Peroxisome proliferator-activated receptor \( \gamma \) agonist pioglitazone prevents the hyperglycemia caused by phosphatidylinositol 3-kinase pathway inhibition by PX-866 without affecting antitumor activity

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Abstract

The phosphatidylinositol 3-kinase (PI3K)/Akt signaling cascade is an important component of the insulin signaling in normal tissues leading to glucose uptake and homeostasis and for cell survival signaling in cancer cells. Hyperglycemia is an on-target side effect of many inhibitors of PI3K/Akt signaling including the specific PI3K inhibitor PX-866. The peroxisome proliferator-activated receptor \( \gamma \) agonist pioglitazone, used to treat type 2 diabetes, prevents a decrease in glucose tolerance caused by acute administration of PX-866. Our studies have shown that pioglitazone does not inhibit the antitumor activity of PX-866 in A-549 non-small cell lung cancer and HT-29 colon cancer xenografts. In vitro studies also showed that pioglitazone increases 2-[\( ^{14} \)C]deoxy-D-glucose uptake in L-6 muscle cells and prevents inhibition of 2-deoxyglucose uptake by PX-866. Neither pioglitazone nor PX-866 had an effect on 2-deoxyglucose uptake by PX-866. In vivo imaging studies using \(^{18} \)F]2-deoxyglucose (FDG) positron emission tomography showed that pioglitazone increases FDG accumulation by normal tissue but does not significantly alter FDG uptake by A-549 xenografts. Thus, peroxisome proliferator-activated receptor \( \gamma \) agonists may be useful in overcoming the increase in blood glucose caused by inhibitors of PI3K signaling by preventing the inhibition of normal tissue insulin-mediated glucose uptake without affecting antitumor activity. [Mol Cancer Ther 2009;8(1):94–100]

Introduction

Phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B) signaling is an important cell survival signaling pathway that mediates the activity of many receptor and protein tyrosine kinases (1). A hallmark toxicity of inhibition of PI3K/Akt signaling is an increase in blood glucose due to inhibition of insulin signaling (2, 3) leading to insulin insensitivity and decreased glucose uptake by muscle and fat cells (4). Knockout of the p110\( \alpha \) catalytic subunit of class I PI3K is embryonic lethal in mice due dysfunctional angiogenesis (5). However, mice with a heterozygous kinase-dead p110\( \alpha \) knockin have viable offspring that display a diabetic phenotype with defective insulin signaling and increased blood glucose, as do mice with a knockout of the regulatory p85 subunit of PI3K (6, 7). Mice with a knockout of the Akt2 isoform, a downstream target activated by PI3K, are viable and also display a diabetic phenotype (8). This role of Akt2 in preventing diabetes is conserved in humans as shown in individuals with a mutationally inactivated Akt2 who exhibit severe insulin resistance manifested in diabetes (9). Together, these studies establish that PI3K/Akt signaling plays an important role in insulin signaling and is conserved throughout mammals.

In addition to this role in normal physiology, the PI3K/Akt signaling pathway has been shown to regulate several fundamental aspects of tumorigenesis including uncontrolled proliferation, angiogenesis, and resistance to apoptosis (10, 11). The PI3K/Akt pathway is frequently activated in human tumors by suppression or deletion of the PTEN tumor suppressor or through mutational activation or amplification of PI3K (12, 13). Thus, inhibition of the PI3K/Akt signaling is an attractive target for agents to inhibit tumor growth, and several inhibitors of PI3K or Akt have or will soon be introduced into clinical trial as antitumor agents (14). Disturbances in glucose metabolism have been observed in patients receiving some early examples of agents that inhibit PI3K/Akt signaling. UCN-01, which inhibits Akt signaling as well as cell cycle-dependent kinases, caused elevated glucose, which had to be managed by insulin injection (15). More recently, clinical studies with rapamycin derivatives, which inhibit mammalian target of rapamycin, a downstream target of Akt, have also shown hyperglycemia as an adverse event (16). Thus, disrupted glucose metabolism may become an important on-target dose-limiting toxicity for agents.
that inhibit PI3K/Akt/mammalian target of rapamycin signaling.

