BM 17.0744: A Structurally New Antidiabetic Compound With Insulin-Sensitizing and Lipid-Lowering Activity

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BM 17.0744 (2,2-dichloro-12-(*p*-chlorophenyl)-dodecanoic acid) is a substance from a group of ω-substituted alkyl carboxylic acids with the general formula, ring–spacer–carboxylic acid. With BM 17.0744—a compound structurally unrelated to thiazolidinediones—antihyperglycemic and antihyperinsulinemic potency has been demonstrated in various animal models of type II diabetes. The antidiabetic effect is independent of the genetic background of the disease, gender, and animal species. The 24-hour blood glucose profile was dose- and time-dependently improved in ob/ob mice after a single and fourth oral administration of 0.3, 1, and 3 mg/kg/d. A dose-dependent reduction of hyperglycemia (10%, 15%, 28%, and 66%) was found in db/db mice after the fifth oral administration of 3, 10, 30, and 100 mg/kg/d. Hyperinsulinemia was reduced dose-dependently in yellow KK mice by 1%, 24%, 34%, and 66% after the fifth oral administration of 0.3, 1, 3, and 10 mg/kg/d. Overall glucose metabolism was predominantly higher in euglycemic-hyperinsulinemic clamp studies in obese fa/fa rats pretreated for 14 days with 10 mg/kg/d BM 17.0744. The data in diabetic and insulin-resistant animals suggest an improvement of insulin action that is supported by enhancement of insulin effects in vitro. There is no evidence of a risk for hypoglycemia in diabetic and metabolically healthy animals. Triglyceride (TG) and cholesterol were reduced in the serum of metabolically healthy rats, as well as serum lipids in db/db mice, which suggests this effect is independent of amelioration of the diabetic status. Lipid-lowering effects in diabetic and healthy animals show an additional property of BM 17.0744. Because of its antidiabetic and lipid-lowering potency, the substance is of great interest in treating the metabolic syndrome. Lipid decreases in rats are associated with a dose-dependent increase in carnitine acetyltransferase activity in the liver to about 100-fold (12.5 mg/kg/d). This together with hepatomegaly in small rodents may indicate peroxisomal proliferation, a phenomenon considered species-specific. Its relevance for humans is well documented for other classes of compounds including fibrates. Specific side effects of insulin sensitizers of the thiazolidinedione type, such as an increase in body weight and heart weight, could not be observed after 4-week oral application of BM 17.0744 in rats. In general, BM 17.0744 was well tolerated in the pharmacological dose range in all species tested.

A REDUCED RESPONSE TO INSULIN plays a central role in the pathogenesis and clinical course of type II diabetes, as well as in the prediabetic status characterized by impaired glucose tolerance.[1] Insulin secretion is enhanced to compensate for insulin resistance, but if secretion is impaired, diabetes becomes overt.[2,3] Enormous efforts worldwide are made to search for compounds that enhance insulin sensitivity.[4] Thiazolidinediones[5] are a class of compounds that reduce hyperglycemia and hyperinsulinemia in parallel in type II animal models.[6] Clinical experience with troglitazone, a thiazolidinedione, demonstrates the relevance of this pharmacological approach in the therapy for insulin-resistant disease in man.[5] Antidiabetic effects were only observed in some type II diabetic patients, so-called responders, which is in line with the heterogeneity of the pathophysiology of type II diabetes but also indicates the need for other drugs to improve insulin action.[4] Adipogenic potency[7,8] and an increase in heart weight[9] have been reported from preclinical studies with thiazolidinediones.

Our aim was to search for new compounds with insulin-potentiating activity. A different toxicological profile can be expected from structurally unrelated compounds, with a chance for more advantages.

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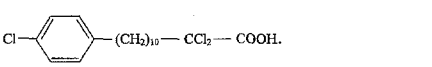
BM 17.0744 was selected for more detailed investigations from a group of ω-substituted alkyl carboxylic acids because of its insulin-potentiating properties in vitro and antidiabetic effects in ob/ob mice. Despite rapid progress in knowledge about the insulin signaling–transduction pathway, the site or sites of reduced insulin sensitivity are not yet known.[10] Animals exhibiting hyperglycemia and/or hyperinsulinemia are useful models for characterizing the insulin-sensitizing potency of compounds. BM 17.0744 was investigated in genetically diabetic male and female mice with regard to its effects on hyperglycemia and hyperinsulinemia. Improvement of insulin action also was investigated in euglycemic-hyperinsulinemic clamp studies in obese fa/fa Zucker rats. A decrease of serum lipids is reportedly accompanied by an increase in liver weight, a phenomenon well known from fibrates.[11] This effect was also investigated in metabolically healthy rats. Because of the known side effects of thiazolidinediones,[7-9] body weight gain and organ weight were determined after a 4-week oral treatment period.

