ELSEVIER

European Journal of Pharmacology 313 (1996) 265–271

ejp

Calmodulin antagonists decrease glucose 1,6-bisphosphate, fructose 1,6-bisphosphate, ATP and viability of melanoma cells

Lea Glass-Marmor, Haya Morgenstern, Rivka Beitner *

Health Sciences Research Center, Department of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel

Received 25 March 1996; revised 21 June 1996; accepted 28 June 1996

Abstract

Glycolysis is known to be the primary energy source in cancer cells. We investigated here the effect of four different calmodulin antagonists: thioridazine (10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylthiophenothiazine), CGS 9343B (1,3-dihydro-1-[1-[(4-methyl-4*H*,6*H*-pyrrolo[1,2-*a*][4,1]-benzoxazepin-4-yl)methyl]-4-piperidinyl]-2*H*-benzimidazol-2-one (1 : 1) maleate), clotrimazole (1-(α-2-chlorotrityl)imidazole) and bifonazole (1-(α-biphenyl-4-ylbenzyl)imidazole), on the levels of glucose 1,6-bisphosphate and fructose 1,6-bisphosphate, the two stimulatory signal molecules of glycolysis, and on ATP content and cell viability in B16 melanoma cells. We found that all four substances significantly reduced the levels of glucose 1,6-bisphosphate, fructose 1,6-bisphosphate and ATP, in a dose- and time-dependent manner. Cell viability was reduced in a close correlation with the fall in ATP. The decrease in glucose 1,6-bisphosphate and fructose 1,6-bisphosphate did not result from the cytotoxic effects of the calmodulin antagonists, since their content was already reduced before any cytotoxic effect was observed. These findings suggest that the fall in the levels of the two signal molecules of glycolysis, induced by the calmodulin antagonists, causes a reduction in glycolysis and ATP levels, which eventually leads to cell death. Since cell proliferation was also reported to be inhibited by calmodulin antagonists, these substances are most promising agents in treatment of cancer by inhibiting both cell proliferation and the glycolytic supply of ATP required for cell growth.

Keywords: Calmodulin antagonist; Glucose 1,6-bisphosphate (glucose-1,6-P_2, Glc-1,6-P_2); Fructose 1,6-bisphosphate (fructose-1,6-P_2, Fru-1,6-P_2); Glycolysis; Melanoma; ATP

1. Introduction

Malignant cells are known to exhibit a high rate of glycolysis even under aerobic conditions. Increased glucose transport and enhanced activities of all three key glycolytic enzymes, namely, hexokinase (predominantly the mitochondrially bound form), phosphofructokinase and pyruvate kinase ('tumor type', type M_2), ensure a high glycolytic capacity (for review, see Eigenbrodt et al., 1985). Glycolysis was found to be the primary energy source in tumor cells, exceeding the capacity of mitochondrial oxidative energy metabolism (Eigenbrodt et al., 1985; Beckner et al., 1990; Fiechter and Gmünder, 1989; Greiner et al., 1994).

Previous studies from our laboratory have revealed that the growth-promoting hormones, insulin and growth factors, stimulate glycolysis by increasing the binding of glycolytic enzymes to cytoskeleton (a rapid and transient effect) and by raising the level of glucose 1,6-bisphosphate (a slower and consistent effect), with a concomitant gradual activation of cytosolic phosphofructokinase, the rate-limiting enzyme of glycolysis (Beitner, 1979, 1990, 1993; Chen-Zion et al., 1992; Livnat et al., 1993, 1994, 1995). The involvement of glucose 1,6-bisphosphate in the mechanism of insulin action was also found in other laboratories (Bauer et al., 1986; Carreras et al., 1988; Katz et al., 1989). Glucose 1,6-bisphosphate is a signal molecule which acts as a potent activator of phosphofructokinase (Hofer and Pette, 1968; Beitner et al., 1978; Beitner, 1985, 1990) and also regulates other key enzymes of glucose metabolism (for reviews, see Beitner, 1979, 1984, 1985, 1990, 1993). Insulin also stimulates binding of hexokinase to mitochondria (Bessman, 1966; Viitanen et al., 1984; Mohan et al., 1989; Chen-Zion et al., 1992), which could be mimicked by Ca^{2+} ions and Ca^{2+}-mobilizing hormones (Bassukevitz et al., 1992). Our experiments have also revealed that all these stimulatory effects of insulin and growth factors on glucose 1,6-bisphosphate levels and glycolysis could be prevented by treatment with calmod-

* Corresponding author. Tel.: 972-3-5318224; fax: 972-3-5351824.