We have reported previously an increase in both blood insulin and glucose in mice treated acutely with the irreversible, specific PI3K inhibitor PX-866, which has recently entered phase I clinical trial (2). Additionally, it was found that the thiazolidinedione peroxisome proliferator-activated receptor γ (PPARγ) agonist pioglitazone, approved for the treatment of type 2 diabetes, prevented the increase in blood glucose caused by PX-866 in animals through reversal of a decreased glucose tolerance (2). However, whether pioglitazone might also antagonize the antitumor effects of a PI3K inhibitor and the mechanism for the reversal of the PI3K-mediated hyperglycemia was not established. We now show that pioglitazone does not inhibit the antitumor activity of PX-866. We also show that PI3K inhibition by PX-866 inhibits insulin-sensitive glucose uptake by normal tissue but not by tumor tissue, and the inhibition can be overcome by pioglitazone. This suggests a mechanism by which pioglitazone may prevent the increase in blood glucose associated with PI3K signaling inhibition without inhibiting antitumor activity.

Materials and Methods

Cells
A-549 human non-small cell lung cancer (NSCLC), HT-29 human colon cancer, and rat L-6 cells, a differentiating myoblast cell line, were obtained from the American Tissue Type Collection. The cells were grown in humidified 95% air, 5% CO2 at 37°C in DMEM with 10% fetal bovine serum or, for the L-6 cells, in Ham’s F-12 medium with 10% fetal bovine serum. L-6 cells were differentiated by transferring confluent cells to DMEM with 2% fetal bovine serum for 4 to 6 days until the fusion of myoblasts into myotubes. All cell lines were tested to be Mycoplasma-free using a PCR ELISA kit (Roche Diagnostics). PX-866 (acetic acid (1S,4E,10R,11R,13S,14R)-4-diallylamino-1-methoxy-10,13-dimethyl-3,7,17-trioxo-1,3,4,7,10,11,12,13,14,15,16,17-dodecahydro-2-oxa-cyclopenta[α]phenanthren-11-yl ester) was dissolved at 10 mg/mL in 5% ethanol in 0.9% NaCl for i.v. administration to

Figure 1. Effect of PX-866 on glucose tolerance and plasma insulin. A, female scid mice were administered (∆) vehicle alone p.o. q.o.d. or (●) PX-866 2.5 mg/kg p.o. for 30 d (15 doses) and glucose tolerance measurements were made 24 h, 5 d, or 12 d after the last dose. Mean ± SE of 6 mice. B, female scid mice were fasted for 16 h and administered (▲) vehicle alone or (●) PX-866 10 mg/kg i.v. or not fasted and administered (∆) vehicle alone or (○) PX-866 10 mg/kg i.v., and plasma insulin was measured. Mean ± SE of 4 mice.
mice and at 5 mg/mL in 5% ethanol in water for p.o. administration by gavage. Pioglitazone hydrochloride and human recombinant insulin were obtained from Sigma. Pioglitazone was administered to mice p.o. by gavage suspended in 0.1% Tween 20 in water. Rabbit purified anti-phospho-Ser 473-Akt antibody, anti-Akt antibody, and anti-Glut4 were obtained from Cell Signaling Technology.

Glucose Measurements and Antitumor Studies

A-549 NSCLC or HT-29 colon cancer cells (f)107 in log-phase growth were injected s.c. into the flanks of female severe combined immunodeficient (scid) mice. When the tumors reached 100 to 200 mm3, the mice were stratified into groups of 6 or 8 animals having approximately equal mean tumor volumes and drug gavage suspended in 0.1% Tween 20 in water. Rabbit purified anti-phospho-Ser473-Akt antibody, anti-Akt antibody, and anti-Glut4 were obtained from Cell Signaling Technology.