MATERIALS AND METHODS

Chemicals

BM 17.0744, troglitazone, and bezafibrate were synthesized in the Department of Chemistry at Boehringer Mannheim (Mannheim, Germany). All other chemicals, salt mixtures, and additives for culture media were purchased in the necessary degree of purity from the usual suppliers.

The chemical structure of BM 17.0744 is as follows:



Cell Cultures

^{14}C-deoxy-D-glucose (DOG) uptake was measured in differentiated 3T3-L1 adipocytes. 3T3-L1 cells were inoculated at a density of 2,000/cm^2 in 24-well tissue culture plates and cultured in Dulbecco's modified Eagle's medium (DMEM) with newborn calf serum and other additives as described by Schmidt et al.[12] In brief, differentiation of 3T3-L1 preadipocytes was performed in serum-free DMEM/F12 (3:1 vol/vol) using fetuin (300 mg/L), isobutylmethylxanthine (44.4 mg/L), corticosterone (34.4 μg/L), and bovine insulin (6 mg/L) for 96 hours, followed by culture in medium without the above-mentioned supplements except insulin until the experiments. For investigating drug effects on ^{14}C-DOG uptake, adipocytes were incubated for 48 hours in DMEM/F12 in the presence of drugs (DMSO 0.1% final concentration) without any additives except sodium ascorbate. The incubation medium was replaced by Krebs-Ringer buffer containing bovine serum albumin (BSA) 1%, increasing insulin concentrations and test compounds for 1 hour ^{14}C-DOG (2 mmol/L, 0.5 μCi) was added in a volume of 10 to 490 μL/well. The uptake was terminated by three rapid washes with ice-cold Krebs-Ringer buffer after an incubation period of 20 minutes. Cell-associated radioactivity was determined by scintillation counting after solubilization in 0.1N NaOH.

Glucose consumption was investigated in primary rat adipocyte cultures. Abdominal fat pads (~20 g) from male Sprague-Dawley rats (body weight, 350 to 400 g) were minced and treated with collagenase (2.5 mg/mL, CLS I, Worthington; Pansystems, Aidenbach, Germany) in Krebs-Ringer buffer (50 mL) at 37°C under 95% O_2/5% CO_2 for 40 to 50 minutes.[13] For glucose consumption, adipocytes (3×10^5c/mL) were incubated in HEPES buffer (10 mmol/L) containing fatty acid–free BSA (4%), glucose (1 mmol/L), insulin at the concentrations indicated in Fig 2, and test compounds dissolved in DMSO (final concentration 0.1% in 24-well tissue culture dishes of 400 mL per well, 37°C and 95% O_2/5% CO_2). Glucose concentrations in the incubation medium were determined using the peridochrome method (test kit; Boehringer Mannheim[14]).

Animals and Treatments

Male ob/ob mice (C57 BL/6J) and male db/db mice (C57 BLKS/J) were purchased from Jackson Laboratories (Bar Harbor, ME), female yellow KK mice (KK-Ay/Ta Jcl) from Clea Japan (Tokyo, Japan), and male Sprague-Dawley rats from Charles River (Kisslegg, Germany).

A minimum of 10 days was allowed for adaptation after arrival. Ob/ob mice were allowed 12 days to adapt to a reversed dark/light cycle (dark phase, 6 AM to 6 PM). All other animals were kept with a 12-hour light/dark cycle with light from 6 AM to 6 PM, housed under environmentally controlled conditions, and kept on standard chow with water ad libitum. The drugs suspended in aqueous sodium carboxymethylcellulose (1%) were administered by gavage (mice, 10 mL/kg; rats, 5 mL/kg) for the periods indicated; the controls received vehicle only.

Blood and Tissue Sampling

Blood samples were collected on day 0 immediately before and at the other time points 2 hours after dosing. For glucose determinations in mice, blood samples were taken from the tail tip. For other parameters, blood was obtained by opening the neck vessels at the end of the experiment. In rats, blood for all parameters was drawn from the retrobulbar vein plexus. Rats were killed 24 hours after the 28th dosing and starvation for 18 hours by neck dissection. Body and organ weight were determined immediately after bleeding. Liver samples were excised rapidly, frozen in liquid nitrogen, and stored at −16°C.

