Endofacial competitive inhibition of the glucose transporter 1 activity by gossypol

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The facilitated exchange of glucose across the membranes of animal cells is mediated by members of the glucose transporter protein family (GLUT/SLC2a; reviewed in Refs. 8 and 20). These transporters have a high degree of stereoselectivity providing for the bidirectional transport of substrate down its concentration gradient. The hexose transporters function to regulate the movement of glucose between the extracellular and intracellular compartments, maintaining a constant supply of glucose available for metabolism (32). GLUT1 is the archetypical member of this family of proteins, the properties of which are being extensively studied in human erythrocytes (8, 9). GLUT1 is present at variable levels in many tissues and is believed to be responsible for basal glucose uptake (5, 12).

Extensive data demonstrate that the glucose transporters also interact with compounds that have no obvious structural similarities to glucose. These compounds include the alkaloid cytochalasin B (21), the chalcone phloretin (29), the diterpene forskolin (22, 27), steroids (25) and anti-estrogenic drugs (1), benzoic acid derivatives (26), and barbiturates (48). We also showed that a group of tyrosine kinase inhibitors that include natural products of the family of flavones and isoflavones and synthetic compounds, such as the tyrphostins, acts as potent blockers of the functional activity of the glucose transporter GLUT1 by directly interacting with the transporter (51). Gossypol is a polyphenolic binaphthyl diterpene naturally present in cottonseed, which has also been shown to block glucose transport in several systems (10, 36). The information regarding this, however, is scarce and even contradictory (17). Gossypol has been shown to exhibit antiespermatogenic, anticancer, antiparasitic, and antiviral activities (4, 30, 34, 54), which may be related to gossypol-induced glucose deprivation. In fact, gossypol as well as phloretin, the tyrphostins and other GLUT1 inhibitors, have characteristics of hydroxylated planar steroids, which may provide the structural basis for GLUTs inhibition. However, gossypol has also been shown to inactivate several protein targets, including intracellular dehydrogenases, reductases, protein kinases, protein phosphatases, steroidogenic adrenal enzymes, cathepsin L, topoisomerase II, and members of the Bcl-2 family of antiapoptotic proteins (3, 6, 14, 16, 24, 31, 58). Noncovalent enzyme-gossypol complexes have also been described for protein kinase C (14), protein kinase A (57), calcineurin (3), lactate dehydrogenase (44), and inositol kinases (31). Rapid binding of gossypol, followed by slow covalent protein modification by Schiff base formation at the NH2-terminal group, results in the inactivation of phospholipase A2 (57). Moreover, gossypol showed inhibitory activity against a wide range of human carcinoma cell lines in culture and in tumor xenograft models (13, 28, 49, 55).

Our results demonstrate that gossypol is a potent inhibitor of the glucose transporter GLUT1. We studied the effect of gossypol on transport using HL-60 cells, Chinese hamster ovary (CHO) cells overexpressing the glucose transporter GLUT1, and human erythrocytes. The characteristics and the specificity of the inhibition, and the results of studies showing that gossypol affects the glucose-displaceable binding of cy-

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tochalasin B to GLUT1 in erythrocyte membranes, indicate that the effect of gossypol on transport is related to direct interaction with GLUT1. Biophysical and kinetic assays provide evidence that there are functionally distinguishable binding sites for phloretin, flavones, and gossypol, and that gossypol interacts with the GLUT1 transporter in a site accessible through the endofacial surface of the protein.

**MATERIALS AND METHODS**

Materials and solutions. Gossypol-acetic acid, apogossypol hexaacetate, nicotinamide, and D-glucose were obtained from Sigma Chemical. Human erythrocytes were purified from freshly outdated blood samples obtained from the Blood Bank Service of the Regional Hospital in Valdivia.

Cell culture of HL-60 and CHO cells. Cells were cultured in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% fetal bovine serum and antibiotics. Cell viability was >95%, as determined by Trypan blue exclusion. CHO cells expressing GLUT1 or the human placental insulin receptor (50) were cultured in IMDM supplemented with 10% fetal bovine serum and 0.25 mg/ml geneticin.