Figure 2. Effect of pioglitazone and PX-866 in mice bearing A-549 NSCLC or HT-29 colon cancer xenografts. A, female scid mice were injected s.c. with 107 A-549 human NSCLC cells. When the tumors were 100 mm3, mice were administered (○) vehicle alone p.o. q.o.d. × 10, (■) pioglitazone 20 mg/kg p.o. q.o.d. × 10, (▲) pioglitazone 20 mg/kg p.o. q.o.d. × 30 and PX-866 2.5 mg/kg p.o. q.o.d. × 30 beginning 7 days before the start of treatment with other drugs. B, female scid mice were injected s.c. with 107 HT-29 human colon cancer cells. On day 11 when the tumors were 180 mm3, mice were administered (○) vehicle alone p.o. q.o.d. × 10, (■) pioglitazone 17.5 mg/kg p.o. q.o.d. × 20, (▲) PX-866 2.5 mg/kg p.o. q.o.d. × 20 and pioglitazone 17.5 mg/kg p.o. q.o.d. × 20. The pioglitazone was given 4 h before PX-866. Mean ± SE of 8 mice per group. *, P < 0.05, compared with control.

Figure 3. Effect of PX-866 and pioglitazone on glucose uptake in normal muscle and tumor cells. A, L-6 muscle cells (top) and A-549 NSCLC cells (bottom) were incubated with 2-[1-14C]deoxy-D-glucose in serum-free medium and cellular accumulation of radioactivity was measured after 1 h in cells treated with insulin, 300 nmol/L, PX-866 100 nmol/L, pioglitazone 10 μmol/L, a combination of PX-866 and pioglitazone, or cytochalasin 10 μmol/L as a positive control to inhibit 2-deoxyglucose uptake. Mean ± SE of 4 experiments. *, P < 0.05, compared with control. B, Western blot showing the expression at the cell membrane of glucose transporters Glut1 and Glut4 in L-6 myoblasts and A-549 cells. Cell membrane proteins were labeled by biotin and collected on streptavidin beads. It was not possible to obtain labeled cell membranes proteins in the absence of insulin because of cell lysis by the reactive biotin. The effects of PX-866 and pioglitazone treatment are shown. Also shown is total cellular phospho-Ser473-Akt and total Akt as a loading control.
administration begun. Mice were dosed daily with pioglitazone p.o. at 17.5 or 20 mg/kg, and PX-866 was administered p.o. every other day at 2.5 mg/kg. On days when pioglitazone was given in combination, it was administered 4 h before PX-866. Animals were weighed weekly and tumor diameters were measured twice weekly at right angles (d_short and d_long) with electronic calipers and converted to volume by the formula: volume = (d_short)^2 × (d_long). Animals were euthanized when the tumor volume reached 2,000 mm³. Blood glucose was measured using the FreeStyle Freedom (Therasense).

**Cellular Glucose Uptake**

Differeniated L-6 myoblasts or A-549 NSCLC cells (10⁷) at 80% confluence were serum-starved overnight in Ham's F-12 medium or DMEM, respectively, and incubated with or without 10 μmol/L pioglitazone for 12 h followed by a further 5 h in serum-free Hanks' buffer (pH 7.4). Fresh Hanks' buffer containing 300 nmol/L insulin, 10 μmol/L pioglitazone, or 100 nmol/L PX-866 in various combinations was added to the cells. Cytochalasin B (10 μmol/L) was used as a positive control to inhibit glucose uptake. For glucose uptake measurements, 0.2 μCi/sample 2-[1-¹⁴C]deoxy-d-glucose (Perkin-Elmer) was added to medium containing 1 mmol/L nonradioactive 2-deoxyglucose. The cells were incubated for 20 min at room temperature with or without 300 nmol/L human recombinant insulin, the medium was aspirated, and the cells were rapidly washed with 4 × 1 mL PBS at 4°C. The cells were solubilized with 1 mL of 0.5 mol/L NaOH and radioactivity was measured by liquid scintillation counting.

