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Ameliorating effect of FK614, a novel nonthiazolidinedione peroxisome proliferator-activated receptor γ agonist, on insulin resistance in Zucker fatty rat

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Abstract

Effect of 3-(2,4-dichlorobenzyl)-2-methyl-*N*-(pentylsulfonyl)-3*H*-benzimidazole-5-carboxamide (FK614), a novel nonthiazolidinedione peroxisome proliferator-activated receptor (PPAR) γ agonist, on glucose tolerance and insulin resistance in peripheral tissues and in liver using Zucker fatty rats (genetically obese and insulin-resistant) was evaluated and compared to other insulin sensitizers. FK614 (0.32, 1 and 3.2 mg/kg), two thiazolidinedione PPAR γ agonists, rosiglitazone (0.1, 0.32, 1 and 3.2 mg/kg) and pioglitazone (1, 3.2 and 10 mg/kg), and a biguanide, metformin (320 and 1000 mg/kg), were orally administered to Zucker fatty rats once a day for 14 days. Zucker fatty rats treated with FK614 and rosiglitazone were subjected to evaluation by oral glucose tolerance test. Ameliorating effect of each compound on peripheral and hepatic insulin resistance was evaluated using a euglycemic–hyperinsulineamic clamp procedure. FK614 and rosiglitazone dose-dependently improved impaired glucose tolerance in Zucker fatty rats. In addition, FK614 dose-dependently ameliorated peripheral and hepatic insulin resistance in Zucker fatty rats, with the degree of its effect in peripheral tissues almost equivalent to that in liver when compared at each dose tested. Similar data indicating ameliorating effects on insulin resistance was obtained for rosiglitazone and pioglitazone. Metformin showed less potent effects than other insulin sensitizers and its effect in liver tended to be greater than that in peripheral tissues. These findings suggest clinical potential for FK614 as a treatment of type 2 diabetes, acting by ameliorating insulin resistance both in peripheral tissues and liver.

Keywords: FK614; Nonthiazolidindione; Insulin sensitizer; Zucker fatty rat; PPAR (peroxisome proliferator-activated receptor) γ

1. Introduction

Insulin resistance is a major pathophysiological factor in the development of type 2 diabetes, occurring in peripheral tissues (muscle and adipose tissues) and liver, leading to reduced glucose uptake and utilization and increased glucose production, respectively. Amelioration of insulin resistance in both the peripheral tissues and liver is thought to be a reasonable treatment of type 2 diabetes. Recently, two thiazolidinedione derivatives, rosiglitazone and pioglitazone, insulin sensitizers available for clinical use in treatment of type 2 diabetes, were shown to improve glycemic control by ameliorating insulin resistance in both peripheral tissues and liver in type 2 diabetic patients (Bajaj et al., 2003; Tiikkainen et al., 2004; Miyazaki et al., 2001a,b, 2002a,b, 2003). Thiazolidinedione derivatives are agonists for peroxisome proliferator-activated receptor (PPAR) γ, a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors that regulates expression of genes closely related to adipocyte differentiation, glucose and lipid metabolism and appear to function as insulin sensitizers mainly through this nuclear receptor activation (Spiegelman, 1998).

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A novel nonthiazolidinedione PPAR γ agonist, 3-(2,4-dichlorobenzyl)-2-methyl-*N*-(pentylsulfonyl)-3*H*-benzimidazole-5-carboxamide (FK614), shows potent antidiabetic effects in *db*/*db* mice, a representative animal model of type 2 diabetes (Minoura et al., 2004). In addition, FK614 improves impaired glucose tolerance in *ob*/*ob* mice without enhancing insulin secretion (Minoura et al., 2004), suggesting it has the ability to improve insulin sensitivity. However, it is not clear whether FK614 ameliorates insulin resistance in peripheral tissues or liver. To answer this question, ameliorating effect of FK614 on insulin resistance in the peripheral tissues and liver was investigated in Zucker fatty rat, an animal with insulin resistance in both the peripheral tissues and liver (Terrettaz and Jeanrenaud, 1983). FK614 was compared with two thiazolidinedione PPAR γ agonists, rosiglitazone and pioglitazone, and a biguanide, metformin, whose mechanism of action is thought to involve suppression of endogenous glucose production mainly by the liver (Cusi et al., 1996; Inzucchi et al., 1998; Stumvoll et al., 1995). Evaluation using a euglycemic–hyperinsulineamic clamp procedure indicated that FK614, as well as rosiglitazone and pioglitazone, potently ameliorates both peripheral and hepatic insulin resistance in Zucker fatty rats.

2. Materials and methods

2.1. Compounds

FK614, rosiglitazone (5-(4-{2-[methyl(2-pyridinyl)amino]ethoxy}benzyl)-1,3-thiazolidine-2,4-dione) and pioglitazone (5-[4-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl]-2,4-thiazolidinedione) were all synthesized at Fujisawa Pharmaceutical Co., Ltd. Metformin (1,1-dimethylbiguanide hydrochrolide) was purchased from Sigma (St. Louis, MO, USA).

2.2. Animals and treatment

2.2.1. Animals

Male Zucker fatty (*fa*/*fa*) and lean littermates Zucker lean (*FA*/*?*) were purchased from Japan SLC, Inc. (Shizuoka, Japan) at 11 or 12 weeks of age. Animals were maintained on standard laboratory chow and water ad libitum.

All experimental procedures using animals were performed under the guidelines of the Animal Experiment Committee of Fujisawa Pharmaceutical Co., Ltd.