Determination of Blood, Serum, and Tissue Parameters

The blood glucose level was measured using the hexokinase method without deproteinization.[15] Triglyceride (TG) and cholesterol in serum were assayed enzymatically according to Siedel et al[16] and Wahlefeld,[17] respectively. Nonesterified fatty acid (NEFA) levels were measured using acylcoenzyme A (acylcoA) synthase and acylCoA oxidase with palmitic acid as a standard. In this method, the resulting hydrogen peroxidase forms a red dye in the presence of peroxidase. For the described assays, test kits and enzymes from Boehringer Mannheim were used and performed on an EPOS 5060 (Eppendorf, Hamburg, Germany).

Serum insulin levels in rats were determined using a radioimmunoassay (Insulin RIA 100; Pharmacia, Uppsala, Sweden) with a rat insulin standard (Incstar, Stillwater, MN), and in mice using an ELISA insulin test kit (Boehringer Mannheim).

The carnitine acetyltransferase level was measured in total liver homogenate by an established procedure.[18]

Euglycemic Glucose Clamp Study

Male obese fa/fa rats (HsdHlr:Zucker-fa; Harlan, Indianapolis, IN) were housed individually under the above-mentioned conditions. Under anesthesia with penthrane/ethrane (1:1 vol/vol, 4 to 5 mL per animal), two catheters were inserted into the right jugular vein and one into the left femoral artery, tunneled subcutaneously, exteriorized at the back of the head, and filled with saline. Euglycemic clamp studies were performed after a postoperative recovery period of at least 4 days and a pretreatment period of 14 to 16 days with BM 17.0744 (10 mg/kg/orally) or vehicle. The animals were aged 14 and 18 weeks, and the mean body weight was 523 ± 8.0 g (n = 5) for the BM 17.0744 group and 542 ± 14.5 g (n = 5) for the control group at the start of clamp studies. The clamp studies were performed at the same time in the morning. Extension tubes from infusion pumps were attached to venous catheters for administration of saline/BSA (1%) during phase I between 0 and 45 minutes. A priming dose of insulin 9 mU/kg/min (porcine; Sigma, Deisenhofen, Germany) between 45 and 60 minutes (phase II) and a maintenance dose of 3 mU/kg/min between 60 and 95 minutes (phase III) were administered. D-[3-^{3}H]-glucose was added to both saline and insulin for determination of overall glucose metabolism using the equation described for tracer-determined glucose clearance.[19] Endogenous glucose production was calculated according to the method used by Burnol et al.[20] A priming dose of 5 μCi/animal was followed by continuous infusion of 0.05 μCi/min. To maintain blood glucose at individual levels measured during the adaptation period (phase I), variable amounts of 20% dextrose solution were infused through the remaining jugular catheter. Glucose concentrations in arterial blood were measured at intervals of 15 minutes in phase I and 5 minutes in phases II and III.

Blood samples were taken from the femoral catheter for determination of serum insulin at 0, 30, 45, 60, and 90 minutes and glucose specific activity at 15, 30, 45, 60, and 90 minutes. Glucose was determined according to the hexokinase/glucose-6-phosphate dehydrogenase method[15] using enhanced enzyme activities of 3.4 and 1.7 U, respectively, in the test mixture during clamp study. The insulin level was measured by RIA (Pharmacia Insulin RIA 100). For glucose specific activity, 100 μL serum was deproteinized with perchloric acid. One aliquot was used for liquid scintillation counting and the other for glucose determination according to the hexokinase method.

Statistical Analysis

Results are expressed as the mean ± SEM or SD. The Mann-Whitney test was used to verify differences compared with vehicle-treated controls.

RESULTS

Glucose Uptake and Consumption in Adipocytes

Insulin-stimulated ^{14}C-DOG uptake was investigated in 3T3-L1 adipocytes (Fig 1) and glucose consumption in primary rat adipocytes (Fig 2). In both test systems, the concentration-response curve for insulin was shifted to the left by both BM 17.0744 and troglitazone, which may indicate enhancement of insulin sensitivity. The maximum uptake of DOG was increased by the test compounds in 3T3-L1 cells, which could be evidence of increased responsiveness.

