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Taxol (paclitaxel) induces a detachment of phosphofructokinase from cytoskeleton of melanoma cells and decreases the levels of glucose 1,6-bisphosphate, fructose 1,6-bisphosphate and ATP

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Abstract

Glucose utilization through glycolysis, which is the primary energy source in cancer cells, is known to be controlled by allosteric regulators, as well as by reversible binding of glycolytic enzymes to cytoskeleton. Here we report of a novel mechanism of action of taxol (paclitaxel; Baccatin III *N*-benzyl-β-phenylisoserine ester), the anti-microtubule agent with remarkable anticancer activity. We show that taxol affects both levels of regulation of glycolysis in melanoma cells; it decreases the levels of glucose 1,6-bisphosphate and fructose 1,6-bisphosphate, the two allosteric stimulatory signal molecules of glycolysis, and also causes a detachment of phosphofructokinase (ATP: D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11), the rate-limiting enzyme of glycolysis, from the cytoskeleton of B16 melanoma cells. These effects of taxol were dose-dependent, and preceded the decrease in ATP levels and cell viability. Thus, taxol not only inhibits the essential dynamic processes of microtubule network, but also reduces glycolysis, through the novel mechanisms described here.

Keywords: Taxol (paclitaxel); Glycolysis; Melanoma; Cytoskeleton; Glucose 1,6-bisphosphate; Fructose 1,6-bisphosphate

1. Introduction

Cancer cells are characterized by a high rate of glycolysis, and glucose utilization through glycolysis is their primary energy source (Eigenbrodt et al., 1985; Fiechter and Gmünder, 1989; Beckner et al., 1990; Greiner et al., 1994). Glycolysis is known to be controlled by allosteric regulators such as glucose 1,6-bisphosphate and fructose 1,6-bisphosphate (for reviews, see Beitner, 1979, 1984, 1985, 1990), as well as by reversible binding of the glycolytic enzymes to cytoskeleton (Arnold and Pette, 1968; for reviews, see Clarke et al., 1985; Beitner, 1993; Pagliaro, 1993; Bereiter-Hahn et al., 1997). All glycolytic enzymes bind to cytoskeleton except hexokinase, which binds reversibly to mitochondria, where it is linked to oxidative phosphorylation (Gots et al., 1972; Gots and Bessman, 1974; Viitanen et al., 1984; Mohan et al., 1989; Adams et al., 1991).

We have previously found that calmodulin antagonists, and especially clotrimazole and bifonazole (the antifungal drugs with calmodulin antagonistic activity), are most effective in treatment of melanoma. They act by reducing energy metabolism in different cellular compartments, which eventually leads to melanoma cell death (Glass-Marmor et al., 1996; Glass-Marmor and Beitner, 1997; Penso and Beitner, 1998; for review, see Beitner, 1998). Based on these experiments, we postulated that this may be a general mechanism of action of anticancer drugs. In the present experiments, we studied whether a reduction in energy metabolism is involved in the mechanism of action of taxol (paclitaxel; Baccatin III *N*-benzyl-β-phenylisoserine ester). Taxol is a novel anti-microtubule agent with remarkable anticancer activity (for reviews, see Rowinsky and Donehower, 1991; Foa et al., 1994). We studied here the effect of taxol in B16 melanoma cells on the levels of glucose 1,6-bisphosphate and fructose 1,6-bisphosphate, the two allosteric stimulatory signal molecules of glycolysis. We also investigated the effect of taxol on cyto-

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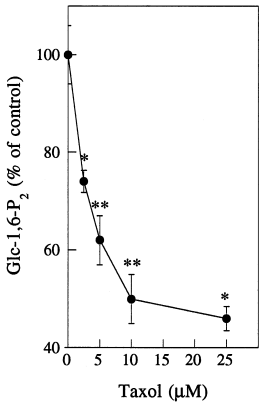