2.2.2. Drug administration

All drugs were suspended or dissolved in 0.5% (*w*/*v*) methylcellulose in water and given orally to rats at a volume of 5 ml/kg of body weight. Drug administration was started at 14 weeks of age and drugs were administered once a day for 14 days. Zucker fatty rats were given 0.32, 1 or 3.2 mg/kg FK614 or 0.1, 0.32, 1 or 3.2 mg/kg rosiglitazone to perform the glucose tolerance test. In addition, FK614, rosiglitazone, pioglitazone and metformin were administered to Zucker fatty rats to evaluate effect of each drug on insulin resistance by means of a euglycemic–hyperinsulinaemic clamp procedure. Doses tested were 0.32, 1 and 3.2 mg/kg for FK614, 0.1, 0.32 and 1 mg/kg for rosiglitazone, and 1, 3.2 and 10 mg/kg for pioglitazone in experiment 1, plus 320 and 1000 mg/kg for metformin and 3.2 mg/kg for rosiglitazone in experiment 2. In each experiment, vehicle (0.5% (*w*/*v*) methylcellulose in water) was administered to Zucker lean (lean control) and control Zucker fatty (fatty control) rats.

2.3. Glucose tolerance test

Glucose tolerance test was performed after overnight fasting, on the day after final drug treatment. Rats received orally 2 g/kg glucose (5 ml/kg) and blood sample was taken before, 30, 60, 120 and 180 min after glucose loading via tail vein using heparinized glass capillaries.

2.4. Euglycemic–hyperinsulinaemic clamp procedure

The clamp procedure was performed according to methods described by Tominaga et al. (1992) and Vettor et al. (1994) with minor modifications. After overnight fasting, on the day after final drug treatment, rats were anesthetized with pentobarbital given intraperitoneally (50 mg/kg) and anesthesia was maintained by giving additional pentobarbital until the end of the clamp procedure. Ventilation was performed by inserting a catheter into the respiratory tract. Catheters were inserted in both sides of femoral veins for insulin and glucose infusions and one more catheter was cannulated into one femoral artery for blood sampling. Body temperature was kept at 37∼38 °C by using a heating pad and monitoring with a rectal probe. Infusion of [^{14}C]–glucose (D-[U–^{14}C]glucose, 310 mCi/mmol, Amersham LIFE SCIENCE, Amersham, U.K.) comprised a priming bolus (10 μCi/0.1 ml in saline) and constant infusion (25 μCi/ml in saline × 0.4 ml/h) was started after stabilizing body temperature. At 55 and 60 min after starting infusion of [^{14}C]–glucose, blood sample was taken, then basal plasma glucose level was determined in whole blood sample using a glucose analyzer ANTSENSE II (Bayer-Sankyo Co., Ltd., Tokyo, Japan) by an immobilized enzyme membrane/H_2O_2 electrode method. After obtaining plasma samples, basal plasma insulin level was measured and [^{14}C]–glucose radioactivity was determined as described below. Infusion of insulin (Humulin R, Eli Lilly Japan Co., Ltd., Hyogo, Japan) diluted with saline was performed 60 min after starting infusion of [^{14}C]–glucose and the time at which infusion of insulin was started was designated 0 min. Insulin was intravenously infused at rates of 180 (0∼3 min), 120 (3∼6 min), 75 (6∼10 min) and 60 mU/kg/min (10∼min). Blood samples were drawn at 4-min intervals for determination of plasma glucose level from 10 min after starting infusion of insulin. Glucose solution (40% *w*/*v*) was infused at an adequate rate to maintain plasma glucose level at approximately 100 mg/dl. After confirming that plasma glucose level was maintained at approximately 100 mg/dl (after more than about 60 min), three consecutive samples of blood were collected for determination of plasma glucose, insulin levels, and radioactivity before the end of the clamp procedure.

A 50 μl plasma sample was mixed with 75 μl of 0.15 M $Ba(OH)_2$ and 75 μl of 0.15 M $ZnSO_4$, then centrifuged after leaving at room temperature for 20 min to obtain supernatant. A 150 μl supernatant was applied to AG anion exchange resin (AG2-X8, Bio-Rad Laboratories, CA, USA) in disposable small columns (0.6 × 1.5 cm, Bio-Spin Disposable Chromatography Columns, Bio-Rad Laboratories) and [^{14}C]–lactate was trapped in AG2

resin. After applied sample had been drained through the resin, 0.75 ml of distilled water was applied twice to elute [^{14}C]–glucose. Radioactivity of the eluate mixed with aqueous scintillant (Aquazol-2, Packard Instrument Company Inc., Meriden, CT, USA) was counted by using a liquid scintillation analyzer (1900TR or 2200CA, Packard Instrument Company Inc., CT, USA)).

2.5. Analytical methods

For samples obtained during the glucose tolerance test, plasma glucose was determined by a mutarotase•glucose oxidase method using a commercial kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma glucose level was determined in whole blood sample using a glucose analyzer ANTSENSE II by an immobilized enzyme membrane/H_2O_2 electrode method during the clamp procedure as described above. Plasma immunoreactive insulin was measured by a commercial insulin enzyme-linked immunosorbent assay kit (Morinaga Institute of Biological Science, Kanagawa, Japan) using rat insulin standards for all samples.