PII S0014-2999(96)00526-2

ulin antagonists (Beitner, 1993; Chen-Zion et al., 1992, 1993; Bassukevitz et al., 1992; Livnat et al., 1993, 1994, 1995). These results strongly suggest that Ca^{2+}/calmodulin is involved in the stimulatory action of insulin and growth factors on glycolysis, which supplies energy for cell growth. Calmodulin is a multifunctional Ca^{2+} binding protein that has been implicated in the regulation of numerous cellular events, including that of normal and abnormal cell proliferation (Veigl et al., 1984; Hait and Lazo, 1986; Rasmussen and Means, 1987; Reddy, 1994). Calmodulin antagonists were reported to inhibit cellular proliferation of various cells (Hait and Lee, 1985; Ford et al., 1989; Susuki et al., 1986; Mac Neil et al., 1993; Hait et al., 1994), including melanoma (Ito and Hidaka, 1983; Mac Neil et al., 1984; Al-Ani et al., 1988).

Based on all these findings we reasoned that calmodulin antagonists may reduce glycolysis in melanoma cells, thereby decreasing the ATP supply required for the cancer cell proliferation. In the present research we investigated the effects of different calmodulin antagonists on the levels of the two signal molecules of glycolysis, glucose 1,6-bisphosphate and fructose 1,6-bisphosphate, in B16 melanoma cells in culture. Fructose 1,6-bisphosphate is the product of phosphofructokinase reaction, and its levels are elevated in cancer cells (Eigenbrodt et al., 1985). Fructose 1,6-bisphosphate is also, similarly to glucose 1,6-bisphosphate, an allosteric activator of phosphofructokinase and pyruvate kinase. We also studied here the effects of calmodulin antagonists on the concentration of ATP and on the viability of B16 melanoma cells. From the constantly growing list of calmodulin antagonists, we have chosen the following drugs: thioridazine (10-[2-(1-methyl-2-piperidyl)ethyl]-2-methylthiophenothiazine), an antipsychotic phenothiazine, clotrimazole (1-(α-2-chlorotrityl)imidazole) and bifonazole (1-(α-biphenyl-4-ylbenzyl)imidazole), the antifungal azole derivatives, that were recently recognized as calmodulin antagonists (Hegemann et al., 1993; Mac Neil et al., 1993), and CGS 9343B (1,3-dihydro-1-[1-[(4-methyl-4*H*,6*H*-pyrrolo[1,2-*a*][4,1]-benzoxazepin-4-yl)methyl]-4-piperidinyl]-2*H*-benzimidazol-2-one (1 : 1) maleate), a potent and more selective inhibitor of calmodulin activity (Norman et al., 1987).

2. Materials and methods

2.1. Materials

Thioridazine hydrochloride was obtained from Taro Pharmaceutical Co., Haifa, Israel. Clotrimazole and bifonazole were purchased from Sigma Chemical Co. CGS 9343B was obtained from Ciba-Geigy Corporation, Summit, NJ, USA.

Other chemicals and enzymes were either from Sigma Chemical Co. or from Boerhinger 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 calmodulin antagonists for different times and concentrations. Thioridazine was dissolved in water, clotrimazole and bifonazole were dissolved in water containing acidified 6% polyethylene glycol 400. CGS 9343B was dissolved in water containing 2% ethanol. The appropriate solvents were added to the controls.

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

The cells were washed twice with ice cold PBS, placed on ice and 1.5 ml HCl 0.05 N was added to the dish. 10 min later, the cells were scraped with a rubber policeman and collected in a centrifuge tube at 0°C. Samples (10 μl) were removed for protein determination. Cells were mixed with 120 μl of 70% cold perchloric acid containing 130 mM EDTA. After 5 min the extract was neutralized with KOH. The precipitated potassium perchlorate was removed by centrifugation at 4°C for 15 min at $5000 \times g$, and the clear supernatant was used for determination of glucose 1,6-bisphosphate, fructose 1,6-bisphosphate and ATP.

2.5. Measurements of glucose 1,6-bisphosphate, fructose 1,6-bisphosphate and ATP levels

Glucose 1,6-bisphosphate was measured by the fluorometric method of Passonneau et al. (1969); glucose 1,6-bisphosphate, being a cofactor of phosphoglucomutase reaction, which was coupled with glucose-6-phosphate dehydrogenase and $NADP^+$. The buffer consisted of 50 mM imidazole-HCl, pH-7, containing 1 mM magnesium acetate, 0.1 mM EDTA, and 0.01% bovine serum albumin. First phosphoglucomutase (4 U/ml) was activated in the buffer for 1 h. The reaction mixture contained: 15 μM glucose-1-phosphate, 2 mU/ml of activated phosphoglucomutase, 0.05 mM $NADP^+$ and 1 U/ml glucose-6-phosphate dehydrogenase in 1 ml buffer. The reaction was started by adding 2–50 μl from the extract. Calculation was made from a standard of the glucose 1,6-bisphosphate between 10^{-8}–5×10^{-9} M.

Fructose 1,6-bisphosphate and ATP were measured by the method of Lowry et al. (1964).

