on logarithmic coordinates). Table 1 shows the

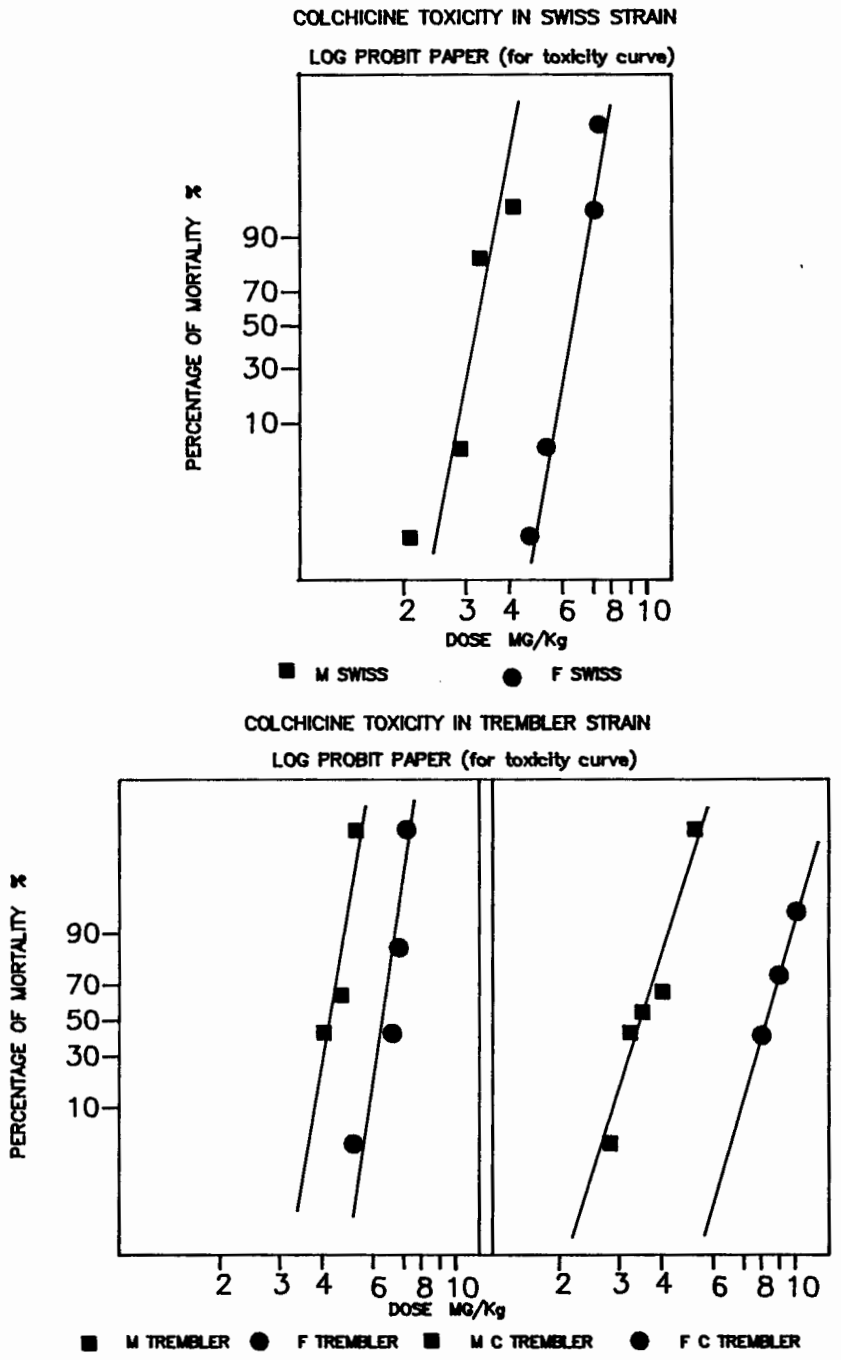

FIG. 1. Lethality profile of SWISS and TREMBLER strains with colchicine. After log-probit transformation % of deaths is plotted against logarithm of doses. M = male, F = female, C = control.