2.6. Calculations

Hepatic glucose production and glucose disappearance rate (peripheral glucose utilization) were calculated using Steele's equation (Steele, 1959). Percentage of suppression of hepatic glucose production by insulin was calculated as follows: Percentage of suppression of hepatic glucose production by insulin = (hepatic glucose production during basal state − hepatic glucose production during clamp state)/hepatic glucose production during basal state × 100. Percentage of improvement in insulin sensitivity in peripheral tissues and liver in rats treated with each drug was calculated by considering mean value of peripheral glucose utilization and percentage of suppression of hepatic glucose production by insulin, respectively, with lean control designated as 100 and fatty control 0.

2.7. Statistical analysis

Data are presented as mean ± S.E.M. Student-*t* or Aspin–Welch test was used to determine significance of differences between lean control and fatty control rats. Dunnett's multiple comparisons were used between fatty control and drug-treated fatty rats. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of FK614 and rosiglitazone on glucose tolerance in Zucker fatty rats

To evaluate the effect of FK614 on impaired glucose tolerance compared to rosiglitazone, Zucker fatty rats were orally treated with FK614 (0.32, 1 or 3.2 mg/kg) or rosiglitazone (0.1, 0.32, 1 or 3.2 mg/kg) once a day for 14 days, then the glucose tolerance test was performed on the day after final drug treatment. When 2 g/kg glucose was orally loaded into control Zucker fatty (fatty control) and lean littermate (lean control) rats after overnight fasting, plasma glucose levels at all time points in fatty control rats were significantly higher than those in lean control rats (Fig. 1A, B). Therefore, area under the curve for blood glucose levels in fatty control rats was significantly higher than that in lean control rats (Fig. 1C). In other words, glucose tolerance was impaired in Zucker fatty rats. Both FK614 and rosiglitazone dose-dependently reduced plasma glucose levels and area under the curve for plasma glucose levels and so improved impaired glucose tolerance in Zucker fatty rats. Area under the curve for plasma glucose levels in fatty rats treated with 1 or 3.2 mg/kg FK614 was 76% or 74% and with 1 or 3.2 mg/kg rosiglitazone was 79% or 72% of that in fatty control rats, respectively. Plasma insulin levels at all time points in fatty control rats were significantly higher than those in lean control rats (Fig. 1D, E) and so area under the curve for plasma insulin levels in fatty control rats was significantly higher than that in lean control rats (Fig. 1F). Both drugs dose-dependently reduced plasma insulin levels at all time points and area under the curve for plasma insulin levels. Area under the curve for plasma insulin levels in fatty rats treated with 1 or 3.2 mg/kg FK614 was 33% or 26% and with 1 or 3.2 mg/kg rosiglitazone was 44% or 37% of that in fatty control rats, respectively. Therefore, at higher doses (1 or 3.2 mg/kg), FK614 more potently reduced insulin demand than rosiglitazone to maintain improved glucose tolerance.

3.2. Effects of FK614, rosiglitazone, pioglitazone and metformin on insulin resistance in Zucker fatty rats—evaluation by euglycemic–hyperinsulinaemic clamp procedure

To investigate the effects of FK614, two thiazolidinedione PPAR γ agonists, rosiglitazone and pioglitazone, and an anti-diabetic biguanide, metformin, on insulin resistance in Zucker fatty rats, two experiments were performed separately. In experiment 1, FK614, rosiglitazone and pioglitazone were evaluated at doses of 0.32, 1 or 3.2 mg/kg, 0.1, 0.32 or 1 mg/kg and 1, 3.2 or 10 mg/kg, respectively. In experiment 2, rosiglitazone at a higher dose, 3.2 mg/kg, and metformin at 320 or 1000 mg/kg were evaluated. In each experiment, rats were orally treated with each drug once a day for 14 days and the clamp procedure was performed on the day after final drug treatment.

3.2.1. Body weight, plasma glucose and insulin levels during basal and clamp state (Table 1)

FK614, rosiglitazone and pioglitazone showed a tendency to increase body weight. In contrast, metformin showed a tendency to decrease body weight at a dose of 1000 mg/kg. All drugs dose-dependently reduced plasma glucose and insulin levels during basal state (fasting plasma glucose and insulin levels under pentobarbital anesthesia), which were significantly higher in fatty control rats than those in lean control rats. In experiment 1, mean values of plasma glucose levels during clamp state in all groups ranged from 103 to 112 mg/dl so that plasma glucose level during the clamp state in each group was almost the same as normal plasma glucose level. In experiment 2, mean values of plasma glucose levels during the clamp state in all groups ranged from 103 to 128 mg/dl, with levels during the clamp state slightly higher in fatty control rats and rats treated with 320 mg/kg metformin (127 ± 11 and 128 ± 13 mg/dl, respectively) than in other groups. However, no significant difference was observed between lean and fatty control rats or between fatty control rats and drug-treated fatty rats in experiment 2. Plasma insulin level during the clamp state in fatty control rats was significantly higher than that in lean control rats in both experiment 1 and 2. In addition, no significant difference was observed between fatty

