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Microtubule-active drugs suppress the closure of the permeability transition pore in tumour mitochondria

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Abstract We report the effects of anticancer drugs, inhibitors of microtubule organisation, on the mitochondrial permeability transition pore (PTP) in Ehrlich ascites tumour cells. Taxol (5–20 μM) and colchicine (100–500 μM) prevented closing of the cyclosporin A-sensitive PTP. No taxol or colchicine effects on oxidative phosphorylation were observed in the range of concentrations used. We suggest that either membrane-bound tubulin per se can be part of PTP and/or the attachment of mitochondria to the microtubular network is essential for PTP regulation. The taxol inhibition of PTP closure, mediated through interaction with the cytoskeleton, sheds new light on the cytotoxic properties of this anticancer drug.

Key words: Permeability transition pore; Mitochondria; Taxol; Colchicine; Cyclosporin A; Calcium; Tumour cell

1. Introduction

Ca^{2+}-induced permeability transition of the inner mitochondrial membrane is characterised by a Ca^{2+}-induced increase in the membrane permeability for small ions and molecules due to the opening of a large transmembrane pore (permeability transition pore, PTP). As a result, the accumulated cations, in part Ca^{2+} ions, are released from the matrix compartment (reviewed in [1,2]). Usually, PTP appears in mitochondria after Ca^{2+} accumulation above a critical level. This can be prevented by a number of natural agents such as adenine nucleotides [3] and polyamines [4], and can also be selectively blocked by the immunosuppressive drug cyclosporin A (CysA) [5]. The permeability transition phenomenon was observed in isolated mitochondria of normal differentiated cells [1,2]. Mitochondria of tumour cells were found to accumulate and retain a large amount of Ca^{2+} without permeability transition [6–8]. Our recent studies have shown that (i) the CysA-sensitive PTP opening can easily occur in mitochondria of Ehrlich ascites tumour cells (EATC) in situ, and (ii) the Ca^{2+}-induced permeability transition is spontaneously reversible in this type of cells [9–11].

Inasmuch as mitochondria in situ are connected with cytoskeleton elements [12,13], the question arises whether the cytoskeleton rearrangement has any effects on the mitochondrial functions. This question is of special interest because recent studies give evidence that PTP is a key element in the process of cell death, and it has been proposed that one of the reasons for the Ca^{2+}-induced cell damage could be the disruption of cytoskeleton structures during permeability transition [14–16].

In the present paper we study the effects of cytoskeleton inhibitors on permeability transition in EATC mitochondria. It was found that the microtubule assembly-disassembly inhibitors, taxol and colchicine, are strong suppressors of restoration of low permeability of the inner mitochondrial membrane after the Ca^{2+}-induced PTP opening.

2. Material and methods

EATC cultivated in the peritoneal cavity of Swiss albino mice were harvested 7–9 days after transplantation, washed twice and resuspended in Hanks' minimal essential medium buffered with HEPES at pH 7.4, in a stock suspension containing about 10^8 cells/ml. For the experiments, 100–150 μl of cell suspension was added to 2.0 ml of the incubation medium and the cells were permeabilised by the addition of 30–40 μM digitonin. Mitochondria were isolated from the cells pretreated with digitonin according to Randall et al. [17] as described previously [18].

Rates of transmembrane calcium fluxes in permeabilised cells and in mitochondrial suspensions were determined by monitoring pCa with a Ca^{2+}-sensitive electrode and the mitochondrial membrane potential with a tetraphenylphosphonium (TTP^+)-sensitive electrode in the presence of 1 μM TTP^+. The oxidative phosphorylation rate was