Fig. 1. Dose–response curve of the effect of taxol on glucose 1,6-bisphosphate (Glc-1,6-P_2) levels in B16 melanoma cells. Cells were incubated for 90 min with different concentrations of taxol. The solvent was added to the control in the appropriate concentration. 100% Glc-1,6-P_2 refers to 1.65 ± 0.09 (nmol/mg protein). Each point is the mean $\pm$ S.E.M of 2–3 experiments, which were performed in triplicate. $^{*}P < 0.005$ $^{**}P < 0.05$ (each point vs. the appropriate control).

skeleton-bound phosphofructokinase (ATP: D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11), the rate-limiting enzyme of glycolysis, ATP content and cell viability in B16 melanoma cells.

2. Materials and methods

2.1. Materials

Taxol (paclitaxel), was obtained from Calbiochem. Other chemicals and enzymes were either from Sigma or from Boehringer Mannheim. Tissue culture reagents were purchased from Biological Industries, Beit Haemek, Israel.

2.2. Cell culture

B16 F10 mouse melanoma cells were grown in RPMI-1640 medium supplemented with 10% fetal calf serum and antibiotics, at 37°C in humidified atmosphere at 5% CO_2 and 95% air. Cells were passaged two to three times weekly.

2.3. Treatment of culture

Melanoma cells (8×10^5 cell ml^{-1}) were seeded in tissue culture plates (10 cm). After 48 h, cells were washed twice with phosphate buffer saline (PBS). Then the cells were incubated at 37°C in PBS containing 5 mM glucose in the absence and presence of taxol at different concentrations. Taxol was dissolved in 50% polyoxyethylated castor oil (Cremophor EL), and 50% dehydrated alcohol. Taxol was further diluted in PBS before administration. As it was shown by others (Nygren et al., 1995), we also found that the solvent had an effect on our parameters, and therefore, the solvent was added to the controls at the appropriate concentrations.

2.4. Extraction and determination of glucose 1,6-bisphosphate, fructose 1,6-bisphosphate and ATP

Glucose 1,6-bisphosphate, fructose 1,6-bisphosphate and ATP were extracted from B16 melanoma cells, as described previously (Glass-Marmor et al., 1996). Glucose 1,6-bisphosphate was measured by the fluorometric method of Passonneau et al. (1969); fructose 1,6-bisphosphate and ATP were measured by the method of Lowry et al. (1964).

2.5. Separation and assay of bound and soluble phosphofructokinase

Separation of cytoskeleton-bound and soluble phosphofructokinase from B16 melanoma cells was described previously (Glass-Marmor and Beitner, 1997). Cytoskeleton-bound phosphofructokinase was assayed as described previously (Lilling and Beitner, 1990).

2.6. Cell viability determination

After incubation in absence and presence of taxol, the cells were harvested with trypsin (0.25%)-EDTA (0.05%) and centrifuged for 10 min at 270 g. The precipitated cells were suspended in PBS and counted in a hemocytometer (Neubauer). Cell viability was determined by trypan blue dye exclusion.

2.7. Protein measurement

Protein was measured by the method of Bradford (1976) with crystalline bovine serum albumin as a standard.

3. Results

The results presented in Fig. 1 show the effect of taxol on glucose 1,6-bisphosphate levels in B16 melanoma cells.

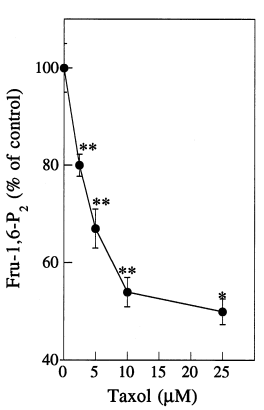

Fig. 2. Dose–response curve of the effect of taxol on fructose 1,6-bisphosphate (Fru-1,6-P_2) levels in B16 melanoma cells. Cells were incubated for 90 min with different concentrations of taxol. The solvent was added to the control in the appropriate concentration. 100% Fru-1,6-P_2 refers to 14.5 ± 0.3 (nmol/mg protein). Each point is the mean $\pm$ S.E.M of 2–3 experiments which were performed in triplicate. $^{*}P < 0.005$ $^{**}P < 0.05$ (each point vs. the appropriate control).