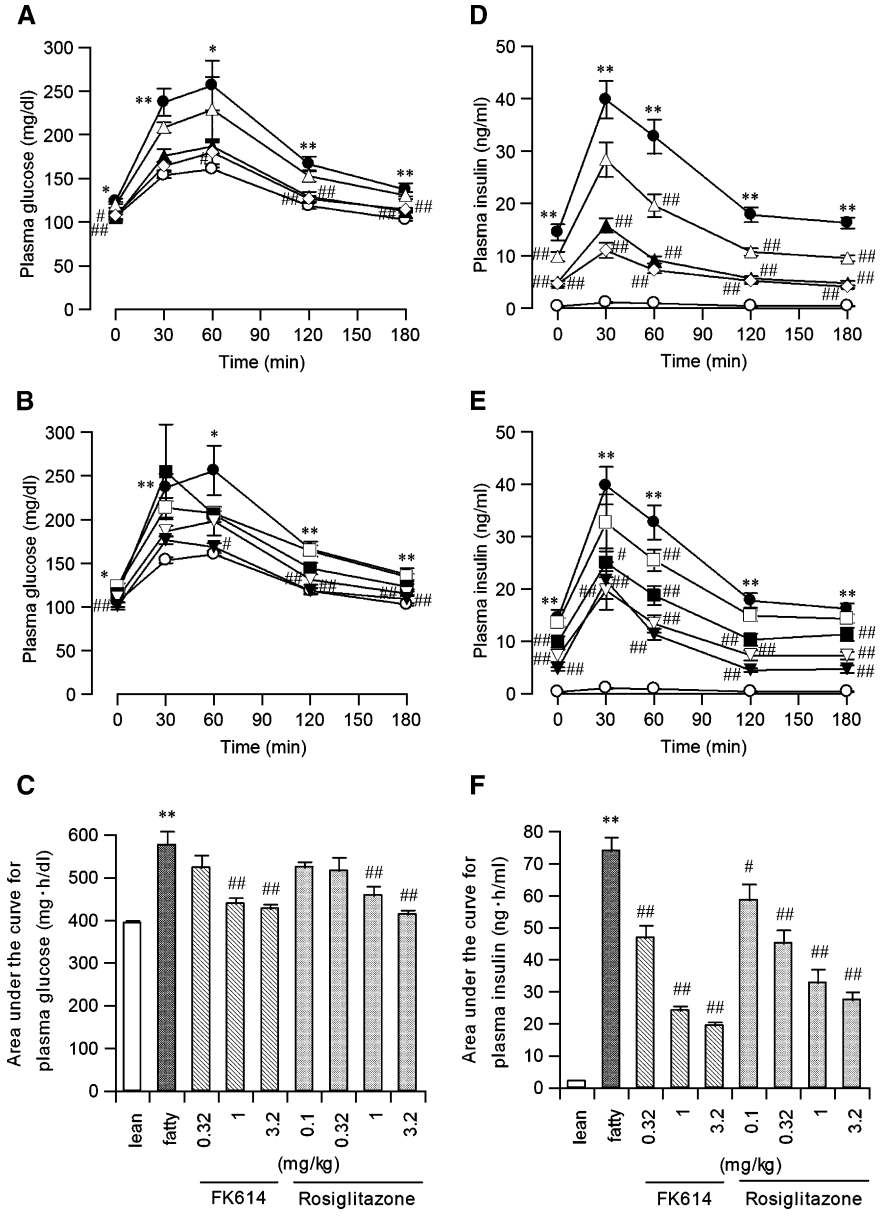

Fig. 1. Effects of FK614 (A, D) and rosiglitazone (B, E) treatment on plasma glucose (A, B), plasma insulin (D, E), and AUC for plasma glucose (C) and plasma insulin (F) during the oral glucose tolerance test in Zucker fatty rats. ○ Lean control; ● fatty control; △ fatty FK614 0.32 mg/kg; ▲ fatty FK614 1 mg/kg; ◇ fatty FK614 3.2 mg/kg; □ fatty rosiglitazone 0.1 mg/kg; ■ fatty rosiglitazone 0.32 mg/kg; ▽ fatty rosiglitazone 1 mg/kg; and ▼ fatty rosiglitazone 3.2 mg/kg. Drugs were orally administered for 14 days. On the day after final drug treatment, rats received 2 g/kg glucose (5 ml/kg) orally. Data are shown as mean±S.E.M. ($N=8$). *$P<0.05$, **$P<0.01$ versus lean control. $^{\#}P<0.05$ and $^{\#\#}P<0.01$ versus fatty control.

control rats and drug-treated fatty rats for plasma insulin levels during the clamp state.

3.2.2. Effect on insulin action in peripheral tissues (Fig. 2)

Results for peripheral glucose utilization during the clamp state are shown in Fig. 2. Peripheral glucose utilization in fatty control rats (experiment 1: 6.80±0.24 mg/kg/min; experiment 2: 6.18±0.21 mg/kg/min) was significantly lower than that in lean control rats (experiment 1: 14.55±0.75 mg/kg/min; experiment 2: 12.72±0.46 mg/kg/min). Thus, insulin-dependent peripheral glucose utilization was decreased in Zucker fatty rats. FK614 dose-dependently increased peripheral glucose utilization and the value in rats treated with 1 or 3.2 mg/kg (11.12±0.40 or 12.44±0.58 mg/kg/min, respectively) was significantly higher than that in fatty control rats. Rosiglitazone or pioglitazone also increased peripheral glucose utilization and significant increases were observed in rats treated with 1 or 3.2 mg/kg rosiglitazone (8.86±0.43 (experiment 1) or 11.24±0.40 (experiment 2) mg/kg/min, respectively) and 3.2 or 10 mg/kg pioglitazone (9.83±0.36 or 12.99±0.55 mg/kg/min, respectively). Although metformin significantly increased peripheral glucose utilization at 1000 mg/kg (7.17±0.19 mg/kg/min), its effect was much weaker than that of other drugs.

