Endofacial competitive inhibition of the glucose transporter 1 activity by gossypol

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Submitted 5 October 2008; accepted in final form 11 April 2009

Pérez A, Ojeda P, Valenzuela X, Ortega M, Sánchez C, Ojeda L, Castro M, Cárcamo JG, Rauch MC, Concha II, Rivas CI, Vera JC, Reyes AM. Endofacial competitive inhibition of the glucose transporter 1 activity by gossypol. Am J Physiol Cell Physiol 297: C86–C93, 2009. First published April 22, 2009; doi:10.1152/ajpcell.00501.2008.—Gossypol is a natural disesquiterpene that blocks the activity of the mammalian facilitated hexose transporter GLUT1. In human HL-60 cells, which express GLUT1, Chinese hamster ovary cells overexpressing GLUT1, and human erythrocytes, gossypol inhibited hexose transport in a concentration-dependent fashion, indicating that blocking of GLUT1 activity is independent of cellular context. With the exception of red blood cells, the inhibition of cellular transport was instantaneous. Gossypol effect was specific for the GLUT1 transporter since it did not alter the uptake of nicotinamide by human erythrocytes. Gossypol affects the glucose-displaceable binding of cytochalasin B to GLUT1 in human erythrocyte ghost in a mixed noncompetitive way, with a Ki value of 20 μM. Likewise, GLUT1 fluorescence was quenched ~80% by gossypol, while Stern-Volmer plots for quenching by iodide displayed increased slopes by gossypol addition. These effects on protein fluorescence were saturable and unaffected by the presence of α-glucose. Gossypol did not alter the affinity of α-glucose for the external substrate site on GLUT1. Kinetic analysis of transport revealed that gossypol behaves as a noncompetitive inhibitor of α-glucose for the external substrate site on GLUT1. Kinetic analysis of transport revealed that gossypol behaves as a noncompetitive inhibitor of zero-trans (substrate outside but not inside) transport, but it acts as a competitive inhibitor of equilibrium-exchange (substrate inside and outside) transport, which is consistent with interaction at the endofacial surface, but not at the exofacial surface of the transporter. Thus, gossypol behaves as a quasi-competitive inhibitor of GLUT1 transport activity by binding to a site accessible through the internal face of the transporter, but it does not, in fact, compete with cytochalasin B binding. Our observations suggest that some effects of gossypol on cellular physiology may be related to its ability to disrupt the normal hexose flux through GLUT1, a transporter expressed in almost every kind of mammalian cell and responsible for the basal uptake of glucose.

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 flavonoids and isoflavonoids 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 disesquiterpene 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 antispermatic, anti-invasive, 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 structures, which may provide the structural basis for GLUT1 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-
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/mmole, NEN-DuPont), [3H]methylglucose (specific activity 86.7 Ci/mmole, NEN-DuPont), or L-[14C-carbonyl]nicotinamide (specific activity, 35 mCi/mmole, 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 310 nm as emission wavelength (5-nm slits). Red blood cells were suspended for 10% fetal bovine serum and antibiotics. Cell viability was 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.

**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 methyglucose 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 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's specificity 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