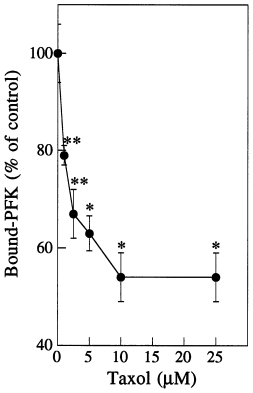

Fig. 3. Dose–response curve of the effect of taxol on cytoskeleton-bound phosphofructokinase (PFK) in B16 melanoma cells. Cells were incubated for 90 min with different concentrations of taxol. The solvent was added to the control in the appropriate concentration. 100% activity of bound phosphofructokinase was 70 ± 5 (mU/mg protein). Each point is the mean $\pm$ S.E.M of 2–3 experiments which were performed in triplicate. $^{*}P < 0.005$ $^{**}P < 0.05$ (each point vs. the appropriate control).

It can be seen that taxol induced a dose-dependent decrease in the levels of glucose 1,6-bisphosphate. Similarly, as shown in Fig. 2, taxol also reduced the levels of fructose 1,6-bisphosphate in melanoma cells, in a concentration-dependent manner.

Fig. 3 shows that taxol also exerted a dose-dependent decrease in cytoskeleton-bound phosphofructokinase in the melanoma cells. Phosphofructokinase activity was assayed in these experiments under maximal (optimal) conditions (pH 8.2), in which the enzyme is not sensitive to allosteric effectors (Beitner et al., 1978). Therefore, changes in the levels of allosteric regulators would not be expressed in its activity. Taxol induced a concentration-dependent reduction in cell viability (Fig. 4). The results presented in Fig. 5 show that the taxol-induced decrease in the levels of glucose 1,6-bisphosphate, fructose 1,6-bisphosphate, and cytoskeleton-bound phosphofructokinase preceded the decrease in cell viability. Fig. 5 also reveals that there was a close relationship between ATP levels and cell viability: taxol at low concentration (2.5 μM) had no effect on ATP levels and cell viability, whereas at a concentration of 25 μM, it induced a drop in ATP levels by 25%, and about 50% reduction in viable cells. The reduction in cell viability results most probably from the combined action of taxol on microtubule network and inhibition of glycolysis.

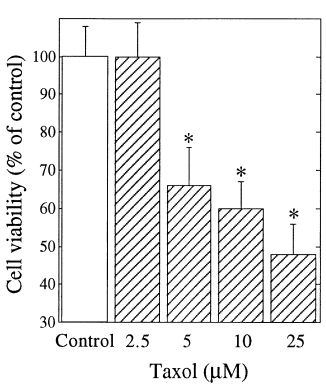

Fig. 4. Effect of taxol on cell viability of B16 melanoma cells. Cells were incubated for 90 min with different concentrations of taxol. The solvent was added to the control in the appropriate concentration. 100% cell viability refers to 5×10^6 cell ml^{-1}. Each point is the mean $\pm$ S.E.M of 2–3 experiments which were performed in triplicate. $^{*}P < 0.05$ (each point vs. the appropriate control).