3.2.3. Effect on insulin action in liver (Fig. 3)

Hepatic glucose production during basal (fasting) state in fatty control rats (experiment 1: 7.62±0.47 mg/kg/min; experi-

Table 1
Body weight, plasma glucose and insulin levels during basal and clamp state

Group		N	Body weight (g)	Plasma glucose (mg/dl)		Plasma insulin (ng/ml)	
				Basal	Clamp	Basal	Clamp
Experiment 1							
Zucker lean (lean control)		8	292.0±6.8	132±5	106±1	1.8±0.1	346.6±36.8
Zucker fatty (fatty control)		9	493.7±12.5[a]	177±10[a]	112±3	26.0±5.4[a]	614.6±89.3[b]
+FK614	0.32 mg/kg	8	515.4±10.2	164±5	107±1	21.3±2.3	525.8±83.1
+FK614	1 mg/kg	8	507.2±12.2	140±4[c]	103±1[d]	11.2±1.5[c]	408.5±37.4
+FK614	3.2 mg/kg	7	531.9±17.2	120±8[c]	106±2	8.3±1.0[c]	420.1±36.4
+rosiglitazone	0.1 mg/kg	8	503.4±13.2	176±5	112±5	24.9±2.6	568.7±75.1
+rosiglitazone	0.32 mg/kg	8	503.9±15.4	167±9	103±1[d]	21.8±3.1	636.2±95.3
+rosiglitazone	1 mg/kg	8	528.1±14.1	152±6	105±1	13.6±1.9	516.4±93.3
+pioglitazone	1 mg/kg	8	515.9±16.0	155±7	105±1	16.1±2.4	470.7±23.3
+pioglitazone	3.2 mg/kg	8	525.7±14.4	139±3[c]	104±1	11.9±0.8[c]	466.1±22.0
+pioglitazone	10 mg/kg	7	527.7±17.4	123±3[c]	106±1	7.9±0.8[c]	376.3±41.0
Experiment 2							
Zucker lean (lean control)		8	299.9±4.4	135±7	104±2	1.9±0.2	429.5±39.3
Zucker fatty (fatty control)		8	519.8±6.9[a]	192±6[a]	127±11	41.9±3.5[a]	838.3±126.0[b]
+metformin	320 mg/kg	9	516.9±7.8	205±13	128±13	33.9±3.0	682.1±44.4
+metformin	1000 mg/kg	9	495.4±9.3	157±3[d]	103±2	18.3±2.3[c]	746.8±144.7
+rosiglitazone	3.2 mg/kg	9	556.7±6.3[c]	132±4[c]	106±2	11.2±0.6[c]	499.5±19.4

Each drug was orally administered to Zucker lean and fatty rats (14 weeks) for 14 days. Euglycemic–hyperinsulinaemic clamp procedure was performed after overnight fasting on the day after final drug treatment. Data are shown as mean±S.E.M.

[a] $P<0.01$ versus lean control.
[b] $P<0.05$ versus lean control.
[c] $P<0.01$ versus fatty control.
[d] $P<0.05$ versus fatty control.

ment 2: 7.14±0.16 mg/kg/min) was significantly higher than that in lean control rats (experiment 1: 5.82±0.23 mg/kg/min; experiment 2: 5.64±0.21 mg/kg/min). No influence on hepatic glucose production during basal state was observed in any rats treated with a drug, except for rats treated with 3.2 mg/kg rosiglitazone, in which hepatic glucose production during basal state (8.28±0.17 mg/kg/min) was significantly higher than that in fatty control rats. In lean control rats, hepatic glucose production was markedly suppressed by insulin infusion during clamp state (experiment 1: from 5.82±0.23 to 1.23±0.40 mg/kg/min; experiment 2: from 5.64±0.21 to 1.74±0.61 mg/kg/min) and percentage of suppression of hepatic glucose production by

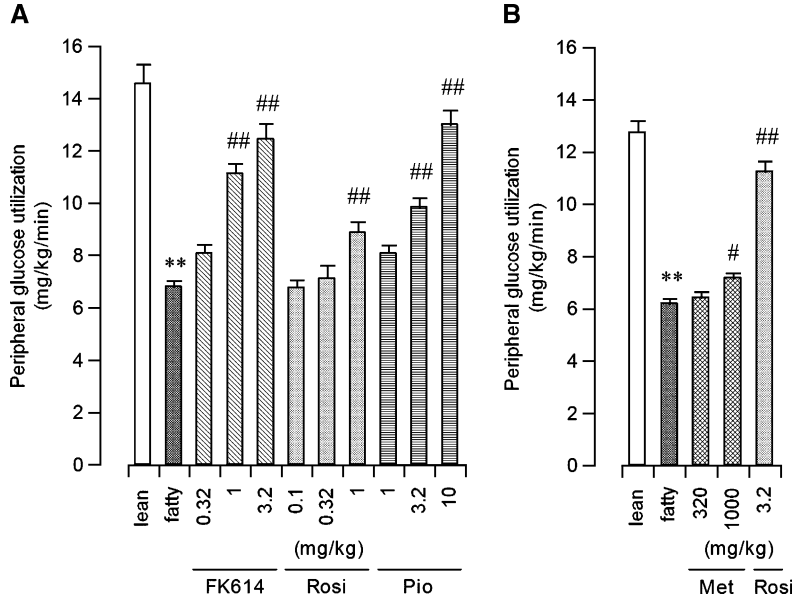

Fig. 2. Effects of FK614, rosiglitazone, pioglitazone and metformin on peripheral glucose utilization during clamp state. Drugs were orally administered for 14 days. Euglycemic–hyperinsulinaemic clamp procedure was performed after overnight fasting on the day after final drug treatment. Data are shown as mean±S.E.M. ($N=7–9$). Rosi, rosiglitazone; Pio, pioglitazone; and Met, metformin. $^{**}P<0.01$ versus lean control. $^{\#}P<0.05$ and $^{\#\#}P<0.01$ versus fatty control.