2.6. Cell viability determination

After incubation in the absence and presence of calmodulin antagonists, the cells were harvested with trypsin (0.25%)-EDTA (0.05%) and centrifuged for 10 min at $270 \times g$. The precipitated cells were suspended in PBS and counted in a hemozitometer (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 the different calmodulin antagonists on glucose 1,6-bisphosphate levels in B16 melanoma cells. It can be seen that all four compounds decreased the levels of glucose 1,6-bisphosphate in a concentration-dependent manner. Fig. 2 shows that all four compounds also exerted a dose-dependent reduction in the levels of fructose 1,6-bisphosphate in melanoma cells. The results presented in Fig. 3 show the effects of the calmodulin inhibitors on ATP levels in the

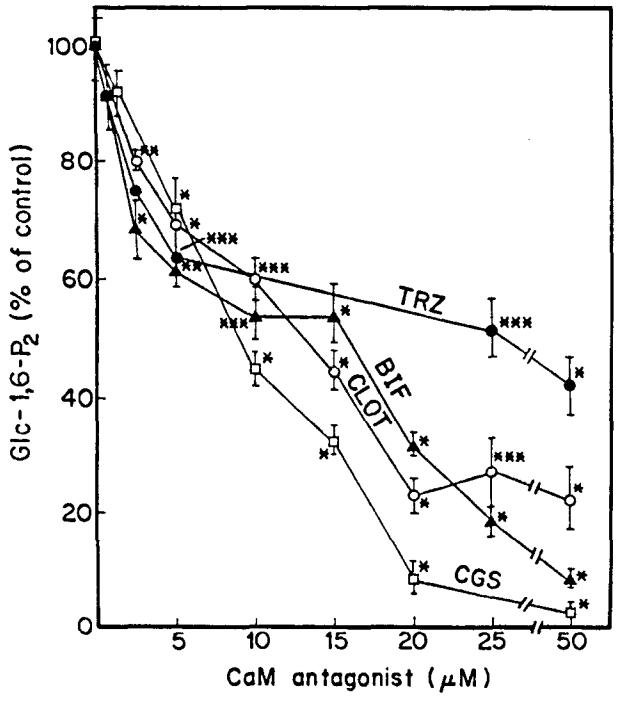

Fig. 1. Dose-response curves of the effects of the calmodulin (CaM) antagonists: thioridazine (TRZ), clotrimazole (CLOT), bifonazole (BIF) and CGS 9343B on glucose 1,6-bisphosphate (Glc-1,6-P_2) levels in B16 melanoma cells. Cells were incubated for 1 h with and without the calmodulin antagonists. 100% glucose 1,6-bisphosphate refers to 1.08 ± 0.07 (nmol/mg protein). Each point is the mean ± S.E. of 2–3 separate experiments which were performed in triplicate. * $P < 0.005$, ** $P < 0.05$, *** $P < 0.01$.

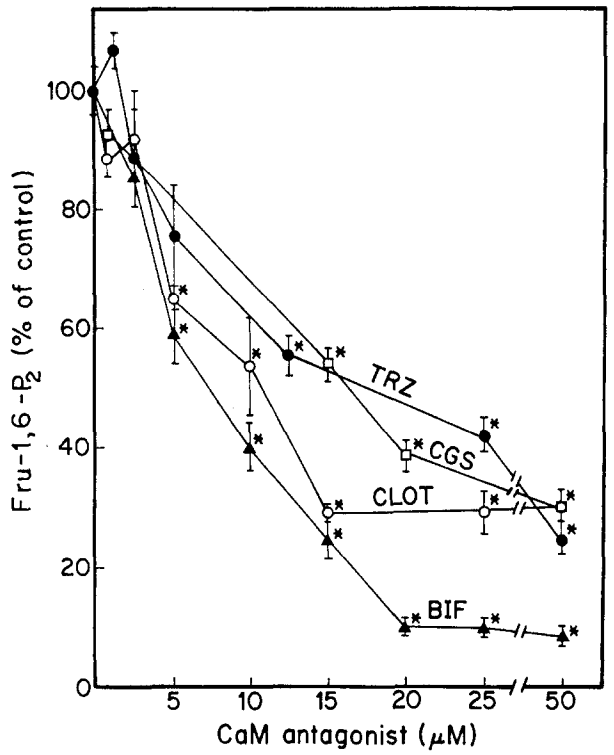

Fig. 2. Dose-response curves of the effects of the calmodulin (CaM)-antagonists: thioridazine (TRZ), clotrimazole (CLOT), bifonazole (BIF), and CGS 9343B on fructose 1,6-bisphosphate (Fru-1,6-P_2) levels in B16 melanoma cells. Cells were incubated for 1 h with and without the calmodulin antagonists. 100% fructose 1,6-bisphosphate refers to 12.73 ± 1.01 (nmol/mg protein). Each point is the mean ± S.E. of 2–3 separate experiments which were performed in triplicate. * $P < 0.005$.