Uptake assays. Uptake assays were performed as previously described (41, 52). Briefly, cells were incubated at room temperature in incubation buffer containing 2-[1,2-3H(N)]deoxy-D-glucose (specific activity 26.2 Ci/mmol, NEN-DuPont), [3H]methylglucose (specific activity 86.7 Ci/mmol, NEN-DuPont), or t-[14C-carbonyl]nicotinamide (specific activity, 35 mCi/mmol, Sigma Chemical) and adequate concentrations of the respective unlabeled compounds for the times indicated in the figures.

Net sugar efflux experiments (Sen-Widdas assays). The exit rates of D-glucose from erythrocyte human cells were monitored photometrically using a Perkin-Elmer spectrofluorimeter LS-50 with a temperature-controlled and monitored cuvette using 650 nm as excitation and emission wavelengths (5-nm slits). Red blood cells were suspended for at least 2 h in solutions containing 100 mM D-glucose. The cells were then recentrifuged to obtain a thick suspension and were kept at 4°C until required. Aliquots of prewarmed cells suspension (3 μl) were added to a fluorescence cuvette containing 3 ml of saline solution also prewarmed at 30°C. The cell suspensions were mixed thoroughly, and photometric monitoring was started within 5 s of mixing. Data points (1,000) were collected at a rate of 0.2–2 points per second, depending on the time course of exit. With D-glucose present in the cytosol, after an initial 2- to 3-s period of cell swelling due to osmotic equilibration, the rate of shrinkage was a linear function of the rate of glucose loss from the cells. In all cases, the time courses of D-glucose exit were fitted to the time course of exit. With D-glucose present in the cytosol, after an initial 2- to 3-s period of cell swelling due to osmotic equilibration, the rate of shrinkage was a linear function of the rate of glucose loss from the cells. In all cases, the time courses of D-glucose exit were fitted to the nonlinear regression routine of SigmaPlot 8.0 software program. Inhibitors were diluted from fresh prepared stock solutions directly into the saline medium for cell dilution, in such a way that the rates of glucose exit were examined without preincubation of the cells with the inhibitors.

Fluorescence measurements. Human erythrocyte membranes stripped of peripheral proteins by alkaline treatment were prepared from washed red blood cells as previously described (41). Fluorescence measurements were performed on a Perkin-Elmer spectrofluorimeter LS-50. Protein fluorescence of stripped membranes was excited at 295 nm to monitor interference from protein tyrosine groups. Slit widths for excitation and emission monochromators were adjusted to give optimal changes without notorious bleaching of the sample. Protein concentration was typically 15 μg/ml. Additions were made directly to the cell without removing it from the cell holder, maintaining a constant rate of stirring. Dilution never exceeded 10%, and in each experiment the amount of quenching was corrected for the dilution factor. Fluorescence intensities are reported as relative values corrected for wavelength variations in detector response. Gossypol at 1 μM absorbs weakly at 295 nm (A295 = 0.023) and insignificantly over 310 nm. The sesquiterpene therefore produces apparent quenching due to attenuation of the exciting light at 295 nm but leaves emission over 310 nm unaffected. This inner filter effect was corrected as suggested (7). Fractional quenching was calculated as (F0−F)/F0, where F is the fluorescence at a given quencher concentration and F0 corresponds to the fluorescence before any addition. Plots of fractional quenching as a function of concentration were used to calculate apparent affinities (K1_app) and maximal quenching (Qmax), by nonlinear regression to simple hyperbolic functions of saturable binding to a single class of binding sites.

For iodide quenching experiments, potassium iodide was freshly prepared as a 5 M stock solution in saline containing 0.1 mM Na2S2O3 to prevent formation of iodine ion, and KCl was used to maintain a constant ionic strength. Quenching was analyzed according to the standard Stern-Volmer relationship F0/F = 1 + KSV[Q], where F0 is the fluorescence in the absence of the quencher, F is the fluorescence at molar concentration of quencher [Q], and KSV is the Stern-Volmer quenching constant.