insulin was 76.6±6.4% and 66.6±7.8% in experiment 1 and 2, respectively. In contrast, only a weak suppression of hepatic glucose production by insulin was observed in fatty control rats (experiment 1: from 7.62±0.47 to 5.82±0.45 mg/kg/min; experiment 2: from 7.14±0.16 to 6.18±0.21 mg/kg/min) and percentage of suppression of hepatic glucose production by insulin was 23.1±5.5% and 13.2±3.5% in experiment 1 and 2, respectively. Hepatic glucose production during clamp state in Zucker fatty rats was reduced in a dose-dependent manner by treatment with FK614, therefore percentage of suppression of hepatic glucose production by insulin was increased. Significant changes in hepatic glucose production during clamp state and percentage of suppression of hepatic glucose production by insulin were observed in rats treated with 1 or 3.2 mg/kg FK614 (hepatic glucose production during clamp state: 3.29±0.61 or 2.56±0.43 mg/kg/min; percentage of suppression of hepatic glucose production by insulin: 54.9±7.6% or 63.8±5.6%, respectively), compared to values in fatty control rats. Rosiglitazone or pioglitazone also dose-dependently improved insulin action in liver and significant changes in hepatic glucose production during clamp state and percentage of suppression of hepatic glucose production by insulin were observed in rats treated with 1 or 3.2 mg/kg rosiglitazone (hepatic glucose production during clamp state: 3.64±0.43 (experiment 1) or 3.86±0.29 (experiment 2) mg/kg/min; percentage of suppression of hepatic glucose production by insulin: 46.2±5.9% (experiment 1) or 53.1±3.7% (experiment 2), respectively) or 3.2 or 10 mg/kg pioglitazone (hepatic glucose production during clamp state: 3.95±0.31 or 2.61±0.77 mg/kg/min; percentage of suppression of hepatic glucose production by insulin: 46.7±3.5% or 67.1±8.9%, respectively). Effect of metformin on insulin action in liver was weaker than that of other drugs; however, a significant increase in percentage of suppression of hepatic glucose production by insulin (31.9±6.9%) was observed in rats treated with 1000 mg/kg of metformin.