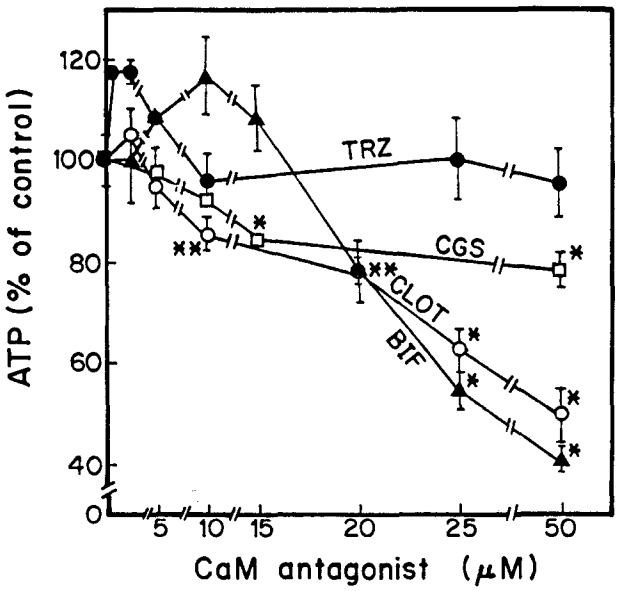

Fig. 3. Dose-response curves of the effects of the calmodulin (CaM) antagonists: thioridazine (TRZ), clotrimazole (CLOT), bifonazole (BIF), and CGS 9343B on ATP levels in B16 melanoma cells. Cells were incubated for 1 h with and without the calmodulin antagonists. 100% ATP refers to 42.5 ± 3.58 (nmol/mg protein). Each point is the mean ± S.E. of 2–3 separate experiments which were performed in triplicate. * $P < 0.005$, ** $P < 0.05$.

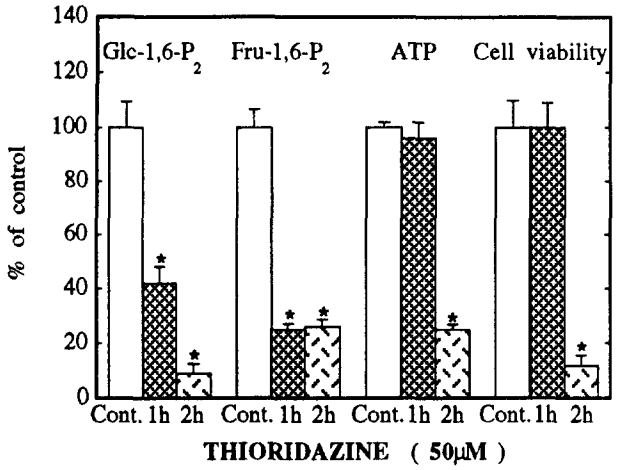

Fig. 4. Effect of thioridazine on cell viability and its relation to the levels of ATP, glucose 1,6-bisphosphate (Glc-1,6-P_2) and fructose 1,6-bisphosphate (Fru-1,6-P_2). Cells were incubated with and without 50 μM thioridazine for 1 h and 2 h. 100% cell viability refers to 4×10^6 cell ml^{-1}; 100% ATP, glucose 1,6-bisphosphate and fructose 1,6-bisphosphate levels refer to 42.5 ± 3.58, 1.08 ± 0.07, and 12.73 ± 1.01 (nmol/mg protein), respectively. Values are the means ± S.E. of 2–3 separate experiments which were performed in triplicate. * $P < 0.005$.

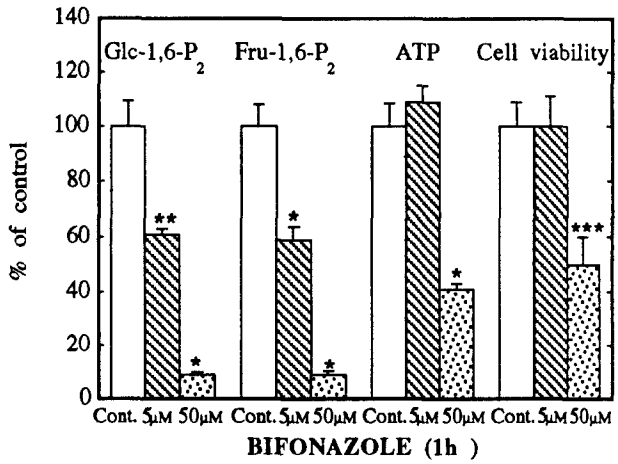

Fig. 6. Effect of bifonazole on cell viability and its relation to the levels of ATP, glucose 1,6-bisphosphate (Glc-1,6-P_2) and fructose 1,6-bisphosphate (Fru-1,6-P_2). Cells were incubated with and without 5 μM and 50 μM bifonazole for 1 h. 100% cell viability refers to 4×10^6 cell ml^{-1}; 100% ATP, glucose 1,6-bisphosphate and fructose 1,6-bisphosphate levels refer to 42.5 ± 3.58, 1.08 ± 0.07, and 12.73 ± 1.01 (nmol/mg protein), respectively. Values are the means ± S.E. of 2–3 separate experiments which were performed in triplicate. * $P < 0.005$, ** $P < 0.05$, *** $P < 0.01$.