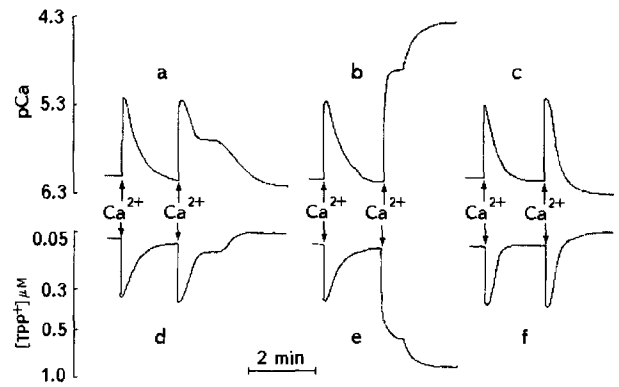

Fig. 1. Effect of taxol and cyclosporin A on the mitochondrial Ca^{2+} fluxes (curves a, b, c) and the membrane potential (curves d, e, f) in permeabilised Ehrlich ascites tumour cells (EATC). The incubation medium contained EATC (2.5 mg protein/ml), 60 mM NaCl, 5 mM KH_2PO_4, 4 mM succinate, 5 mM HEPES (pH 7.5) and (in d, e, f) 1 μM TPP^+ in addition. The cells were permeabilised by addition of 35 μM digitonin. Additions of 25 μM Ca^{2+} are shown by arrows. Taxol and CysA were added 1 min before Ca^{2+} loading. Curves a and d: no addition; curves b and e, taxol 10 μM; curves c and f, taxol 10 μM+CysA 0.4 μM.

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Abbreviations: PTP, permeability transition pore; EATC, Ehrlich ascites tumour cells; CysA, cyclosporin A; TPP^+, tetraphenylphosphonium

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monitored by outer medium alkalisation with a H^+-selective electrode as described previously [19].

The chemicals used were from Sigma, except TPP^+ (Aldrich), cyclosporin A (a gift from Sandoz, Basel, Switzerland). CysA (base) was dissolved in absolute ethanol. Ehrlich cell line (EATC) was generously provided by Dr. F. Lavelle (Rhone-Poulenc Rorer).

3. Results

Fig. 1 shows the effects of taxol on the mitochondrial Ca^{2+} fluxes and Ca^{2+}-induced change in the inner mitochondrial membrane potential ($\Delta\Psi_m$) of permeabilised EATC. After an addition of Ca^{2+} to the EATC suspension, Ca^{2+} accumulates in the mitochondrial matrix (Fig. 1a) and a transient drop in the membrane potential (Fig. 1d) takes place. It can be noticed that the Ca^{2+} influx rate decreases after the second Ca^{2+} pulse, due to transitory PTP opening during Ca^{2+} accumulation. In the presence of taxol after the first Ca^{2+} pulse, the Ca^{2+} influx and $\Delta\Psi_m$ response do not change significantly. However, after the second Ca^{2+} pulse, Ca^{2+} accumulation in mitochondria is no longer observed and Ca^{2+} efflux from the mitochondrial matrix occurs (Fig. 1b). Under these conditions, the membrane potential collapses (Fig. 1e).

It should be noted that in the presence of a PTP blocker, CysA, the effect of taxol is eliminated, and full Ca^{2+} accumulation and $\Delta\Psi_m$ restoration take place (Fig. 1c,f). These effects indicate that, under the conditions used, when PTP is opened in the inner mitochondrial membrane taxol prevents its closing.

The effects of taxol and colchicine on PTP closing and the oxidative phosphorylation rate of isolated EATC mitochondria were also tested. The rate of ADP phosphorylation was evaluated on the basis of the $\cdot H^+$ consumption rate from the outer medium (Fig. 2a,b,c) and $\Delta\Psi_m$ dissipation was used as an indicator of PTP opening (Fig. 2d,e,f).

Experiments on the isolated mitochondria have shown that neither taxol (Fig. 2c) nor colchicine (Fig. 2b) affects the ADP phosphorylation rate measured before addition of Ca^{2+}. However, both inhibitors suppress the membrane potential restoration after the Ca^{2+} loading (Fig. 2e,f). Thus, these inhibitors prevent PTP closing in both permeabilised EATC and isolated mitochondria.