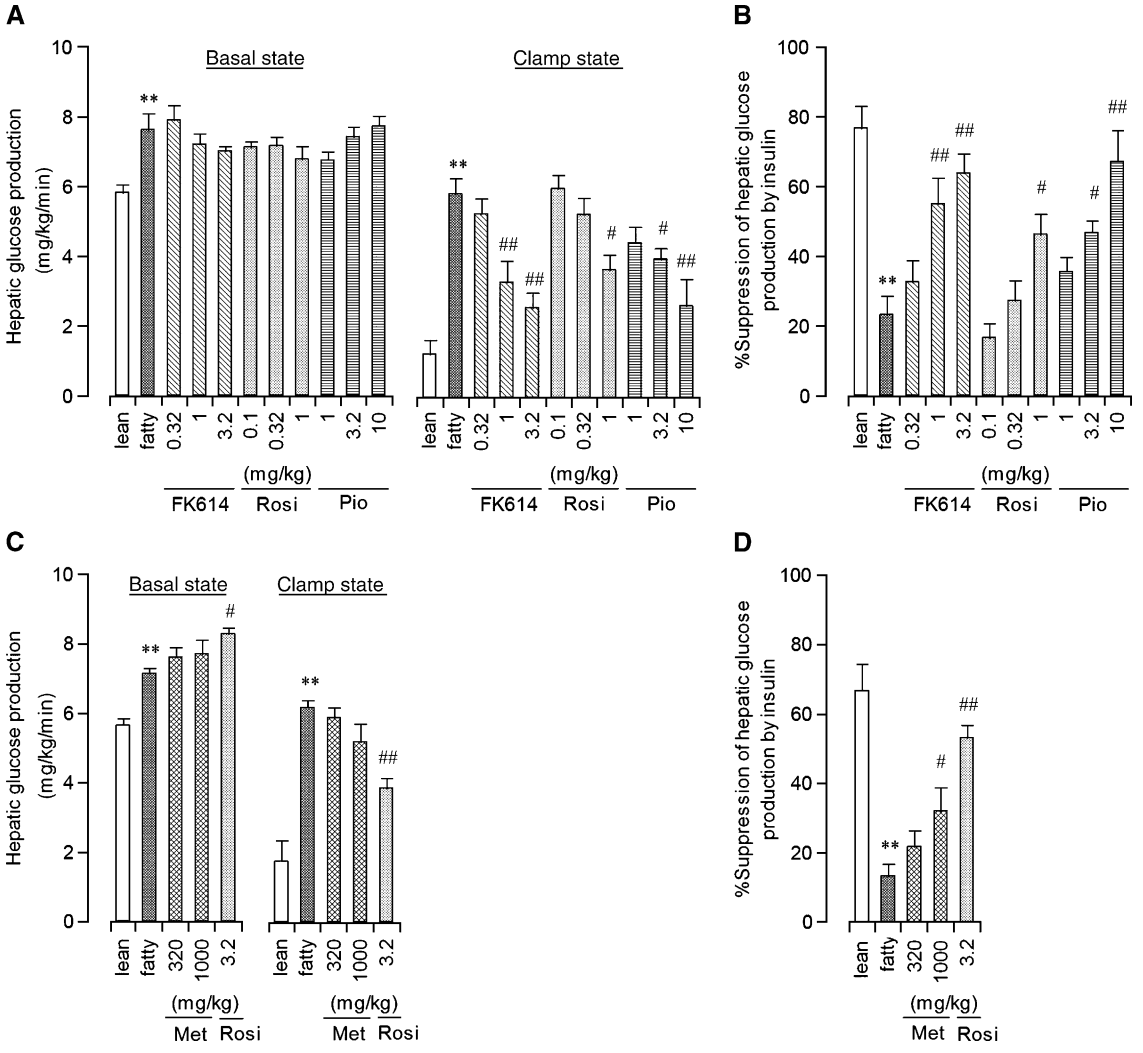

Fig. 3. Effects of FK614, rosiglitazone, pioglitazone and metformin on insulin action in liver. (A, C) Hepatic glucose production during basal and clamp state. (B, D) Percentage of suppression of hepatic glucose production by insulin. (A, B) Experiment 1. (C, D) Experiment 2. Drugs were orally administered for 14 days. Euglycemic–hyperinsulinaemic clamp procedure was performed after overnight fasting on the day after final drug treatment. Data are shown as mean±S.E.M. (N=7–9). Rosi, rosiglitazone; Pio, pioglitazone; and Met, metformin. **P<0.01 versus lean control. $^{\#}P$<0.05 and $^{\#\#}P$<0.01 versus fatty control.

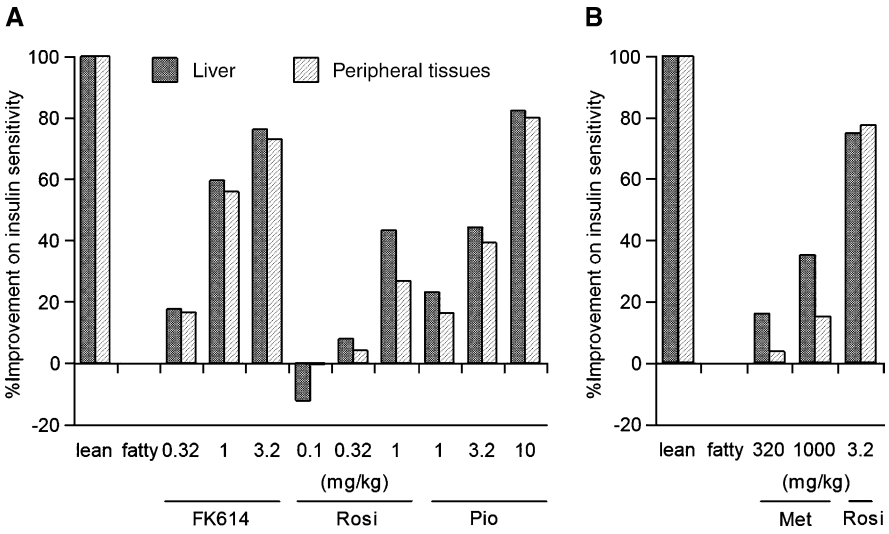

Fig. 4. Percentage of improvement by FK614, rosiglitazone, pioglitazone and metformin on insulin sensitivity in peripheral tissues and liver. (A) Experiment 1. (B) Experiment 2. Drugs were orally administered for 14 days. Euglycemic–hyperinsulinaemic clamp procedure was performed after overnight fasting on the day after final drug treatment. Percentage of improvement of insulin sensitivity in peripheral tissues and liver in rats treated with each drug was calculated by considering mean value of peripheral glucose utilization and percentage of suppression of hepatic glucose production by insulin, respectively, with lean control designated as 100 and fatty control 0. Rosi, rosiglitazone; Pio, pioglitazone; and Met, metformin.

3.2.4. Improvement on insulin sensitivity in peripheral tissues and liver (Fig. 4)

As described in Materials and methods, percentage of improvement in insulin sensitivity in peripheral tissues and liver in rats treated with each drug was calculated by considering the mean value of peripheral glucose utilization and percentage of suppression of hepatic glucose production by insulin, respectively, with lean control designated as 100 and fatty control 0. FK614 improved insulin sensitivity equally in peripheral tissues and liver at each dose tested (0.32 mg/kg: 16.5% and 17.5%; 1 mg/kg: 55.8% and 59.4%; and 3.2 mg/kg: 72.9% and 76.1%, respectively). Pioglitazone also improved insulin sensitivity equally in peripheral tissues and liver at each dose tested (1 mg/kg: 16.3% and 23.1%; 3.2 mg/kg: 39.1% and 44.2%; and 10 mg/kg: 79.9% and 82.2%, respectively). Percentage of improvement by rosiglitazone in liver (43.2%) tended to be slightly higher than that in peripheral tissues (26.7%) at a dose of 1 mg/kg, while rosiglitazone improved insulin sensitivity equally in peripheral tissues and liver (77.5% and 74.8%, respectively) at a dose of 3.2 mg/kg. Percentage of improvement in liver by metformin was higher than that in peripheral tissues at both doses of 320 and 1000 mg/kg (liver: 16.0% and 35.1%; peripheral tissues: 3.6% and 15.1%, respectively).