**Western Blotting for Plasma Membrane Glucose Transporters**

Cells were prepared and treated with agents as described for the glucose uptake measurements before being incubated with 0.5 mg/mL Sulfo-NHS-Biotin (Pierce) in PBS for 30 min at 4°C to label cell surface proteins. The cells were washed with PBS containing 100 mmol/L glycine at 4°C to quench unreacted biotin before being harvested in 200 μL lysis buffer containing 50 mmol/L Tris (pH 7.5), 1% Triton X-100, 0.5% NP-40, 2 mmol/L EDTA, 150 mmol/L NaCl, 20 μmol/L aprotinin, 20 μmol/L leupeptin, 0.2 mmol/L NaF, 0.2 mmol/L sodium pyrophosphate, and 0.2 mmol/L sodium vanadate. Plasma membrane bound proteins were captured by 4 h room temperature incubation with 2 units streptavidin bound to agarose beads (Pierce). The streptavidin beads were then washed four times with lysis buffer by centrifugation. Streptavidin-bound proteins were solubilized by boiling in NuPage LDS sample buffer (Invitrogen) with 0.05 mol/L DTT and separated by SDS-PAGE before being transferred to a polyvinylidene fluoride membrane, preincubated with a blocking buffer (Invitrogen) in PBS for 30 min at 4°C to label cell surface proteins. The cells were washed with PBS containing 100 mmol/L glycine at 4°C to quench unreacted biotin before being harvested in 200 μL lysis buffer containing 50 mmol/L Tris (pH 7.5), 1% Triton X-100, 0.5% NP-40, 2 mmol/L EDTA, 150 mmol/L NaCl, 20 μmol/L aprotinin, 20 μmol/L leupeptin, 0.2 mmol/L NaF, 0.2 mmol/L sodium pyrophosphate, and 0.2 mmol/L sodium vanadate. Plasma membrane bound proteins were captured by 4 h room temperature incubation with 2 units streptavidin bound to agarose beads (Pierce). The streptavidin beads were then washed four times with lysis buffer by centrifugation. Streptavidin-bound proteins were solubilized by boiling in NuPage LDS sample buffer (Invitrogen) with 0.05 mol/L DTT and separated by SDS-PAGE before being transferred to a polyvinylidene fluoride membrane, preincubated with a blocking buffer of 137 mmol/L NaCl, 2.7 mmol/L KCl, 897 mmol/L CaCl₂, 491 mmol/L MgCl₂, 3.4 mmol/L Na₂HPO₄, 593 mmol/L KH₂PO₄, and 5% bovine serum albumin, and incubated overnight with anti-Glut1 (Chemicon) or anti-Glut4 (Cell Signaling) polyclonal antibody. Detection was performed using donkey anti-rabbit IgG peroxidase-coupled secondary antibody and the Renaissance chemiluminescence system on Kodak X-Omat Blue ML films (Eastman Kodak).

**In vivo Glucose Uptake**

In vivo glucose uptake was measured by [¹⁸F]2-deoxyglucose (FDG) positron emission tomography (PET) imaging. scid mice with 300 mm³ s.c. A-549 NSCLC xenografts in the left back shoulder were dosed 7 days with 9 μg/kg pioglitazone. The mice were fasted overnight and injected with 100 μCi FDG by tail vein 45 min before imaging. A whole-animal field of view was obtained over 10 min on a micro-PET R4 (Concorde Microsystems) under 2% isoflurane inhalation anesthesia. Maximum tissue (tumor, hind leg muscle) was measured in triplicate with standardized