B16 melanoma cells. It can be seen that the azole derivatives, clotrimazole and bifonazole, markedly reduced ATP levels in a concentration-dependent manner. On the other hand, thioridazine and CGS 9343B, exerted a very small reduction in ATP levels in cells incubated for 1h. Longer incubation (2 h) at high concentration (50 μM) of these compounds was required to induce a significant decrease in ATP levels (Fig. 4 and Fig. 5). There was a close relationship between ATP levels and cell viability (Fig. 4 and Fig. 5 and Fig. 6 and Fig. 7). A marked decrease in ATP by about 40% or more, induced by the calmodulin antagonists, led to a significant reduction in viable cells that resulted in marked cell detachment from culture plates.

The results presented in Fig. 4 and Fig. 5 and Fig. 6 and Fig. 7 clearly show that the decrease in the levels of glucose 1,6-bisphosphate and fructose 1,6-bisphosphate did not result from the cytotoxic effects of the calmodulin antagonists, since their concentration was already significantly reduced by all four compounds before any cytotoxic effect was observed, in 100% viable cells.

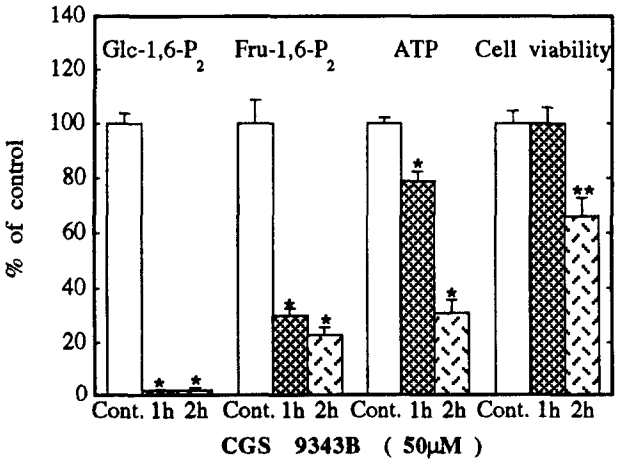

Fig. 5. Effect of CGS 9343B on cell viability and its relation to the levels of ATP, glucose 1,6-bisphosphate (Glc-1,6-P_2) and fructose 1,6-bisphosphate (Fru-1,6-P_2). Cells were incubated with and without 50 μM CGS 9343B for 1 h and 2 h. 100% cell viability refers to 4×10^6 cell ml^{-1}; 100% ATP, glucose 1,6-bisphosphate and fructose 1,6-bisphosphate levels refer to 42.5 ± 3.58, 1.08 ± 0.07, and 12.73 ± 1.01 (nmol/mg protein), respectively. Values are the means ± S.E. of 2–3 separate experiments which were performed in triplicate. * $P < 0.005$, ** $P < 0.05$.

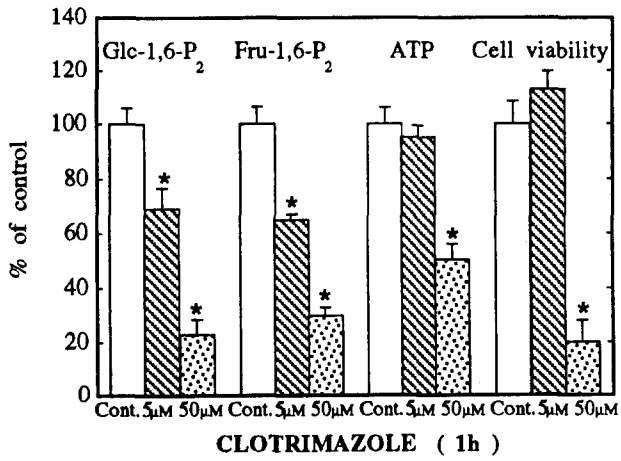

Fig. 7. Effect of clotrimazole on cell viability and its relation to the levels of ATP, glucose 1,6-bisphosphate (Glc-1,6-P_2) and fructose 1,6-bisphosphate (Fru-1,6-P_2). Cells were incubated with and without 5 μM and 50 μM clotrimazole for 1 h. 100% cell viability refers to 4×10^6 cell ml^{-1}; 100% ATP, glucose 1,6-bisphosphate and fructose 1,6-bisphosphate levels refer to 42.5 ± 3.58, 1.08 ± 0.07, and 12.73 ± 1.01 (nmol/mg protein), respectively. Values are the means ± S.E. of 2–3 separate experiments which were performed in triplicate. * $P < 0.005$.