4. Discussion

This study showed that FK614 improves impaired glucose tolerance and ameliorates insulin resistance in Zucker fatty rat, demonstrating that FK614 has the ability to improve insulin sensitivity. In addition, the ameliorating effect of FK614 on insulin resistance was observed both in peripheral tissues and liver. Thiazolidinedione PPAR γ agonists, rosiglitazone and pioglitazone, which are in clinical use as insulin-sensitizing antidiabetic agents, also ameliorated both peripheral and hepatic insulin resistance in Zucker fatty rat in this study. Insulin resistance profoundly contributes to the pathophysiology of type 2 diabetes and peripheral and hepatic insulin resistance induces reduced glucose uptake and utilization in insulin-sensitive peripheral tissues (muscle and adipose tissues) and increased glucose production in liver, respectively, leading to hyperglycemia. Therefore, amelioration of both peripheral and hepatic insulin resistance is likely to provide good glycemic control in type 2 diabetes. Both rosiglitazone and pioglitazone improve glycemic control by ameliorating insulin resistance both in peripheral tissues and liver in type 2 diabetic patients (Bajaj et al., 2003; Tiikkainen et al., 2004; Miyazaki et al., 2001a,b, 2002a,b, 2003). These findings indicate the potential for FK614 as an efficacious treatment of type 2 diabetes.

In this study using Zucker fatty rats, ameliorating effects of pioglitazone and rosiglitazone on insulin resistance were clearly observed both in peripheral tissues and liver, with the degree of effect in liver appearing to be equivalent to that in peripheral tissues. However, while a number of clinical studies using the euglyacemic–hyperinsulinaemic clamp technique indicate that thiazolidinedione PPAR γ agonists potently ameliorate peripheral insulin resistance, it remains controversial whether thiazolidinedione PPAR γ agonists ameliorate hepatic insulin resistance. Inzucchi et al. (1998) reported that troglitazone, the first thiazolidinedione PPAR γ agonist to receive approval for clinical use for treatment of type 2 diabetes, had no effects on endogenous glucose production in type 2 diabetic patients; however other reports showed that it suppressed basal hepatic glucose production (Maggs et al., 1998; Suter et al., 1992). Recently, several reports on clinical effects of pioglitazone and rosiglitazone, available thiazolidinedione PPAR γ agonists in clinical use today, show that pioglita-

zone improves hepatic insulin sensitivity and rosiglitazone reduces basal hepatic glucose production in addition to increasing hepatic insulin sensitivity (Bajaj et al., 2003; Miyazaki et al., 2001a,b, 2002a, 2003). In addition to the present study, other preclinical studies indicate that pioglitazone and rosiglitazone improve hepatic insulin sensitivity in different rodent models (Brand et al., 2003; Sugiyama et al., 1990). These findings suggest that thiazolidinedione PPAR γ agonists ameliorate both peripheral and hepatic insulin resistance in type 2 diabetic patients, therefore the novel nonthiazolidinedione PPAR γ agonist, FK614, is also likely to improve both peripheral and hepatic insulin sensitivity in type 2 diabetic patients.

To our knowledge, this is the first report in which effect of metformin on insulin resistance in Zucker fatty rats has been investigated using the euglycemic–hyperinsulinaemic clamp procedure. Coinciding with previous reports that metformin improves glycemic control primarily by suppressing hepatic glucose production in type 2 diabetes (Cusi et al., 1996; Inzucchi et al., 1998; Stumvoll et al., 1995), an ameliorating effect of metformin on hepatic insulin resistance was obviously observed in Zucker fatty rats; however, only a very weak effect on peripheral insulin resistance was observed. Therefore, Zucker fatty rats may be a more suitable rodent model for predicting effect of novel antidiabetic agents in type 2 diabetic patients than neonatal streptozotocin induced-diabetic rats, in which metformin significantly increased insulin-mediated glucose disposal, but had no effect on basal hepatic glucose production and suppression of hepatic glucose production by insulin (Rossetti et al., 1990). Metformin and rosiglitazone failed to suppress basal hepatic glucose production in Zucker fatty rats. This may be due to weak enhancement of basal hepatic glucose production in Zucker fatty rats compared to control lean rats.

Since FK614 is able to act as a partial agonist and as the mode of PPAR γ activation of FK614 is different from thiazolidinedione derivatives (Fujimura et al., unpublished data), FK614 may induce different effects from thiazolidinedione derivatives in vivo. We demonstrated that hemodilution by FK614 was less potent than rosiglitazone so far (Minoura et al., 2004). Also, despite its partial agonistic effect, FK614, as well as rosiglitazone caused a potent and effective hypoglycemic effect in diabetic *db*/*db* mice (Minoura et al., 2004). This study also demonstrated that FK614, as well as thiazolidinedione derivatives, causes a potent and effective suppression on hepatic and peripheral insulin resistance in Zucker fatty rats. Additional differences derived from its partial agonistic effect may be characterized.

In conclusion, a novel nonthiazolidinedione PPAR γ agonist, FK614, as well as thiazolidinedione PPAR γ agonists ameliorated both peripheral and hepatic insulin resistance in Zucker fatty rats, suggesting FK614 is likely to be efficacious for treatment of type 2 diabetes.

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