**RESULTS**

**Gossypol inhibits the transport of hexoses in HL-60 and CHO cells.** We determined the dose dependence from the effect of gossypol on the transport of methylglucose and deoxyglucose in HL-60 cells. We have previously shown that HL-60 cells express the glucose transporter GLUT1 and efficiently transport hexoses (53). We used a 30-s uptake assay to determine the kinetic constants of transport. When gossypol was added at the beginning of the uptake assay, it inhibited the uptake of deoxyglucose and methylglucose by HL-60 cells in a dose-dependent manner (Fig. 1, A and B). Fifty percent inhibition was observed at ~30 μM.

To test whether the effect of gossypol on transport is cell independent, we next analyzed its effect on the uptake of methylglucose and deoxyglucose in stably transfected CHO cells overexpressing GLUT1. The GLUT1-transfected CHO cells have an increased capacity to take up hexoses compared with control cells stably transfected with a plasmid carrying the cDNA for the human placental insulin receptor. Gossypol caused a dose-dependent inhibition of the uptake of methylglucose and deoxyglucose in the GLUT1-expressing cells as well as the control cells (Fig. 1, C and D). In both cell lines, 50% inhibition of uptake was observed at ~30 μM gossypol. These results show that the effect of gossypol on the activity of GLUT1 is independent of the cell context in which the transporters are expressed. The simplest interpretation of these results is that gossypol interferes with transport by directly interacting with the sugar transport protein.
Gossypol inhibits the transport of methylglucose and deoxyglucose in human erythrocytes. GLUT1 is especially abundant in human erythrocytes, and most of the information on the structure and function of GLUT1 has been obtained using the erythrocyte transporter (8). Confirming the data obtained in the HL-60 and CHO cell lines, gossypol inhibited the uptake of methylglucose and deoxyglucose in human erythrocytes in a dose-dependent manner, with 50% inhibition observed at ~30 μM (Fig. 1, E and F). After incubation with a high concentration of gossypol, thorough washing of the red blood cells in a gossypol-free media restored the transport activity to >95% of controls, indicating that the inhibition was reversible (data not shown). The specificity of the effect of gossypol on hexose transport was confirmed in experiments that showed that gossypol failed to inhibit the transport of nicotinamide by the red blood cells (Fig. 1G). Moreover, apogossypol, an analog that is devoid of the 8,8'-aldehyde group, was unable to inhibit hexose uptake in the red blood cells (not shown). These results diminish the possibility that the decrease of transport in the presence of gossypol was due to an unspecific effect.

The difference between red blood cells and the HL-60 and CHO cell lines was that the effect of gossypol in human erythrocytes was time dependent, because no inhibition was seen when gossypol was added along with the radioactive substrate at the beginning of the transport assay (data not shown). Haspel et al. (17) were also unable to detect any decrease in transport of deoxyglucose in human erythrocytes with the simultaneous addition of gossypol. However, we did find that a short preincubation of the cells with gossypol for a period of 2 min was enough to attain full effect at any particular gossypol concentration. This difference may be attributed to the particular lipid composition of the erythrocyte cell membrane, which could affect gossypol diffusion through the plasma membrane. Therefore, red blood cells were incubated with gossypol for 10 min before transport assays.

Gossypol inhibits cytochalasin B binding to erythrocyte membranes. Gossypol unspecific effect of altering membrane fluidity (42) may explain the alteration of membrane permeability to hexoses. Therefore, to demonstrate the specificity of gossypol interaction with GLUT1, we analyzed gossypol’s effect on the binding of radiolabeled cytochalasin B to the hexose transporters present in purified human erythrocyte ghosts. Cytochalasin B binds to the glucose transporter in a n-glucose-displaceable manner (50). Increasing concentrations of gossypol efficiently competed for the n-glucose-sensitive cytochalasin B binding sites present in the erythrocyte membranes (Fig. 2A). Approximately 30 μM gossypol inhibited the binding of 0.1 μM cytochalasin B by 50%, and total inhibition of binding was observed at 100 μM gossypol, thus suggesting that gossypol effectively interacts with GLUT1. As anticipated, apogossypol had no effect on cytochalasin B binding (Fig. 2A). Further analysis revealed that gossypol displaced cytochalasin B in a mixed noncompetitive manner (Fig. 2B). The linearity of both the slopes and the intercepts on the y-axis in the secondary plot (Fig. 2C) demonstrates gossypol as a linear noncompetitive blocker of cytochalasin B binding to the erythrocyte ghosts. When either lines, slope, or intercept is extrapolated to zero, the value of the inhibition constant for gossypol was estimated at ~20 μM. Overall, the data are consistent with the direct interaction of gossypol with the erythrocyte glucose transporter.

