Report

Uptake of Colchicine, a Microtubule System Disrupting Agent, by Isolated Rat Hepatocytes

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The possible mechanism of hepatic uptake of colchicine (CLC), a microtubule system disrupting agent, was examined using isolated rat hepatocytes. The existence of a carrier-mediated active transport system for CLC was demonstrated. This transport system is saturable, is affected by metabolic inhibitors (dinitrophenol, KCN) and a SH-group blocker (p-hydroxymercuribenzoic acid but not N-ethylmaleimide), and is sensitive to temperature. Ouabain, an inhibitor of Na+,K+-ATPase, does not affect the transport system of CLC.

KEY WORDS: colchicine; hepatic uptake; isolated hepatocyte; microtubule.

INTRODUCTION

Colchicine (CLC) is a disrupting agent of microtubules which causes metaphase arrest. CLC binds specifically to free tubulin, a structural subunit of the microtubules, and further, preformed tubulin–CLC complex interacts with microtubules. In consequence, the elongation of microtubules is inhibited, while at the other end of the polymer, the process of microtubule assembly occurs (1). Finally, CLC-induced microtubule depolymerization inhibits cell mitosis.

Intracellular and membrane transport of several substances is mediated by the microtubular system (2–7), and CLC or other microtubular poisons are used as a probe to study these transport phenomena. The effect of CLC on the intracellular disposition and excretion through the bile canalicular membrane of several compounds has been studied in the perfused rat liver (4–6). However, few studies exist on CLC uptake into cultured cells (8–10), and there are no data on CLC uptake by the isolated hepatocytes. CLC uptake into Chinese hamster ovary cells is nonsaturable and unaffected by the sulfhydryl reagents and ouabain, which are potent inhibitors of various mediated transport systems (9). On the other hand, some metabolic inhibitors, such as cyanide, azide, and dinitrophenol, stimulate CLC uptake into the Chinese hamster ovary cells. This finding indicates the presence of an energy-dependent carrier-mediated drug-efflux system for the maintenance of a drug-permeability barrier.

CLC serves as a specific marker of the microtubular system, to test whether an agent is transported by a microtubule-mediated system. The purpose of our study was to elucidate the possible mechanism of hepatic uptake of CLC using isolated rat hepatocytes.

MATERIALS AND METHODS

3H-Colchicine (CLC) (32.5 Ci/mmol) was purchased from the New England Nuclear Co. (Boston, Mass.). Unlabeled CLC was purchased from Sigma Chemical Co. (St. Louis, Mo.). Phthalic acid diisobutyl ester (d = 1.041) was obtained from Tokyo Kasei Kogyo Co., Tokyo. Collagenase (150–300 U/mg) was obtained from the Wako Pure Chemical Industries, Ltd., Osaka, Japan. All other reagents were of analytical grade.

Isolated hepatocytes were prepared by collagenase perfusion according to the procedure of Berry and Friend (12) as modified by Baur et al. (13). Adult male, Wistar rats, 180–200 g, were used as liver donors. Animals were maintained in a controlled environment. After isolation, hepatocytes were suspended in ice-cold Hanks buffer (8 g NaCl, 0.4 g KCl, 0.14 g CaCl2, 0.213 g MgCl2, 6H2O, 0.205 g MgSO4, 7H2O, 0.078 NaH2PO4, 0.151 g Na2HPO4, 0.35 g NaHCO3, and 0.9 g glucose per liter of distilled water), oxygenated with 95% O2–5% CO2, pH 7.4. Viability was assessed by trypan blue exclusion, and only hepatocytes with greater than 90% viability were used for experiments.

Isolated hepatocytes were suspended in the incubation buffer at a final cellular protein concentration of approximately 2 mg/ml. After preincubation at 37°C for 10 min, uptake was initiated by the addition of radiolabeled substrate (tracer experiments) or by the addition of an aliquot of hepatocyte suspension to the prewarmed (37°C) buffer solutions of CLC, containing a wide range of CLC concentrations (0.1 to 100 μM). One hundred-microliter aliquots of cell suspension were removed at 20, 40, 60, and 120 sec and 15 and 30 min and were transferred to 400-μl microcentrifuge
tories, Richmond, Calif.). Bovine serum albumin was used as the standard.

All experiments were performed in three to five separate liver-cell preparations. Statistical significance was analyzed by Student’s t test.

RESULTS AND DISCUSSION

The time course of tracer CLC uptake into the isolated rat hepatocytes is presented in Fig. 1. The uptake rate of CLC is linear up to 60 sec. Incubation of hepatocytes at a low temperature (0°C) dramatically decreased the uptake of CLC. When the incubation time exceeds 60 sec, the transport of CLC is no longer linear (Fig. 1b). Therefore, the values of \( V_0 \) were estimated from the slope of the linear regression line obtained during the 60-sec incubation period with simultaneous correction for the nonspecific binding to the cell surface ("zero-time" uptake). The relationship between the \( V_0 \) and the substrate concentration, presented as an Eadie–Hofstee plot (Fig. 2), shows that the uptake of CLC is a saturable process. The \( K_m \) and \( V_{max} \) values for that process were 28.0 ± 5.7 \( \mu M \) and 1670 ± 200 pmol/min/mg, respectively.

Equilibrium binding experiments were performed with rat liver 100,000g cytosols (Fig. 3), to determine the binding parameters of CLC to its specific intracellular binder, tubu-

![Fig. 1. Time course of the uptake of \(^3\)H-colchicine by isolated rat hepatocytes. (a) The tracer CLC concentration was used. The uptake was measured at 37 and 0°C and was expressed as the cell-to-medium concentration ratio. (b) Concentration-dependent uptake of CLC. Each point and vertical bar represent the mean ± SE of three to five independent experiments. The uptake is expressed as the amount taken up per milligram of protein.](image-url)