Antidiabetic Effects in Animal Models

Enhancement of insulin action should result in an improvement of the diabetic status in animal models of type II diabetes. The effect of BM 17.0744 on blood glucose over a 24-hour observation period was investigated in male ob/ob mice after single (Fig 3A) and multiple (Fig 3B) administration. To obtain detailed information on blood glucose in the postprandial phase, animals adapted to a reversed dark/light cycle were used and blood sampling was started at 7 AM.

After a single administration, an increase in blood glucose was observed in the control group during the 24-hour observation period. In drug-treated animals, lower blood glucose values were found predominantly in the high-dosage groups. This effect showed dose-dependency 24 hours after the first treatment. Statistical significance ($P \leq .01$ and higher) was reached in the medium- and high-dosage groups at 8 and 4 hours, respectively, and thereafter. After the fourth dosing, all blood glucose values were lower in drug-treated animals versus controls, even in the group treated with the low dose of 0.3 mg/kg/d (1 hour, $P \leq .01$; 8 hours, $P \leq .05$). In groups treated with 1 mg/kg/d (1, 5, 8, and 24 hours, $P \leq .01$; 2, 3, 4, and 6 hours, $P \leq .05$; 7 hours, $P \leq .001$) and 3 mg/kg/d (1, 5, 7, and 8 hours, $P \leq .001$; 2, 4, and 6 hours, $P \leq .05$; 3 and 24 hours, $P \leq .01$), nearly normal blood glucose values were observed during the 24-hour observation period.

To broaden the in vivo relevance of the antidiabetic effect of

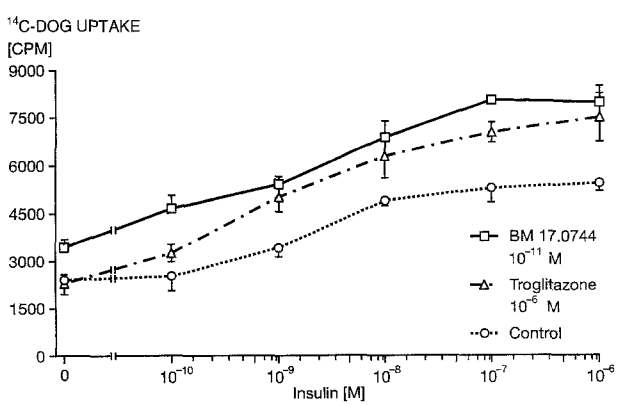


Fig 1. Insulin concentration-response curve of ^{14}C-DOG uptake in 3T3-L1 adipocytes without and with BM 17.0744 and troglitazone dissolved in DMSO (final concentration, 0.1%). Adipocytes were incubated in DMEM for 48 hours in the presence of the drugs without insulin, followed by stimulation with insulin for 1 hour and ^{14}C-DOG exposure for 20 minutes. Data are the mean ± SD for cpm/culture dish of 3-4 independent experiments.

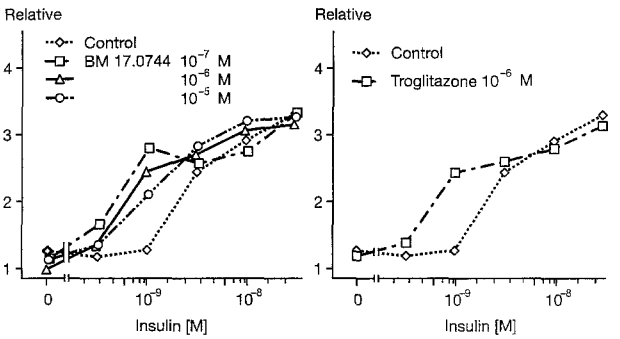


Fig 2. Insulin-dependent glucose consumption in primary rat adipocyte cultures in HEPES buffer with glucose (1 mmol/L) and other additives in the absence and presence of BM 17.0744 and troglitazone dissolved in DMSO (final concentration, 0.1%). Glucose concentrations in incubation medium were measured after an incubation period of 3 hours. The means of 3 independent cultures relative to solvent-treated drug and insulin-free cultures are given.

BM 17.0744, investigations on diabetic status were performed in db/db and yellow KK mice, animals with a different genetic background of the disease.

BM 17.0744 was administered to female yellow KK mice in a dose range of 0.3 to 10 mg/kg/d over a period of 5 days. Blood glucose values were 304 ± 7 to 305 ± 9 mg/dL in the different experimental groups after randomization before the first administration.