Quenching of protein fluorescence by gossypol in stripped membranes. To gather independent proof of gossypol interaction with GLUT1, fluorescence spectroscopy experiments were conducted to characterize how gossypol interacts with the erythrocyte glucose transporter. When excited at 295 nm, peripheral protein-depleted erythrocyte purified membranes
displayed a fluorescence emission spectrum characterized by a maximum at 334 nm resulting from tryptophan fluorescence. The addition of gossypol to purified membranes resulted in a marked fluorescence quenching (Fig. 2D); as expected, apogossypol does not quench GLUT1 fluorescence (data not shown). Gossypol quenched the fluorescence emission intensity immediately after mixing, and the intensity did not change after that. The emission intensity after the rapid noncovalent binding of gossypol was concentration dependent (Fig. 2E). The concentration of protein was 15 μg/ml. E: concentration dependence of the fluorescence changes observed with gossypol. The line through the data points of fractional quenching of purified membranes (●) by gossypol is a best-fit curve to a simple saturable component, with $K_d$ and maximal quenching ($Q_{max}$) values of 0.1 μM and 0.83, respectively. The data of fractional quenching of tryptophanamide (○) by gossypol was fitted by linear regression. F: Stern-Volmer iodide quenching plots of purified transporter. Fluorescence at 340 nm was measured at 20°C (excitation wavelength at 295 nm), with slits set at 5 nm. The plots are in the absence (●) or presence of 0.1 (○) or 0.5 μM gossypol (▲). G: concentration dependence of the effect of gossypol on the quenching of the transporter by iodide. The values of Stern-Volmer quenching constant ($K_{SV}$) calculated from the slopes of quenching by iodide of the transporter are represented as function of the concentration of gossypol. The line through the data points is a best-fit curve to a simple saturable component, with a $K_d$ value of 0.2 μM and a limiting $K_{SV}$ of 2.6 × 10^{-3} M^{-1} at saturating concentrations of gossypol. For fluorescence determinations, the results are from single experiments representative of three or four separate experiments for each condition. AU, arbitrary units.
Gossypol inhibited glucose exits from human erythrocytes. The inhibition of the rate of glucose exits into glucose-free medium was studied at 30°C. Gossypol inhibited the exit of solutions by varying concentrations of gossypol in the external medium. The inhibition of the rate of glucose exits into glucose-free medium in the absence (●) or presence of 3 μM (○) or 6 μM (▲) gossypol. C: Sen-Widdas plot of d-glucose exit data at different concentrations of glucose in the external medium in the absence (●) or presence of 1 μM cytochalasin B (○) or 5 μM phloretin (▲). D: Hanes-Woolf plot of the effect of gossypol on the substrate concentration dependence for zero-trans uptake of methylglucose in the absence (●) or presence of 3 (○) or 6 μM (▲) gossypol. Varying slopes and convergence on the abscissa in the plots are indicative of noncompetitive inhibition. E: secondary plot of the effect of gossypol on the substrate dependence for zero-trans uptake of methylglucose. F: Hanes-Woolf plot of the effect of gossypol on the substrate concentration dependence for methylglucose exchange in the absence (●) or presence of 1 (○) or 3 μM (▲) gossypol. Equal and parallel slopes in the plots are indicative of competitive inhibition. G: secondary plot of the effect of gossypol on the substrate dependence for methylglucose exchange. Results are from single experiments representative of three or four separate experiments for each condition.

Gossypol inhibits glucose exits from human erythrocytes. The inhibition of the rate of glucose exits into glucose-free solutions by varying concentrations of gossypol in the external medium was studied at 30°C. Gossypol inhibited the exit of d-glucose in human erythrocytes in a dose-dependent manner, with 50% inhibition observed at ~3 μM (Fig. 3A). The specificity of the gossypol’s effect on transport was confirmed in experiments that showed that apogossypol failed to inhibit the exit of d-glucose by the red blood cells. Thus, although 30 μM gossypol completely obliterated d-glucose exit, <15% of inhibition was observed at the highest apogossypol concentration tested.