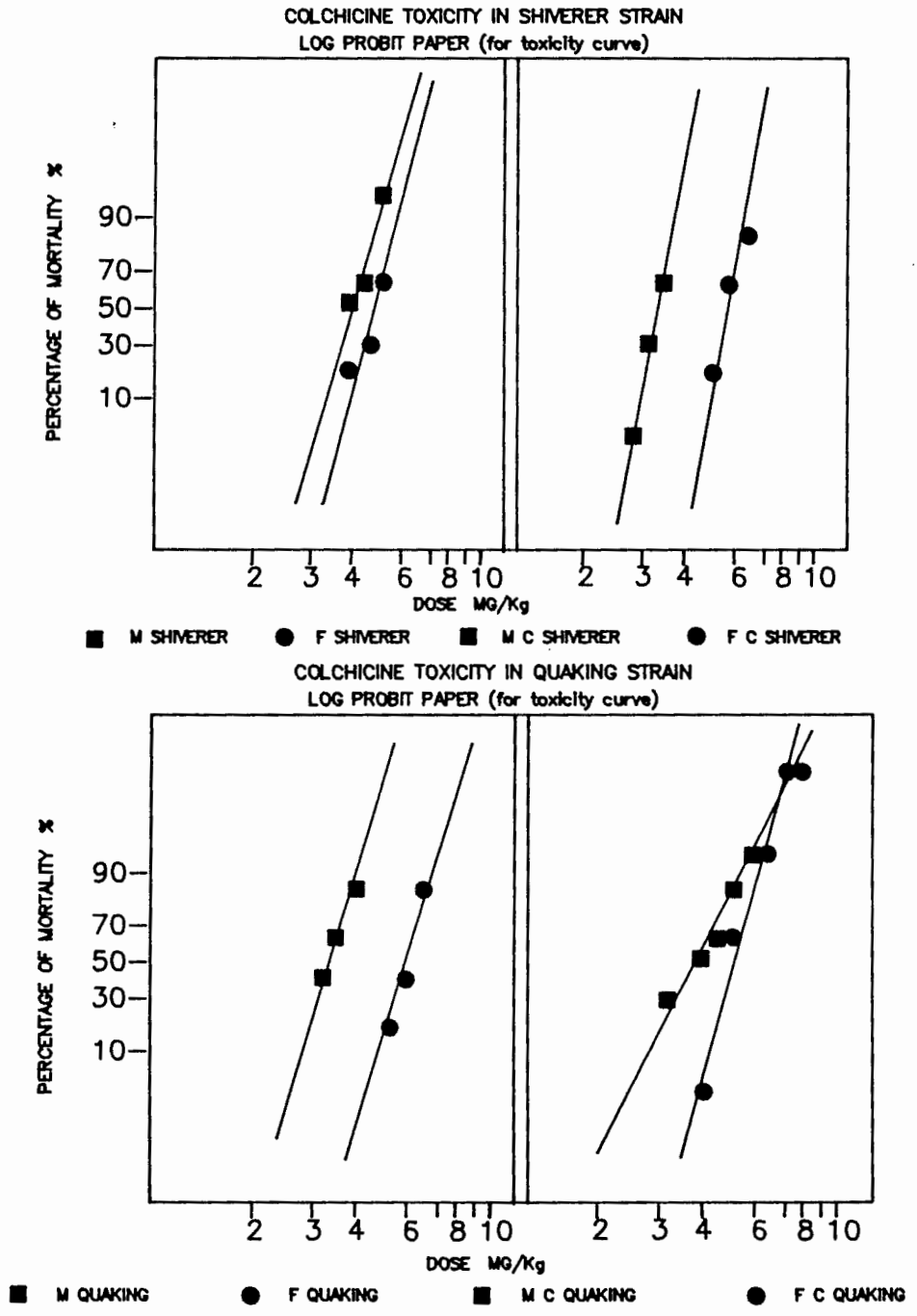

FIG. 2. Lethality profile of SHIVERER and QUAKING strains with colchicine. After log-probit transformation % of deaths is plotted against logarithm of doses. M = male, F = female, C = control.

linear regression equations calculated for each strain. Table 2 presents the various values calculated for LD50 and LD10. The variations observed can be classified into two categories: variations between the sexes, variations within the same strain between a mutant and its control. Preliminary analysis of variance of LD50 values using strain and sex as criteria showed a difference of sensitivity to colchicine between the sexes. Except in the Shiverer mutant. Females were always more resistant than males. This first result allowed us to go further in our statistical analysis separating the males from the females. Global comparison of all regression lines for males and females shows that they are not parallel both for males and females with statistical significance ($p < 0.01$). The same method used to compare the difference of sensitivity to colchicine in the same strain between the mutant and its control led to the following results: regression lines are distinct for Trembler females and their controls and for Quaking males and their controls ($p < 0.05$ see Table 1). But for Trembler the slopes are both distinct and parallel, while for Quaking there is absence of parallelism. So the statistical difference available for the slope is no more significant for comparison of lethal doses of Quaking males.

DISCUSSION

The dysmyelination of peripheral nerves (Brown and Seed, 1945) observed during colchicine intoxication demonstrates the neurotoxicity of colchicine. Colchicine has already been used to study axonal transport in both central and peripheral pathways (Edström and Hanson, 1979; Wiesniewsky *et al.*, 1968).

Because it is an intracellular poison, the use of colchicine as a pharmacological tool is particularly valuable for the study of myelin assembly. The injection of colchicine into the vitreous of the rabbit during development induces an alteration in the concentration of basic myelin protein and in the activity of 2',3' phosphodiesterase (Matthieu *et al.*, 1981). Townsend *et al.*(1984) demonstrated that colchicine inhibits the appearance of myelin sulfatides, while the synthesis and appearance of myelin cerebrosides is not modified. The simultaneous use of neurological mutants with myelin defects of variable severity and colchicine constitutes an ideal combination for the study of myelin assembly.

The data obtained in this work , together with well known *in vitro* effects of colchicine on microtubules described in literature, are presented as being of interest from the point of view of using the *in vivo* colchicine toxicological model as a pharmacological tool. This toxicity is expressed by the determination of the LD50 and LD10 and the slope of the lethal curve. They revealed a different sensitivity according to sex in two of the strains and this must be taken into account in all studies in which colchicine is administered systemically and not directly *in situ*. Neurobiologists using colchicine as an investigational tool in these mutants should not use toxicological data determined from the Swiss strain. These data would lead to an underestimation of the toxic dose required. Because of the low percentage of colchicine present in the CNS as shown by pharmacokinetic data (Bennett *et al.*, 1981). The effective toxic dose needs to be carefully calculated. Our results also demonstrate the greater sensitivity of male mice in comparison with female mice. Any study using colchicine as a pharmacological tool with these strains must take this disparity between the sexes into account.

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MS# 1605