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**Fig. 1.** Dose dependence of the effect of gossypol on the transport of hexoses and nicotinamide by HL-60 cells, Chinese hamster ovary (CHO) cells, and human erythrocytes. Transport was measured using 30-s uptake assays in the presence of the indicated concentrations of gossypol. Uptake of methylglucose (OMG) was measured in HL-60 cells (A), in transfected CHO cells overexpressing glucose transporter 1 (GLUT1) (●, C), in transfected CHO cells overexpressing the human placental insulin receptor (●, C), and in human erythrocytes (E). Uptake of deoxyglucose (DOG) was measured in HL-60 cells (B), in transfected CHO cells expressing GLUT1 (●, D), in transfected CHO cells overexpressing the human placental insulin receptor (●, D), and in human erythrocytes (F). Transport of nicotinamide (NAM) was measured in human erythrocytes (G). The data represent means ± SD of four independent determinations.
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, apo-gossypol 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), p-glucose did not alter the quenching, and no significant shifts in emission were observed (data not shown). The $K_d$ and $Q_{max}$ for this saturable process were 0.1 $\mu$M and 0.83, respectively. The high $Q_{max}$ for gossypol contrasts with the lower effect of p-glucose or cytochalasin B on sugar transport fluorescence ($Q_{max}$ 0.12–0.41) (15, 19, 37), and is comparable to the effect of pentobarbital ($Q_{max}$ 0.75) (19). In contrast, the quenching of tryptophan fluorescence by gossypol (Fig. 2E) exhibited a linear dependence consistent with collisional quenching.

Our fluorescence results suggest that a significant fraction of the fluorescence of tryptophanyl residues in the transporter is effectively quenched by gossypol. Three possible explanations for this are 1) most of the six tryptophan residues are situated on the protein surface; 2) the protein structure is flexible enough to allow gossypol to reach most of the residues; or 3) protein structural changes induced by gossypol interaction with the transporter may alter the environment of the tryptophanyl residues to promote intramolecular quenching, because a variety of functional groups present in proteins are able to quench tryptophan fluorescence. While none of these possibilities are definitive, our data strongly suggest that the gossypol-induced fluorescence quenching is the result of specific ligand binding instead of unspecific collisional quenching, and that the six tryptophan residues of the sugar transporter are accessible to gossypol.

Gossypol increases the quenching of purified sugar transport protein fluorescence by iodide. Hydrophilic quenchers are expected to influence preferentially the fluorescence of accessible aqueous exposed tryptophan residues. Pawagi and Deber (37) used quenching of the purified erythrocyte glucose transporter with iodide to sense protein conformational changes induced by several transporter ligands. They found that the effect of iodide on purified transporter fluorescence can be explained by collisional quenching. Most importantly, they described that p-glucose reduced quenching by iodide, while the addition of cytochalasin B had little effect on fluorescence quenching. Our studies confirmed these observations (data not shown). We chose this approach for our study to further characterize how gossypol binds to GLUT1, because the results are expressed as relative values that are unaffected by the internal filter effect due to the absorbance of gossypol. Gossypol modified the ability of iodide to induce quenching of the intrinsic tryptophan fluorescence of purified sugar transport protein. Figure 2F shows Stern-Volmer plots for KI quenching
of transporter fluorescence in the absence and presence of gossypol, with fluorescence emission monitored at 340 nm. These experiments demonstrated that gossypol, in clear contrast to the effect of D-glucose, actually increased the ability of KI to quench tryptophan transporter fluorescence. The increase in accessibility was dependent on the gossypol concentration up to a Ki value obtained through analysis of gossypol-induced quenching of protein fluorescence. The observed gossypol-dependent saturable increase in the slope of the Stern-Volmer plot sup-

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 Kd 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 Ki 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 Kd 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 (sub-
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-\textit{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-\textit{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 \( 7 \ \mu M \) (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 \( 3 \ \mu M \) (Fig. 3G). Therefore, gossypol produced the same pattern of transport inhibition as cytochalasin B; it acted as a noncompetitive inhibitor of zero-\textit{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 \( \alpha \)-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 \( K_{\text{d app}} \) value for gossypol-induced \( W \)-quenching (0.1 \( \mu M \)) relative to \( K_{\text{i app}} \) value for inhibition of transport (8 – 20 \( \mu M \)). 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-\textit{trans} conditions because return of the substrate site 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 site 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 Bcl-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 chemoresistance (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.

GRANTS

This work was supported by Grants 1020908 (to A. M. Reyes) and 1060198 (to A. M. Reyes) from Fondo Nacional de Desarrollo Cientı ´fico y Tecnolı ´gico (FONDECYT), Chile.

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