Serum ceruloplasmin oxidase activity in Li^+ treated mice

Treatment	Route of administration	Dose (mEq/kg)	Period	No. of mice	Ceruloplasmin (mg/100 ml of serum)
Li_2CO_3	os	0.58/day	120 days	20	12.1 ± 0.4
Controls (H_2O)	os	—	120 days	20	13.5 ± 0.8
LiCl	i.p.	20	2 h	10	19.5 ± 1.6
NaCl	i.p.	20	2 h	8	14.0 ± 0.4

Each value of ceruloplasmin represents the mean ± SEM.

group, and this difference was found to be statistically significant ($t = 3.333$, $p < 0.01$). The same result was obtained comparing LiCl-treated mice with normal control animals ($t = 3.353$, $p < 0.01$).

It is interesting to recall that the dose of Li_2CO_3 used in the long-term experiment is in the range of that employed in manic-depressive psychosis[3]. In our previous reports with this schedule of Li_2CO_3 administration, we obtained inhibition of aconitase[10] and activation of succinate dehydrogenase[11] and fumarase[12] in cerebral tissues of mice. The patterns of oral and i.p. absorption of Li_2CO_3 and LiCl are similar[13], reaching measurable plasma levels rapidly and with a slow movement of Li^+ into the brain. The results of the present study indicate that the activation of ceruloplasmin by Li^+ is probably a function of the concentration achieved by the ion in blood.

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Effect of Colchicine on Polymerization of Tubulin from Rats, Mice, Hamsters and Guinea-Pigs[1]

T. J. Fitzgerald and D. G. Mayfield

School of Pharmacy, Florida A & M University, Tallahassee (Florida 32307, USA), 19 August 1975.

Summary. Colchicine-inhibition of polymerization of tubulin from rats, mice, golden hamsters and guinea-pigs was studied to determine if species differences in tubulin sensitivity to colchicine might parallel species variation in colchicine toxicity. It was found that polymerization of tubulin is nearly equally sensitive to colchicine in all four species.

It is well known that the golden hamster (*Mesocricetus auratus*) is highly resistant to the toxic effects of the alkaloid colchicine as compared to other species of rodents[2]. Among the hypotheses suggested for this phenomenon has been the possibility of a unique or different metabolic or excretory pathway for colchicine in the hamster, but this now seems unlikely in view of recent investigations[3,4]. Midgeley et al.[5] have demonstrated that the hamster has a cellular resistance to colchicine as revealed by the 100-fold higher dose of colchicine required to inhibit mitosis in hamster tissues. Since it is generally felt that colchicine's toxic effects are a result of interaction of colchicine with microtubule protein (tubulin) and breakdown of microtubules in various tissues, it is of interest to ask if hamster tubulin polymerization is more resistant to blockade by colchicine than polymerization of tubulin from species more susceptable to colchicine. In order to make this comparison we have determined the ability of colchicine to inhibit polymerization of tubulin from rats, mice, hamsters, and guinea-pigs.

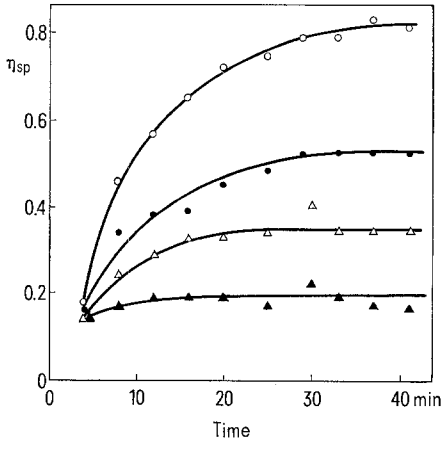

Fig. 1. Development of viscosity in hamster brain extracts with time and the effect of colchicine. Control (○); colchicine, 2.2×10^{-7} *M* (●); colchicine, 6.6×10^{-7} *M* (△); colchicine, 2.15×10^{-5} *M* (▲).