Blood glucose was unaffected, but at the end of the experiment, a dose-dependent reduction in insulin was found for dosage groups with 1 to 10 mg/kg/d BM 17.0744 (Fig 4).

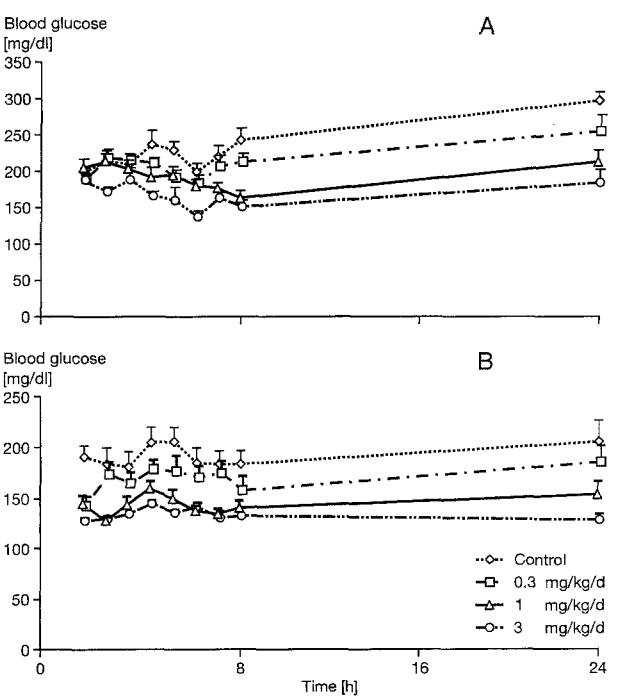


Fig 3. Twenty-four-hour blood glucose profile in male ob/ob mice housed under a reversed dark/light cycle after the first (A) and fourth (B) oral dosing of BM 17.0744 in aqueous sodium carboxymethylcellulose. The controls were treated with the same amount of vehicle (mean ± SEM, n = 10 per group).

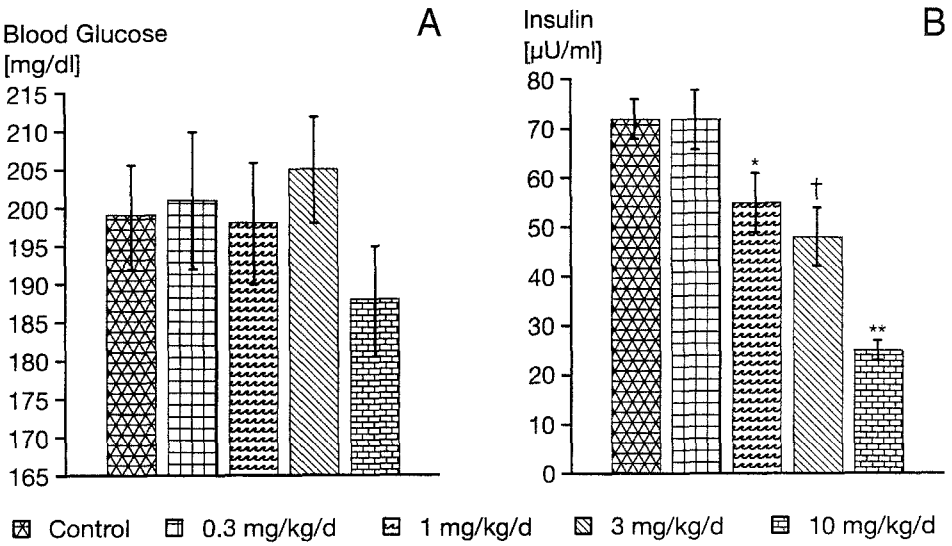


Fig 4. Blood glucose and serum insulin in female yellow KK mice 2 hours after the fifth oral administration of BM 17.0744 in aqueous sodium carboxymethylcellulose. The control was treated with vehicle (mean ± SEM, n = 10 per group; Mann-Whitney test, *$P \leq .05$, †$P \leq .01$, **$P \leq .001$).

The db/db mice had a metabolic status characterized by high blood glucose and relatively low serum insulin, which is more a reflection of the situation in late-stage type II diabetes in man. The drug was administered to male db/db mice in a dose range of 3 to 100 mg/kg/d over a period of 5 days. Blood glucose values were 500 ± 16 to 503 ± 15 mg/dL in the different experimental groups after randomization before the first administration.