To determine whether gossypol and d-glucose share a common binding site on the external face of the transporter, the effect of gossypol on the inhibition of glucose exit was tested by using increasing concentrations of d-glucose in the external medium. Figure 3B shows the Sen-Widdas plot of glucose exit in the absence or presence of gossypol. The value of $-K_{in}$ of the external glucose site of the GLUT1 transporter is evaluated from the intersect on the x-axis (46). In the absence of gossypol, we calculated a value of 2 mM for the affinity of glucose for the external site, which is consistent with Basketter and Widdas (2), and was not affected by the addition of gossypol at levels that potently inhibit glucose transport (Fig. 3B). The exit rate was slower in the presence of gossypol without affecting the affinity for glucose of the glucose binding domain located exofacially on the transporter. Control experiments were done using cytochalasin B and phloretin (at their respective $K_{i}$ values), cytochalasin B interacts with the transporter at the cytoplasmic face, reversibly and with high affinity, while phloretin binds to the transporter at the external site in a competitive way with d-glucose. As expected, only phloretin was able to modify the $K_{in}$ value for glucose at the GLUT1 transporter external site (Fig. 3C).

Characterization of the glucose transporter inhibition by gossypol. The absence of a typical competitive inhibitor effect in Sen-Widdas cis-infinite exit experiments implies that gossypol does not react with the external d-glucose site on the transporter protein. To further analyze this, we used both zero-trans (no substrate inside) and equilibrium exchange (substrate inside and outside) transport protocols to analyze the effect of gossypol. Because gossypol does not inhibit glucose transport when applied directly with the substrate to the outer surface ([17]; this work), we hypothesized that it may interact at the internal or endofacial surface of the transporter. As such, the competitive nature of the interaction would only be revealed in equilibrium exchange experiments where the competing substrate is present at the putative endofacial inhibitor site. In human erythrocytes, cytochalasin B is known to com-
petitively inhibit only at the inside site. Basketter and Widdas (2) and Deves and Krupka (11) showed that kinetically this interaction produces a noncompetitive mode of inhibition in zero-trans entry experiments, but a competitive mode of inhibition in equilibrium exchange transport experiments. In the experiments using gossypol, the inhibitor reduced the $V_{\text{max}}$ for zero-trans methylglucose transport but did not alter the $K_m$ (Fig. 3D). A secondary plot of the slopes against gossypol concentration was linear, with a $K_i$ of 7 μM (Fig. 3E). By contrast, in equilibrium exchange experiments, gossypol increased the $K_m$ value without altering the $V_{\text{max}}$ (Fig. 3F). A secondary plot of the $x$-intercepts against gossypol concentration was linear with a $K_i$ of 3 μM (Fig. 3G). Therefore, gossypol produced the same pattern of transport inhibition as cytochalasin B; it acted as a noncompetitive inhibitor of zero-trans entry and as a competitive inhibitor of equilibrium-exchange experiments. These results provide persuasive evidence indicating that gossypol affects GLUT1 function by a mechanism involving the blocking of the endofacial glucose site on the transporter.

**DISCUSSION**

Gossypol is a potent and reversible inhibitor of the cellular uptake of deoxyglucose and methylglucose, substrates that both enter cells through hexose transporters of the facilitative type. For experimental systems, we used HL-60 cells and human erythrocytes, which express the facilitative hexose transporter GLUT1. We also used stably transfected CHO cells expressing GLUT1. The data from time-course experiments consistently demonstrated that the effect of gossypol was directly related to the inhibition of GLUT1. No preincubation step was necessary to observe gossypol’s effect on the uptake of deoxyglucose and methylglucose in HL-60 or CHO cell lines. In these systems, the effect on transport was instantaneous and maximum when gossypol was added to the uptake assay simultaneously with the test substrate at time zero. The similar dose curves for gossypol’s effect on uptake in the HL-60 cells, transfected CHO cells, and human erythrocytes indicated that the effect was cell independent and likely occurred through the same mechanism in the three cell types analyzed. Our data confirm that gossypol interacts directly with GLUT1 and that this interaction obliterates the transport of hexoses across the cell plasma membrane.