Polymerization of tubulin was measured in high speed supernatant fractions of brain homogenates from the various animals. Animals were killed by cervical dislocation. The brains were removed and homogenized in a cold glass homogenizer with 1.5 volumes (ml) of ice cold PEG buffer (100 m*M* PIPES, 1 m*M* EGTA and 2.5 m*M* GTP; pH 6.94). The homogenate was centrifuged at

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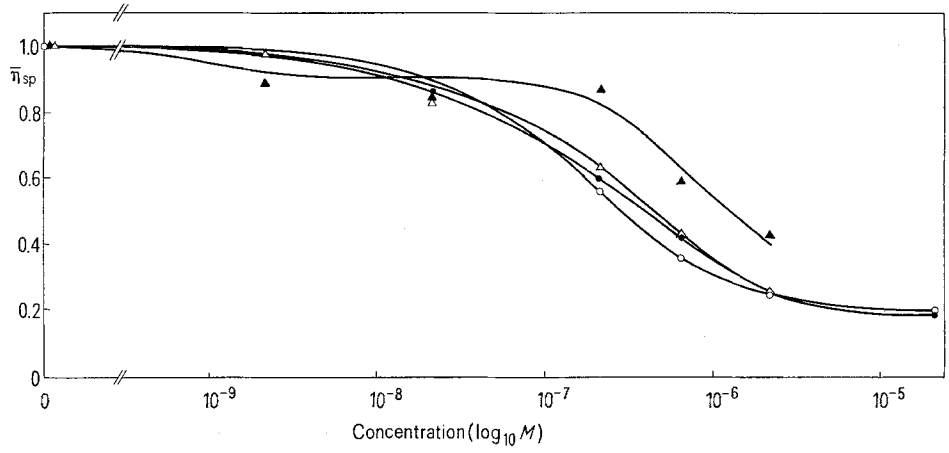

Fig. 2. Colchicine inhibition of polymerization of tubulin from various rodent species. Hamster (○); rat (△); mouse (●); guinea-pig (▲).

20,000 rpm for 30 min at 4 °C (Spinco type 65 fixed angle rotor, 9.0 ml polycarbonate tubes), and the supernatant fraction (henceforth called 'extract') was used for subsequent experiments. The protein concentration (range 8–12 mg/ml) was determined by a modified Biuret assay[6]. All extracts were used within 3 h of their preparation. Ostwald capillary viscometers (Cannon-Manning semimicro viscometer, type 100, Cannon Instrument Co., State College, Pa.) were immersed in a large water bath regulated at 37 ± 0.1 °C, and outflow times were measured using stopwatches calibrated to 0.5 sec. To obtain experimental data 0.6 ml of extract prepared at 4 °C was placed in a viscometer equilibrated at 37 °C and viscosity development was followed as a function of time of incubation. Specific viscosity (η_{sp}) was calculated in terms of outflow times of buffer (OT_b) and extract (OT_e) by the equation

$$\eta_{sp} = (OT_e - OT_b)/(OT_b).$$

For comparison of data from different experiments a normalized specific viscosity ($\overline{\eta}_{sp}$) was obtained from the expression

$$\overline{\eta}_{sp} = \eta_{spi}/\eta_{spc}$$

where η_{spi} represents the maximum specific viscosity achieved at a given concentration of colchicine and η_{spc} represents the maximum specific viscosity obtained in the absence of colchicine.

Viscometry has been shown to reflect biological properties of microtubule polymerization and has been demonstrated to be a rapid, sensitive, and quantitative method for studying the polymerization reaction[7]. A typical viscosity-versus-time curve for hamster brain tubulin and inhibition by colchicine is shown in Figure 1. Similar curves were obtained for each of the other species of animals. Figure 2 illustrates a plot of maximum viscosity obtained versus concentration of colchicine for the various species, and the half-maximal inhibition values obtained from these curves are displayed in the Table along with respective LD_{50} values[8,9]. The data assembled here indicate that colchicine inhibits polymerization of tubulin from each species within a single order of magnitude of concentrations. Since the LD_{50} values vary over nearly three orders of magnitude it seems unlikely that differences in sensitivity of the polymerization reaction of tubulin is the basis for the interspecies difference in colchicine toxicity in these animals. In fact, from these experiments it appears that tubulin isolated from guinea-pig brain is least sensitive to colchicine even though colchicine is most toxic in this species. It is, of course, possible that the intracellular environment may influence the sensitivity of tubulin to colchicine differently in different species in a manner that is not apparent in cell-free extracts. Finally, inability of colchicine to traverse cellular membranes in hamster cells remains an attractive but unproven hypothesis for accounting for this animal's resistance to colchicine.

Half-maximal values for inhibition of tubulin polymerization by colchicine and LD_{50} values for colchicine

Species	Half-maximal inhibition values (M)	LD_{50} (mg/kg, i.p.)
Golden Hamster	3×10^{-7}	470[8]
Rat	5×10^{-7}	4[9]
Mouse	4×10^{-7}	3.5[8]
Guinea-pig	10×10^{-7}	0.5[8]

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