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**Figure 4.** Effect of PX-866 and pioglitazone on FDG-PET imaging in normal tissue and tumor xenografts in mice. A, female scid mice with 300 mm³ s.c. A-549 NSCLC xenografts were administered vehicle alone (control), pioglitazone 20 mg/kg p.o. q.d. × 5 starting 5 d before the study, PX-866 9 mg/kg i.v., or the combination of pioglitazone treatment and PX-866. Four hours later, FDG PET imaging was done as described in Materials and Methods. Dorsal views are shown with the head uppermost. B, signal from muscle was normalized to the signal from the brain in each animal and expressed as a percent of the mean control. Mean ± SE of 4 animals. *; P < 0.05, PX-866 and pioglitazone-treated tumor compared with pioglitazone-treated tumor. C, signal from muscle was normalized to the signal from the brain in each animal and expressed as a percent of the mean control. Mean ± SE of 4 animals. *; P < 0.05, PX-866 and pioglitazone-treated animal compared with the pioglitazone-treated animal.
(3 pixel diameter for tumor, 7 pixels for muscle) two-dimensional circular regions-of-interest on contiguous transverse image projections with ASIProVM software (Concorde Microsystems). Tissue tracer uptake was expressed as a ratio relative to baseline brain FDG uptake measured in triplicate with 7 pixel two-dimensional regions-of-interest on contiguous transverse projections.

Results

PX-866 Decreases Glucose Tolerance in Mice

We have reported previously that a single dose of the PI3K inhibitor PX-866 administered acutely to mice causes a decrease in glucose tolerance (14). We now show that chronic administration of PX-866 to mice over 30 days, to model the chronic administration of PX-866 used clinically, causes an increase in blood glucose from 50 to 220 mg/dL associated with a decrease in glucose tolerance measured 24 h after the last dose in fasted mice. This decrease in glucose tolerance was reversible with a partial recovery 5 days after the last treatment with PX-866 and a complete recovery with a glucose tolerance similar to the control mice by 12 days (Fig. 1A). The decrease in glucose tolerance was accompanied by an increase in serum insulin in both nonfasted and fasted mice, the nonfasted animals having a slower increase in serum insulin than the fasted animals (Fig. 1B). The results suggests that the increase in serum glucose by PI3K inhibition is due to insulin insensitivity as seen in type 2 diabetes and as would be expected from the dependency of insulin activity on PI3K signaling (17).

Pioglitazone Restores Normal Glucose Uptake in Mice Without Affecting Antitumor Activity in Response to PX-866 Treatment

The effect of pioglitazone on the antitumor activity of PX-866 was studied in A-549 NSCLC and HT-29 colon cancer xenografts (Fig. 2A and B). We observed no antitumor activity due to pioglitazone alone and a comparable response between PX-866-treated mice and mice treated with PX-866 and pioglitazone. Mice were allowed unrestricted access to food while treated with vehicle, PX-866, pioglitazone, or a combination of PX-866 and pioglitazone for 30 days (Fig. 2B). Twenty-four hours after the last treatment, blood glucose was measured in each group. The group treated with PX-866 alone had a blood glucose level significantly higher than control (214 compared with 105 mg/dL), whereas both the pioglitazone and combination groups had a glucose levels similar to control (Fig. 2C).

2-Deoxyglucose Uptake in Cells

The effect of PX-866 and pioglitazone on the uptake of the nonmetabolizable 2-14C-2-deoxy-glucose was studied in L-6 muscle cells and A-549 NSCLC cells. Preliminary studies showed that 2-deoxyglucose uptake was linear in both L-6 and A-549 cells for at least 2 h (data not shown). With L-6 muscle cells in serum-free medium, insulin stimulated 2-deoxyglucose uptake, whereas PX-866 decreased the uptake (Fig. 3A, top). Pioglitazone alone did not significantly alter 2-deoxyglucose uptake but blocked the decrease in 2-deoxyglucose uptake caused by PX-866 in the L-6 cells. In contrast, 2-deoxyglucose uptake by A-549 tumor cells was insensitive to insulin, PX-866, pioglitazone, or the combination of PX-866 and pioglitazone (Fig. 3A, bottom). Western blotting following labeling of cell surface proteins with non-cell-permeant biotin showed the presence of the glucose transporter Glut4 at the plasma membrane in differentiated L-6 myoblasts exposed to insulin but not with A-549 NSCLC cells (Fig. 3B). PX-866 inhibited the expression of Glut4 in the L-6 myoblasts, which was reversed by pioglitazone treatment. Glut1 was expressed at the plasma membrane of both L-6 myoblasts and A-549 NSCLC cells. In L-6 myoblasts, Glut1 was decreased by PX-866, an effect that was not reversed by pioglitazone. In A-549 NSCLC cells, Glut1 levels were not affected by either PX-866 or pioglitazone. Glut4 could not be detected in A-549 NSCLC cells. PX-866 inhibited cellular PI3K signaling measured by phospho-Ser473-Akt in both L-6 myoblasts and A-549 NSCLC cells (Fig. 3B).