Fig. 3 illustrates the rates of Ca^{2+} fluxes in the permeabilised EATC as a function of taxol and colchicine concentrations. This figure shows that, when the taxol concentration increases, the Ca^{2+} influx is suppressed whereas the Ca^{2+} efflux increases. A half-maximal effect of taxol on PTP closing was observed at 12 μM (Fig. 3, curve 1). Colchicine has a similar inhibitory effect on PTP closing and on mitochondrial Ca^{2+} fluxes, with a half-maximal effect at approximately 0.25 mM (Fig. 3, curve 2). Before the addition of Ca^{2+} no effect of either taxol or colchicine on the oxidative phosphorylation rate is observed in the tested concentration ranges (Fig. 3, insert).

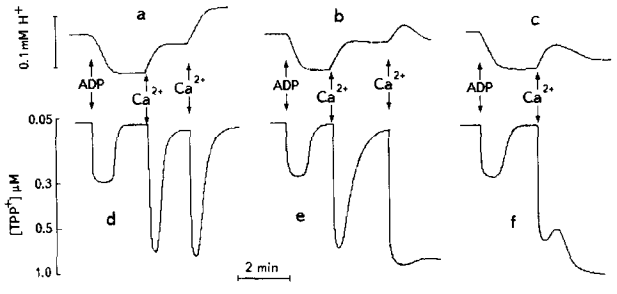

Fig. 2. Effects of taxol and colchicine on ADP and Ca^{2+}-induced H^+ consumption/production (curves a, b, c) and membrane potential response (curves d, e, f) in isolated mitochondria from EATC. The incubation medium contained 100 mM KCl, 4 mM KH_2PO_4, 5 mM succinate, 2 mM HEPES (pH 7.4), 1 μM TPP^+ and mitochondria 0.7 mg protein/ml. Additions of 100 μM ADP and 50 μM $CaCl_2$ are shown by arrows. 20 μM taxol (curves c and f) or 0.5 mM colchicine (curves b and e) were added 1 min before ADP. Curves a and d, no addition.

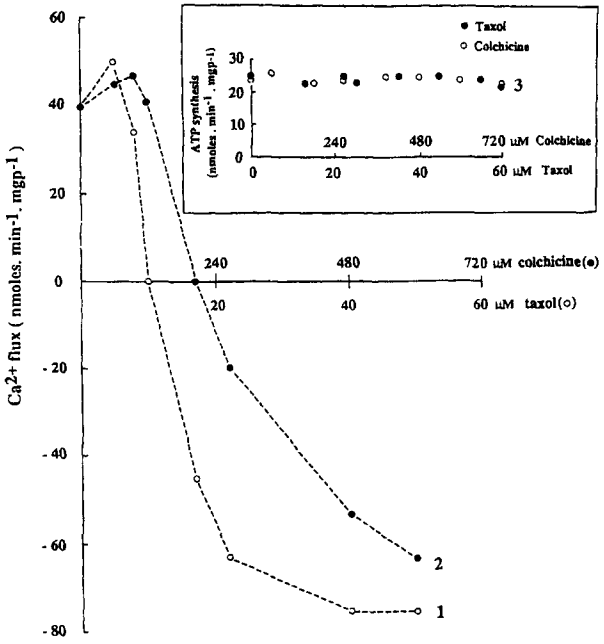

Fig. 3. Rate of the mitochondrial Ca^{2+} fluxes in EATC as a function of taxol and colchicine concentrations. The experimental conditions correspond to those in Fig. 1: curve 1 (○), taxol titration and curve 2 (●), colchicine titration. The positive values of flux correspond to Ca^{2+} influx and negative values to Ca^{2+} efflux. Insert: ADP phosphorylation in the presence of taxol (○) or colchicine (●) before addition of Ca^{2+}.