4. Discussion

The present results (Fig. 1 and Fig. 2) reveal that the antifungal imidazole derivatives, clotrimazole and bifonazole, which were recently reported to display calmodulin antagonistic activity (Hegemann et al., 1993; Mac Neil et al., 1993), caused a significant dose-dependent decrease in the levels of glucose 1,6-bisphosphate and fructose 1,6-bisphosphate in B16 melanoma cells. Their action was similar to that of thioridazine and CGS 9343B, the more selective inhibitor of calmodulin activity (Norman et al., 1987). These results strongly suggest that all four compounds exerted their effects on the two signal molecules of glycolysis, through inhibition of Ca^{2+}/calmodulin activity. The relative potency of the different compounds in reducing glucose 1,6-bisphosphate and fructose 1,6-bisphosphate levels was similar, with a 50% inhibitory concentration (IC_{50}) of approximately 8–18 μM, which is close to their reported IC_{50} for calmodulin inhibition. (The reported IC_{50} for CGS 9343B, thioridazine, bifonazole and clotrimazole is 3.3, 18, 14 and 18.4, respectively.) Antifungal azole derivatives, like many other calmodulin antagonists, were also shown to inhibit cell proliferation (Mac Neil et al., 1993).

The results presented here (Fig. 4 and Fig. 5 and Fig. 6 and Fig. 7) also reveal that at high concentration, all four compounds were cytotoxic, markedly reducing viability of the melanoma cells. This reduction was closely related to the decrease in ATP content. Thioridazine and CGS 9343B required a longer incubation time to exert a cytotoxic effect. The greater cytotoxicity of the azole derivatives may result from their additional actions, e.g., inhibition of cytochrome P-450.

The decrease in glucose 1,6-bisphosphate and fructose 1,6-bisphosphate levels, induced by all four calmodulin antagonists, was not due to the cytotoxic effect of the drugs, because the content of the two regulatory molecules was already significantly reduced under nontoxic conditions, in 100% viable cells (Fig. 4 and Fig. 5 and Fig. 6 and Fig. 7).

Our previous experiments have suggested that Ca^{2+}/calmodulin is involved in the stimulatory action of insulin and growth factors on glucose 1,6-bisphosphate levels and glycolysis in normal cells, that could be inhibited by calmodulin antagonists (Beitner, 1993; Chen-Zion et al., 1992, 1993; Bassukevitz et al., 1992; Livnat et al., 1993, 1994, 1995). Here we show that in B16 melanoma cells, the different calmodulin antagonists markedly decreased the levels of glucose 1,6-bisphosphate, which is a potent activator of phosphofructokinase, the rate-limiting enzyme of glycolysis. The decrease in glucose 1,6-bisphosphate leads to a reduction in the activity of phosphofructokinase, as reflected here by the drastic fall in fructose 1,6-bisphosphate, 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 activators of pyruvate kinase, another key enzyme in glycolysis (for reviews, see Beitner, 1979, 1985). The reduction in glycolysis, which is the primary energy source in malignant cells, leads to the decrease in ATP induced by the calmodulin antagonists. A decrease in ATP is known to cause various cell abnormalities, since ATP is required for many energy-dependent systems (e.g., ion movement), as well as for various phosphorylation processes, and it is well known that a fall in ATP eventually leads to cell death. This is expressed here by the dose- and time-dependent reduction in the viability of B16 melanoma cells induced by the calmodulin antagonists, which correlated with the fall in ATP content.

It has recently become evident that calmodulin antagonists are a new generation of drugs with broad therapeutic applications (for review, see Mannhold and Timmerman, 1992). Calmodulin antagonists were found in our laboratory to be effective drugs in treatment of skin burns and frostbite (Beitner, 1987; Beitner et al., 1989a,b, 1991). They also attenuate skin changes induced by uv radiation (Beitner, 1987; Anthony et al., 1994).

The present results reveal a novel mechanism of action of calmodulin antagonists in B16 melanoma cells. Since Ca^{2+}/calmodulin plays a critical role in both cell proliferation and glycolysis, the primary energy source in cancer cell, calmodulin antagonists are most promising agents for treatment of cancer. The list of compounds which display calmodulin antagonistic activity is growing fast and many drugs used for treatment of different diseases are now being discovered to act as calmodulin antagonists. In addition, the results reported here suggest that a reduction in the levels of glucose 1,6-bisphosphate, fructose 1,6-bisphosphate and ATP could be employed to serve as markers for cancer cell dysfunction to evaluate therapeutic action of anti-cancer drugs.

Acknowledgements

The skillful technical assistance of Mrs H. Ben-Porat is acknowledged with thanks. This work was supported in part by the ALSAM Foundation, Los Angeles, CA, USA; the Health Sciences Research Center; and by the Research Committee, Bar-Ilan University. This paper is part of the Ph.D. thesis of L. Glass-Marmor to be submitted to the Senate of the Bar-Ilan University, Ramat Gan, Israel.

References

Al-Ani, A.M., A.G. Messenger, J. Laury, S.S. Bleehen and S. Mac Neil, 1988, Calcium/calmodulin regulation of the proliferation of human epidermal keratinocytes, dermal fibroblasts and mouse B16 melanoma cells in culture. Br. J. Dermatol. 119, 295.

Anthony, F.A., J.C. Dowdy and M.E. Costlow, 1994, Attenuation of ultraviolet radiation-induced edema and erythema with topical calmodulin and protein kinase C inhibitors, Photodermatol. Photoimmunol. Photomed. 10, 227.