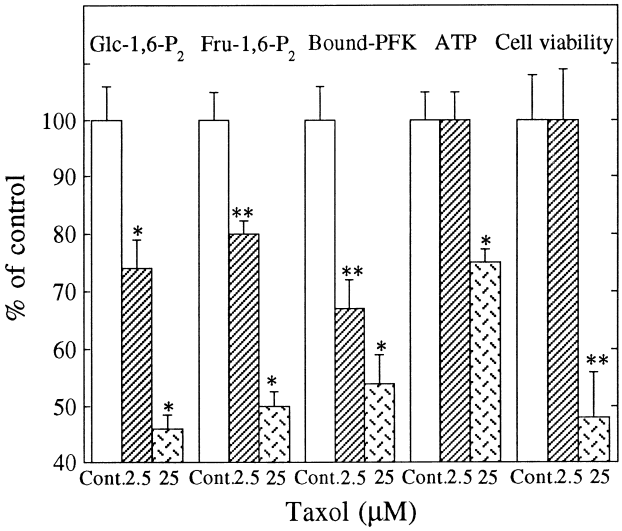

Fig. 5. Effect of taxol on cell viability and its relation to the levels of glucose 1,6-bisphosphate (Glc-1,6-P_2), Fructose 1,6-bisphosphate (Fru-1,6-P_2), the binding of phosphofructokinase (PFK) to cytoskeleton and ATP in B16 melanoma cells. Cells were incubated in the absence and presence of 2.5 μM and 25 μM taxol for 90 min. 100% cell viability refers to 5×10^6 cell ml^{-1}. 100% Glc-1,6-P_2, Fru-1,6-P_2 and ATP levels refer to 1.65 ± 0.09, 14.5 ± 0.3, and 43.5 ± 2.1 (nmol/mg protein), respectively. 100% activity of bound PFK refers to 70 ± 5 (mU/mg protein). Values are the mean $\pm$ S.E.M of 2–3 separate experiments which were performed in triplicate. $^{*}P < 0.005$ $^{**}P < 0.05$ (each point vs. the appropriate control).

4. Discussion

The present results reveal that taxol induced in B16 melanoma cells a dose-dependent decrease in the levels of glucose 1,6-bisphosphate (Fig. 1), which is the chief allosteric regulator of cytosolic glycolysis in extrahepatic tissues (for reviews, see Beitner, 1979, 1984, 1985, 1990, 1993). Glucose 1,6-bisphosphate is a potent allosteric activator of phosphofructokinase, the rate-limiting enzyme of glycolysis (Hofer and Pette, 1968; Beitner, 1979). The taxol-induced decrease in glucose 1,6-bisphosphate, leads to a reduction in the activity of phosphofructokinase, as reflected here by the fall in fructose 1,6-bisphosphate (Fig. 2), which is the product of phosphofructokinase reaction and also an allosteric activator of this enzyme. Both glucose 1,6-bisphosphate and fructose 1,6-bisphosphate are also allosteric activators of pyruvate kinase, another key enzyme in glycolysis (for reviews, see Beitner, 1979, 1985). The decrease in both these allosteric stimulatory

signal molecules of glycolysis, induced by taxol, would lead to a reduction in ATP production through cytosolic glycolysis.

In addition to inducing a reduction in cytosolic glycolysis, taxol also reduced cytoskeletal glycolysis by causing a detachment of phosphofructokinase from cytoskeleton of melanoma cells (Fig. 3). This would reduce the provision of local ATP in the vicinity of the cytoskeleton-membrane, which is required for the dynamic changes of the cell (Beitner, 1993). Glycolytic enzymes were reported to bind not only to actin filaments, but also to tubulin/microtubules (Walsh et al., 1989). The detachment of phosphofructokinase from cytoskeleton induced by taxol (Fig. 3), results most probably from its antimicrotubule activity (taxol promotes assembly of microtubules and inhibits tubulin disassembly).

The taxol-induced decrease in cytoskeletal phosphofructokinase and in the levels of the two allosteric stimulatory signal molecules of glycolysis preceded the reduction in cell viability (Fig. 5). The results indicate that these changes are early events, and not a result of cell death.

The present results reveal a novel mechanism of action of taxol: taxol not only inhibits the essential dynamic processes of the microtubule network, but also reduces glycolysis, the primary energy source of cancer cells.

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