The controls exhibited pronounced hyperglycemia during the whole experiment (day 5, 508 ± 11 mg/dL). Blood glucose was decreased in a dose-dependent manner by BM 17.0744. Hyperinsulinemia was moderate in the control group (108 ± 4 µU/mL) and was not statistically significantly different versus all treatment groups at the end of the experiment. Normoglycemia was not reached even in the high-dosage group. TG and NEFA were reduced dose-dependently (Fig 5).

Obese fa/fa Zucker rats exhibit pronounced hyperinsulinemia, whereas glycemia is normal or only slightly enhanced, indicating extreme insulin resistance. Glucose clamp studies under these pathophysiological circumstances are ideal for demonstrating insulin-sensitizing potency. Euglycemic glucose clamp studies were performed in obese fa/fa rats after a pretreatment period of 2 weeks. Glucose infusion to maintain individual blood glucose values constant during insulin infusion was only required when the priming dose of insulin 9 mU/kg/min (phase II) was administered in vehicle-treated control animals (Table 1). In BM 17.0744–treated rats, the glucose infusion rate was dramatically enhanced during the prime dosing of insulin and during the maintenance dose of insulin 3 mU/kg/min (phase III). The extent of hyperinsulinemia was comparable in both groups in phases II and III. Blood glucose was significantly higher in fa/fa rats pretreated with BM 17.0744 versus vehicle only during the adaptation period (phase I), possibly due to higher endogenous glucose production in phase I. Endogenous glucose production was comparable in both groups during the other phases of the experiment. Overall glucose metabolism was higher in drug-treated animals in phase I and increased more rapidly, reaching statistical significance in phases II and III. The higher glucose infusion rate in this metabolically controlled situation is a strong indication of improvement in insulin action.

Interference of BM 17.0744 With Lipid Metabolism in Healthy Rats

To clarify whether the reduction in TG and NEFA in db/db mice is only mediated by amelioration of the diabetic status or

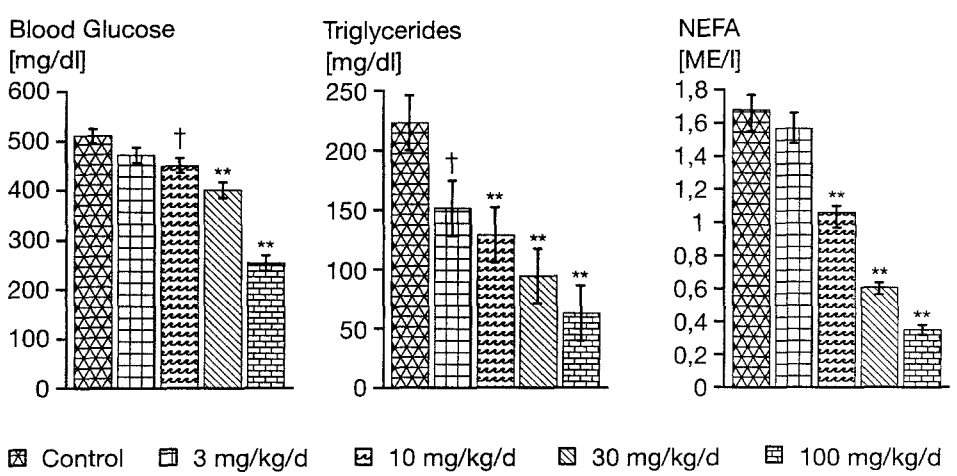


Fig 5. Blood glucose, TG, NEFA in the serum of male db/db mice 2 hours after the fifth oral administration of BM 17.0744 in aqueous sodium carboxymethylcellulose. The control was treated with vehicle (mean ± SEM, n = 10 per group; Mann-Whitney test, †$P \leq .01$, **$P \leq .001$).

Table 1. Euglycemic Glucose Clamp Study in fa/fa Zucker Rats Pretreated With BM 17.0744