FDG Accumulation In vivo

The effect of PX-866 and pioglitazone on the accumulation of FDG measured by PET imaging was studied in normal tissue and A-549 NSCLC xenografts in mice (Fig. 4A). The accumulation of FDG in the tissues was normalized to FDG accumulation in the brain, a high glucose uptake tissue that shows minimal insulin sensitivity (18). PX-866 treatment showed a trend toward decreased FGD uptake in normal tissue, but the effects were not significant (Fig. 4B). Pioglitazone treatment significantly increased FDG accumulation by the normal tissue, an effect that was reversed by PX-866 to a level that was not significantly different to the nontreated control value (Fig. 4B). In A-549 NSCLC xenografts, pioglitazone had no effect on FDG uptake, whereas PX-866 decreased FDG uptake, which was not reversed by pioglitazone (Fig. 4C).

Discussion

A hallmark on-target side effect of PI3K/Akt inhibitors is an increase in blood glucose due to inhibition of insulin signaling (2, 3). The toxicity is similar to type 2 diabetes and could potentially limit the use of such agents, particularly for long-term use. We have investigated ways that might be used clinically to limit this toxicity without inhibiting the antitumor activity of the agents. To inhibit PI3K, we used PX-866, a selective inhibitor of the type 1 PI3K family that has recently entered phase I clinical trial. PX-866 has been shown to down-regulate tumor phospho-Akt and to exhibit antitumor activity in several human tumor xenograft models when administered either i.v. or p.o. (19). Previous studies have shown that PX-866 at the doses used in this study inhibits PI3K measured by phospho-Akt levels in tumor and in normal tissue (20).

Insulin signaling is relayed predominantly through PI3K p110α, although other PI3K class I isoforms have been implicated in specific tissues (4, 21). We have found that prolonged administration of PX-866 to mice decreases

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glucose tolerance and, at the same time, plasma insulin levels increase, suggesting a diminished sensitivity to insulin. This is similar to type 2 diabetes and also similar to the phenotype of mice deficient in the Akt2 isoform that show marked hyperglycemia, hyperinsulinemia, and an impaired ability of insulin to lower blood glucose (8). We found that the thiazolidinedione drug pioglitazone, used to treat type 2 diabetes, reverses the inhibitory effects of both acute and chronic PX-866 administration on glucose tolerance. However, another drug used to treat type 2 diabetes, metformin, did not reverse the decrease in glucose tolerance by PX-866 (2).

Thiazolidinediones are ligands for the PPARγ transcription factor present at high levels in adipose tissue, and they sensitize the body to the effects of insulin (22). An important question remaining to be answered was whether pioglitazone also inhibits the antitumor activity associated with PI3K inhibition by PX-866. PPARγ activation has been reported to have tumor-suppressing effects of its own through experiments using genetic manipulation of the target (23). PPARγ activation by thiazolidinediones, including pioglitazone, has been reported to inhibit the growth of some tumors including A-549 NSCLC xenografts in scid mice and to augment the effects of cytotoxic agents (24, 25). However, this activity could not be confirmed in clinical trials using this class of drugs, which have been largely disappointing (26, 27). In our study, we found that pioglitazone by itself did not affect the growth of A-549 NSCLC or HT-29 colon cancer xenografts and did not block the antitumor effects of PX-866 but did restore insulin signaling in treated mice.