A direct interaction of gossypol with the hexose transporter is strongly supported by the saturable quenching of the intrinsic tryptophan fluorescence data of the purified sugar transport protein. This is also consistent with the binding data indicating that gossypol blocked the glucose-sensitive binding of cytochalasin B to GLUT1 present in human erythrocytes. The affinity of gossypol interaction with the purified transporter, and its lack of dependence on d-glucose binding, is in concord with the results of the hexose transport inhibition experiments. Gossypol may have binding sites on GLUT1, which do not interfere with glucose transport, such that W-quenching and transport inhibition may correspond to unrelated processes. This is consistent with the low $K_d$ app value for gossypol-induced W-quenching (0.1 μM) relative to $K_i$ app value for inhibition of transport (8–20 μM). In human GLUT1, there are six tryptophans. The theoretical three-dimensional templated model of GLUT1 (45) indicates that four of them are close to the external vestibule, another one is close to the narrow translocation channel, and only one tryptophan residue is at the endofacial surface. Thus, tryptophan fluorescence quenching could result from gossypol binding to an exofacial site in GLUT1, with sugar transport inhibition due to interaction with an endofacial site.

We have determined that gossypol is a competitive inhibitor of glucose transport activity, and that, on the basis of a kinetic test, the site of interaction is at the endofacial surface of the transporter. Assuming the traditional mobile carrier model for the transporter, occupation of the endofacial substrate site reduces $V_{\text{max}}$ under zero-trans conditions because return of the substrate to the outside is blocked, and there is no substrate inside to displace the inhibitor. Under equilibrium exchange conditions, internal substrate competitively displaces the inhibitor and the transport $K_m$ is increased. An endofacial site of action is consistent with other aspects of gossypol inhibition of transport activity. The requirement for a preincubation step in human erythrocytes is most likely due to a slow penetration of the compound across the red cell plasma membrane before interacting with the endofacial glucose binding site of the transporter. Several hydrophobic compounds, including cytochalasin B, steroids, isoflavones, and anesthetics also seem to interact with GLUT1 at the endofacial surface (1).

Gossypol, however, was unable to competitively displace cytochalasin B from the carrier, which suggests that gossypol and cytochalasin B binding sites on the endofacial surface of GLUT1 are different. In a simple carrier model, if gossypol and sugar compete for the exit site, as cytochalasin B does, cytochalasin B binding should also be competitively displaced by gossypol. The question is whether gossypol and cytochalasin B actually compete for the same site on the endofacial surface of the transporter. It is not necessary for glucose and cytochalasin B (or gossypol) to bind to the same site to observe competitive inhibition of sugar transport. Cytochalasin B (or gossypol) binding converts the carrier to a state unable to bind sugar. Initial results from mutagenesis studies suggest that the binding site for cytochalasin B is distinguishable from the sugar exit site (X. Valenzuela and A. M. Reyes, unpublished observations). We hypothesize that gossypol binds to the carrier in a state at or close to the sugar exit site, but different from the cytochalasin B binding site. Gossypol binding led the carrier to a sugar transport-incompetent conformation, which explains the competitive character of the inhibition in exchange conditions. Both gossypol and cytochalasin B compete for the sugar-free conformation of the transporter. However, since gossypol and cytochalasin B sites seem to be different, cytochalasin B does not completely block gossypol binding to the carrier (even at high cytochalasin B concentrations). This explains the noncompetitive interaction between cytochalasin B and gossypol.

In a recent study, the classic alternating carrier model of asymmetric glucose transport has been questioned as inconsistent with energy conservation laws (35). Instead, the author suggests that the transporter has two or more fixed interconnected sites for sugar on each membrane side. Ligand flows occur by serial jumps resulting from ligand dissociation and reassociation reactions between the connected vacant sites or nodes in the network. Thus, noncompetitive inhibition of uptake and exit and competitive inhibition of exchange could also be explained if GLUT1 has two fixed sites for sugar and gossypol.
cannot bind to a doubly occupied transporter (e.g., S1.e.S2) but binds to either (S1.e or e.S2) site. Multisite kinetics in combination with site-specific mutations may provide a useful strategy for future investigations into the complex nature of transporter specificity for sugars and other ligands.