Bassukevitz, Y., M. Chen-Zion and R. Beitner, 1992, Ca^{2+}-ionophore A23187 and the Ca^{2+}-mobilizing hormones serotonin, vasopressin, and bradykinin increase mitochondrially bound hexokinase in muscle, Biochem. Med. Metab. Biol. 47, 181.

Bauer, H.P., G. Birkel and H.W. Hofer, 1986, The concentration of glucose-1,6-bisphosphate and other regulatory metabolites, and the activities of enzymes of the glycogen metabolism in the perfused rabbit psoas muscle, Int. J. Biochem. 18, 73.

Beckner, M.E., M.L. Stracke, L.A. Liotta and E. Schiffmann, 1990, Glycolysis as primary energy source in tumor cell chemotaxis, J. Natl. Cancer Inst. 82, 1836.

Beitner, R. 1979, The role of glucose-1,6-diphosphate in the regulation of carbohydrate metabolism in muscle, Trends Biochem. Sci. 4, 228.

Beitner, R. 1984, Control of levels of glucose 1,6-bisphosphate, Int. J. Biochem. 16, 579.

Beitner, R. 1985, Glucose 1,6-bisphosphate - the regulator of carbohydrate metabolism, in: Regulation of Carbohydrate Metabolism, ed. R. Beitner (CRC Press, Inc., Boca Raton, FL) Vol. I, p. 1.

Beitner, R. 1987, Method and composition for the therapeutic and prophylactic treatment of trauma to the skin. U.S. Patent No. 4,654,323; No. 4,777,171 (1988) and No. 4,910,197 (1990).

Beitner, R. 1990, Regulation of carbohydrate metabolism by glucose-1,6-bisphosphate in extrahepatic tissues; comparison with fructose-2,6-bisphosphate, Int. J. Biochem. 22, 553.

Beitner, R. 1993, Control of glycolytic enzymes through binding to cell structures and by glucose 1,6-bisphosphate under different conditions. The role of Ca^{2+} and calmodulin, Int. J. Biochem. 25, 297.

Beitner, R., S. Haberman, J. Nordenberg and T.J. Cohen, 1978, The levels of cyclic GMP and glucose-1,6-diphosphate, and the activity of phosphofructokinase, in muscle from normal and dystrophic mice, Biochim. Biophys. Acta 542, 537.

Beitner, R., M. Chen-Zion, Y. Sofer-Bassukevitz, H. Morgenstern and H. Ben-Porat, 1989a, Treatment of frostbite with the calmodulin antagonists thioridazine and trifluoperazine, Gen. Pharmacol. 20, 641.

Beitner, R., M. Chen-Zion, Y. Sofer-Bassukevitz, Y. Oster, H. Ben-Porat and H. Morgenstern, 1989b, Therapeutic and prophylactic treatment of skin burns with several calmodulin antagonists, Gen. Pharmacol. 20, 165.

Beitner, R., M. Chen-Zion and Y. Bassukevitz, 1991, Effect of the calmodulin antagonist CGS 9343B on skin burns, Gen. Pharmacol. 22, 67.

Bessman, S.P. 1966, A molecular basis for the mechanism of insulin action, Am. J. Med. 40, 740.

Bradford, M.M. 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72, 248.

Carreras, M., A.M. Bassols, J. Carreras and F. Climent, 1988, Effects of vanadate and insulin on glucose 1,6-P_2 and fructose-2,6-P_2 levels in rat skeletal muscle, Biochem. Int. 17, 359.

Chen-Zion, M., Y. Bassukevitz and R. Beitner, 1992, Sequence of insulin effects on cytoskeletal and cytosolic phosphofructokinase, mitochondrial hexokinase, glucose 1,6-bisphosphate and fructose 2,6-bisphosphate levels, and the antagonistic action of calmodulin inhibitors, in diaphragm muscle, Int. J. Biochem. 24, 1661.

Chen-Zion, M., G. Lilling and R. Beitner, 1993, The dual effects of Ca^{2+} on binding of the glycolytic enzymes, phosphofructokinase and aldolase, to muscle cytoskeleton, Biochem. Med. Metab. Biol. 49, 173.

Eigenbrodt, E., P. Fister and M. Reinacher, 1985, New perspectives on carbohydrate metabolism in tumor cells, in: Regulation of Carbohydrate Metabolism, ed. R. Beitner (CRC Press, Boca Raton, FL), Vol. II, p. 141.

Fiechter, A. and F.K. Gmünder, 1989, Metabolic control of glucose degradation in yeast and tumor cells, Adv. Biochem. Eng. Biotechnol. 39, 1.

Ford, J.M., W.C. Prozialeck and W.N. Hait, 1989, Structural features determining activity of phenothiazines and related drugs for inhibition of cell growth and reversal of multidrug resistance, Mol. Pharmacol. 35, 105.

Greiner, E.F., M. Guppy and K. Brand, 1994, Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production, J. Biol. Chem. 269, 31484.