The most evident effect of taxol as an agent preventing PTP closing is shown in Fig. 4. After the addition of an overloading amount of Ca^{2+} to the isolated mitochondria, the Ca^{2+} efflux from the matrix space takes place (Fig. 4a) and the membrane potential drops to zero (Fig. 4d). Under these conditions, addition of ADP, which is an effective PTP blocker [3], results in Ca^{2+} reaccumulation and partial $\Delta\Psi_m$ restoration (Fig. 4b,e). The second Ca^{2+} pulse is followed by full Ca^{2+} accumulation in mitochondria (Fig. 4b,e). However, the addition of taxol before ADP prevents Ca^{2+} reaccumulation, $\Delta\Psi_m$ restoration and PTP closing (Fig. 4c,f).

4. Discussion

We have shown that the antitumour drugs taxol and colchicine, which are inhibitors of microtubule organisation, can affect the mitochondrial PTP state and Ca^{2+} fluxes in permeabilised EATC and in isolated mitochondria. Both inhibitors strongly suppress the PTP closure in tumour mitochondria.

The association of microtubules with mitochondria was already reported by Bernier at al. [20] and Leterrier et al. [13]. They demonstrated the existence of membrane-bound tubulin

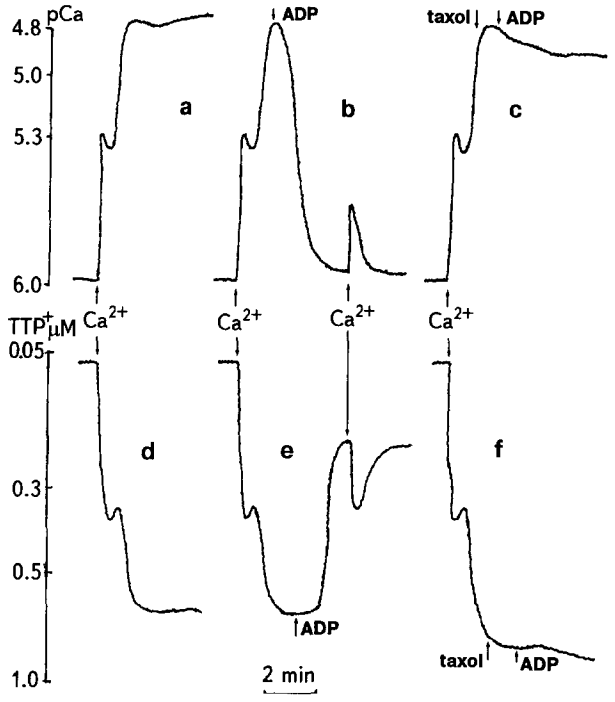

Fig. 4. Prevention by taxol of ADP-dependent Ca^{2+} reaccumulation and membrane potential restoration in EATC mitochondria. Experimental conditions correspond to those in Fig. 2. 10 μM taxol, 25 μM ADP were added at arrows; first addition of Ca^{2+} was 125 μM $CaCl_2$ and the second addition was 25 μM $CaCl_2$. In experiments a, b and c, TPP^+ was omitted. μM ADP addition.

in mitochondria and interaction of microtubule-associated proteins with mitochondrial membranes in vitro. However, the functions of membrane-bound tubulin in mitochondria are not quite clear yet. Our data suggest that either the membrane-bound tubulin per se can be part of PTP or mitochondrial attachment to the microtubular network is an essential feature of PTP regulation.

Since colchicine induces tubulin depolymerisation and taxol stabilises microtubules, we conclude that the whole cycle of tubulin polymerisation-depolymerisation is required for the PTP closure to occur. The reasons for the taxol and colchicine effects on the PTP state could be their interaction with microtubules or with membrane-bound tubulin. The tubulin-mediated PTP regulation can be involved in Ca^{2+} redistribution between cytosol, mitochondria and other intracellular Ca^{2+} stores, in part through luminal communication of intercellular calcium stores as shown by Hainoczky et al. [21].

Our results also support the idea of Hoek et al. [14] that the mitochondria-cytoskeleton interaction during permeability transition can be an essential step in cell death. So, taxol inhibition of PTP closing, mediated through interaction with the cytoskeleton, sheds new light on the cytotoxic properties of this anticancer drug.

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