Our results highlight the ability of GLUT1 to interact with molecules structurally unrelated to glucose. Although the precise nature of the interaction between gossypol and GLUT1 cannot be determined from these experiments, the data indicate that the sesquiterpene interacts with a site that resides on the endofacial face of the transporter, and that this interaction is also responsible for interfering with the binding of cytochalasin B. Other studies have suggested that the glucose transporters possess a nucleotide binding site, and experimental evidence has shown that they can be photolabeled with azido ATP and azido adenosine. Further evidence indicates the ability of ATP and ADP to modulate the functional activity of the glucose transporters (1, 18). We recently demonstrated that several flavonoids of natural origin interact directly with and are efficient and specific blockers of the activity of GLUT1 (50, 51). These observations suggest the existence of a variety of compounds able to interact with the glucose transporters in a highly specific manner that are potentially capable of modulating their functional activity.

Given the presence of GLUT1 in almost all cells and tissues, and the importance of this family of proteins for the provision of cellular nutrients essential for normal cell function, it is reasonable to consider that some of gossypol’s effects on cell proliferation and differentiation may be related to its capacity to inhibit the activity of GLUT1. Inhibition of glucose transport by gossypol in different cell types has been suggested previously by several authors. De Peyster et al. (10) determined that addition of gossypol to rat adipocytes caused a dose-dependent inhibition of the uptake of deoxyglucose, in the absence or presence of insulin; half-maximal effects occurred at ~200 μM gossypol. Although we do not have direct evidence for an effect of gossypol on other members of the glucose transporter family, the data from de Peyster et al. are interesting because adipocytes express GLUT4, an isoform that is translocated to the plasma membrane in response to insulin and is responsible for most of the transport of glucose in insulin-stimulated adipocytes. Nakamura et al. (36) observed that gossypol strongly decreases glucose transport in round rat spermatids, although this was attributed to a decline in cellular ATP levels elicited by the sesquiterpene. However, there is evidence that gossypol inhibits sugar metabolism in spermatooza (38, 47). Our results suggest that this is partially due to inhibition of glucose uptake. There are also conflicting results that we believe reflect particular properties of the different cell types employed. Haspel et al. (17) reported that hexose uptake by human erythrocytes was not affected by the simultaneous addition of low concentrations (10–50 μM) of gossypol. Our present data demonstrate that gossypol inhibits sugar transport in human erythrocytes only when added before the transported substrate. Reyes et al. (43) reported that 50 μM gossypol had no effect on methylglucose uptake on the cell line TM4 derived from mouse testicular cells. However, sugar transport on this cell line was not affected by phloretin, a typical inhibitor of GLUT1, suggesting the expression of a different hexose transporter isoform.

Given the multiple actions of gossypol on the physiology of cells, it is difficult to ascertain what degree of the inhibitory effect on sugar uptake described herein contributes to gossypol antiproliferative activity. Gossypol is a potent inhibitor of Bel-2 family of antiapoptotic proteins (24, 59), more notably Bel-2 and Bel-XL, which are implicated in the development of many human malignancies (39, 40). Overexpression of either of these related proteins affects the mitochondrial-mediated pathway of apoptosis and contributes to the development of chemotherapeutic resistance (23). Interestingly, gossypol enhances the response of tumor cells to radiation and chemotherapy (33, 56). Sugar transport inhibition by gossypol does, however, have implications for the pathophysiology of cancer cells. One of the primary characteristics of these cells is an increased metabolism of glucose. Because cancer cells do not accumulate intracellular stores of glucose in the form of glycogen or fat as does liver and adipose tissue, glucose must be obtained continuously from external sources and transported intracellularly. The mechanism whereby cancer cells increase their ability to take up glucose involves the selective overexpression of GLUT1 (32), a transporter we have shown is inhibited by gossypol.

**REFERENCES**


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