Studies in cells using the nonmetabolizable glucose analogue 2-deoxyglucose showed that PX-866 significantly inhibited glucose uptake in L-6 rat differentiated myotubes and that the inhibition was reversed by pioglitazone. There was no effect of either PX-866 or pioglitazone on glucose uptake by A-549 NSCLC cells. Glut4 is the primary glucose transporter in muscle and fat where it translocates to the cell membrane from intracellular stores in response to insulin (28, 29). The expression of cell membrane Glut1 is not insulin-responsive and is seen in both normal and transformed tissues (30). Early studies showed that cells transformed with an active Ras or Src oncogene showed pronounced up-regulation of the Glut1 high-affinity glucose transporter and that tumors showed dramatically increased glucose uptake, famously noted by Warburg (34, 35). Our current study serves to confirm that cancer cells rely on high-affinity glucose transporters such as Glut1 to maximize glucose uptake, whereas insulin-responsive tissues rely on Glut4. Pioglitazone restores insulin-induced Glut4 translocation in normal tissues without directly affecting cancer cells that rely on more efficient but less regulated mechanisms for glucose uptake. It is unlikely that the antitumor activity of PX-866 relies solely on the decrease in glucose uptake by tumors, but it may contribute to the inhibition of the many other well-known effects of tumor PI3K on tumor cell survival and proliferation or serve as a marker for these processes (1–3).

In summary, we have shown that thiazolidinedione PPARγ agonist pioglitazone can prevent the increase in blood glucose caused by the PI3K inhibitor PX-866. We have also shown that pioglitazone does not inhibit the antitumor activity of PX-866. Neither PX-866 nor pioglitazone in combination with PX-866 showed altered glucose accumulation by tumor cells in vitro. In vivo, a modest inhibition in the FDG glucose was seen, which was not reversed by the addition of pioglitazone. However, PX-866 inhibited insulin-sensitive glucose uptake by normal tissue, and the inhibition was overcome by pioglitazone both in vitro and in vivo, suggesting a mechanism by which pioglitazone is able to prevent the increase in blood uptake with pioglitazone. This is contrary to what was seen in cell culture, a phenomena that may result from the reported differential effects of PX-866 in two- and three-dimensional cultures (31) or the well-established relationship between PI3K signaling and angiogenesis (32). Pioglitazone increased the uptake of glucose by normal tissue, presumably muscle and adipose tissue, which are known to take up glucose in response to insulin (33). PX-866 showed a small inhibition of glucose uptake by normal tissue by itself and reversed the increase by pioglitazone to values that were the same as in untreated animals.

Taken together, the results suggest that PX-866 inhibits insulin signaling, thus blocking the ability of muscle and presumably other normal tissues, to take up glucose, resulting in hyperglycemia. Pioglitazone, a thiazolidinedione, increases sensitivity to insulin through modulation of the PPARγ response or other mechanisms (22). Pioglitazone is able to prevent the decreased glucose uptake by muscle and other normal tissue by PX-866, thus restoring normal blood glucose levels. In contrast to normal tissue, PX-866 decreased glucose uptake by tumor and neither the decreased glucose uptake or the antitumor activity of PX-866 was blocked by pioglitazone. Glut4 is the primary glucose transporter in muscle and fat where it translocates to the cell membrane from intracellular stores in response to insulin (28, 29). The expression of cell membrane Glut1 is not insulin-responsive and is seen in both normal and transformed tissues (30).
glucose associated with PI3K signaling inhibition without altering antitumor activity in vivo.

Disclosure of Potential Conflicts of Interest

L. Kirkpatrick: founder and employee of Prox Pharmaceuticals (now Oncothryone) who has the licensed right to PX-866. G. Powis: founder of Prox Pharmaceuticals and consultant to and stockholder on Oncothyreon. P. Wipf: named as inventor on patent for PX-866.

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Molecular Cancer Therapeutics

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