Hait, W.N. and G.L. Lee, 1985, Characteristics of the cytotoxic effects of the phenothiazine class of calmodulin antagonists, Biochem. Pharmacol. 34, 3973.

Hait, W.N. and J.S. Lazo, 1986, Calmodulin: a potential target for cancer chemotherapeutic agents, J. Clin. Oncol. 4, 994.

Hait, W.N., J.F. Gesmonde and J.S. Lazo, 1994, Effect of anti-calmodulin drugs on the growth and sensitivity of C6 rat glioma cells to bleomycin, Anticancer Res. 14, 1711.

Hegemann, L., S.M. Toso, K.I. Lahijani, G.F. Webster and J. Uitto, 1993, Direct interaction of antifungal azole-derivatives with calmodulin: a possible mechanism for their therapeutic activity, J. Invest. Dermatol. 100, 343.

Hofer, H.W. and D. Pette, 1968, Wirkungen und Wechselwirkungen von Substraten und Effektoren an der Phosphofructokinase des Kaninchen-Skeletmuskels, Hoppe Seyler's Z. Physiol. Chem. 349, 1378.

Ito, H. and H. Hidaka, 1983, Antitumor effect of calmodulin antagonist against MH-134 hepatoma, *Ehrlich ascites* carcinoma and B-16 melanocarcinoma in mice, Eur. J. Cancer Clin. Oncol. 19, 1183.

Katz, A., B.L. Nyomba and C. Bogardus, 1989, Euglycemic hyperinsulinemia increases glucose-1,6-bisphosphate in human skeletal muscle, Int. J. Biochem. 21, 1079.

Livnat, T., M. Chen-Zion and R. Beitner, 1993, Stimulatory effect of epidermal growth factor on binding of glycolytic enzymes to muscle cytoskeleton and the antagonistic action of calmodulin inhibitors, Biochem. Med. Metab. Biol. 50, 24.

Livnat, T., M. Chen-Zion and R. Beitner, 1994, Platelet-derived growth factor (PDGF) rapidly stimulates binding of glycolytic enzymes to muscle cytoskeleton, prevented by calmodulin antagonists, Biochem. Med. Metab. Biol. 53, 28.

Livnat, T., M. Chen-Zion, C. Brodie and R. Beitner, 1995, Rapid stimulatory effect of insulin on binding of glycolytic enzymes to cytoskeleton of C-6 glial cells, and the antagonistic action of calmodulin inhibitors, Endocrine 3, 319.

Lowry, O.H., J.V. Passonneau, F.X. Hasselberger and D.W. Schulz, 1964, Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain, J. Biol. Chem. 239, 18.

Mac Neil, S., S.W. Walker, H.J. Senior, S.S. Bleehen and S. Tomlinson, 1984, Effects of extracellular calmodulin and calmodulin antagonists on B16 melanoma cell growth, J. Invest. Dermatol. 83, 15.

Mac Neil, S., R.A. Dawson, G. Crocker, W.F.G. Tucker, B. Bittiner, J.G. Singleton, T. Hunter and D.F. Tierney, 1993, Antiproliferative effects on keratinocytes of a range of clinically used drugs with calmodulin antagonist activity, Br. J. Dermatol. 128, 143.

Mannhold, R. and H. Timmerman, 1992, Putative therapeutic applications of calmodulin antagonists, Pharm. Weekbl. [Sci.] 14, 161.

Mohan, C., P.J. Geiger and S.P. Bessman, 1989, The intracellular site of action of insulin: the mitochondrial Krebs cycle, in: Current Topics in Cellular Regulation, eds. B.L. Horecker and E.R. Stadtman (Academic Press, New York) p. 105.

Norman, J.A., J. Ansell, G.A. Stone, L.P. Wennogle and J.W.F. Wasley, 1987, CGS 9343B, a novel, potent, and selective inhibitor of calmodulin activity, Mol. Pharmacol. 31, 535.

Passonneau, J.V., O.H. Lowry, D.W. Schulz and J.G. Brown, 1969, Glucose 1,6-diphosphate formation by phosphoglucomutase in mammalian tissues, J. Biol. Chem. 244, 902.

Rasmussen, C.D. and A.R. Means, 1987, Calmodulin is involved in regulation of cell proliferation, EMBO J. 6, 3961.

Reddy, G.P.V. 1994, Cell cycle: regulatory events in $G_1 \rightarrow S$ transition of mammalian cells, J. Cell. Biochem. 54, 379.

Susuki, N., T. Kanno, Y. Nagata and T. Kato, 1986, Inhibition of proliferative growth in glioma cells by calmodulin antagonists, J. Neurosurg. 65, 74.

Veigl, M.L., T.C. Vanaman and W.D. Sedwick, 1984, Calcium and calmodulin in cell growth and transformation, Biochim. Biophys. Acta 738, 21.

Viitanen, P.V., P.J. Geiger, S. Erickson-Viitanen and S.P. Bessman, 1984, Evidence for functional hexokinase compartmentation in rat skeletal muscle mitochondria, J. Biol. Chem. 259, 9679.