Parameter	Phase I: 0-45 min (insulin 0 mU/kg/min)	Phase II: 45-60 min (insulin 9 mU/kg/min)	Phase III: 60-95 min (insulin 3 mU/kg/min)
GIR (mg/kg/min)			
Control	0.00 ± 0.000	0.76 ± 0.038	0.00 ± 0.000
BM 17.0744	0.00 ± 0.000	2.36 ± 0.284*	3.13 ± 0.534*
BG (mg/100 mL)			
Control	136 ± 4.9	136 ± 6.1	124 ± 6.08
BM 17.0744	170 ± 6.2*	174 ± 9.3*	180 ± 6.8*
SI (μU/mL)			
Control	53 ± 1.3	254 ± 9.8	146 ± 6.8
BM 17.0744	36 ± 2.1*	223 ± 5.1	127 ± 5.6
EGP (mg/kg/min)			
Control	4.77 ± 0.192	4.44 ± 0.174	6.16 ± 0.310
BM 17.0744	6.20 ± 0.554	4.46 ± 0.977	6.03 ± 1.020
OGM (mg/kg/min)			
Control	4.77 ± 0.192	5.44 ± 0.146	6.16 ± 0.310
BM 17.0744	6.20 ± 0.554	7.34 ± 0.803*	9.14 ± 0.876*

NOTE. A euglycemic glucose clamp was performed in obese male fa/fa rats pretreated with 10 mg/kg/d BM 17.0744 in aqueous sodium carboxymethylcellulose or vehicle alone for 14 days. Values measured at 65 minutes were included in phase II (mean ± SEM, n = 5 per group).

Abbreviations: GIR, glucose infusion rate; BG, blood glucose; SI, serum insulin; EGP, endogenous glucose production; OGM, overall glucose metabolism.

*$P \leq .05$ by Mann-Whitney test.

an additional direct effect of the drug, metabolically healthy rats were administered BM 17.0744 for 4 weeks. In an earlier experiment with BM 17.0744 in rats at a dose range of 12.5 to 100 mg/kg/d, it was found that a maximal decrease of serum lipids occurred in the group with the lowest dose. Additionally, an increase in liver weight was observed, a phenomenon well known from other lipid-lowering agents. This effect was also expressed maximally in the low-dose group. In the following study, BM 17.0744 was used in the range of 1.5 to 12.5 mg/kg/d. For comparison, bezafibrate was used at a dosage of 25 and 75 mg/kg/d, which can be expected to produce distinct and maximal effects.[11,21]

Both drugs were well tolerated during the administration period of 4 weeks. BM 17.0744 and bezafibrate did not show any effects on blood glucose and serum insulin under fed and starved conditions. TG (Fig 6A) and cholesterol (Fig 6B) were reduced nearly maximally with the lowest dose of 1.5 mg/kg/d. Statistical significance ($P \leq .05$) was reached for both parameters in this dosage group at day 3 of treatment, and improved with dosage and time of application. The lipid-lowering effect was comparable to that of bezafibrate.

At the end of the experiment, the body weight gain was lowest in the high-dosage group of 12.5 mg BM 17.0744/kg/d. In the groups at 3 and 6 mg/kg/d, there was a tendency for lower values. In contrast, bezafibrate had no effect. All organ weights (heart, adrenals, spleen, thyroid gland, and brain) were normal, except for the liver and kidneys, which were elevated. For the liver, this effect was more pronounced, reached statistical significance, exhibited dose-dependency, and was quantitatively comparable with both drugs. Carnitine acetyltransferase activity in the liver increased dose-dependently up to 6 mg/kg/d in BM 17.0744–treated animals. The maximum increase with BM 17.0744 is about twice that obtained with bezafibrate (Table 2).

DISCUSSION

An increase in insulin resistance is a primary feature of type II diabetes.[1,22] Various pathogenetic mechanisms are reported to contribute to insulin resistance, eg, disturbances at different sites of the insulin receptor signal transduction pathway, cellular metabolism of fatty acids, adipose tissue distribution, and life-style (eating habits and physical exercise).[1,23,24] Complex test systems were therefore used to demonstrate an improvement in the insulin response. The shift of the insulin concentration-response curve for DOG uptake and glucose consumption to the left in 3T3-L1 and primary rat adipocytes, respectively, indicates enhancement of insulin action, whereas the higher maximal uptake of DOG in the presence of insulin plus test drugs could be due to enhancement of insulin responsiveness. DOG is a nonmetabolizable glucose derivative. Enhanced DOG uptake suggests an increase in glucose transport capacity under conditions of acute insulin stimulation, as demonstrated in various insulin-responsive target cells.[25,26]

The antihyperglycemic and antihyperinsulinemic effects in type II diabetes models such as ob/ob, db/db, and yellow KK mice demonstrate the in vivo relevance of the insulin-potentiating effects of BM 17.0744 observed in vitro. Reduction of serum insulin after BM 17.0744 treatment as a result of impaired insulin secretion can be excluded, because a reduction

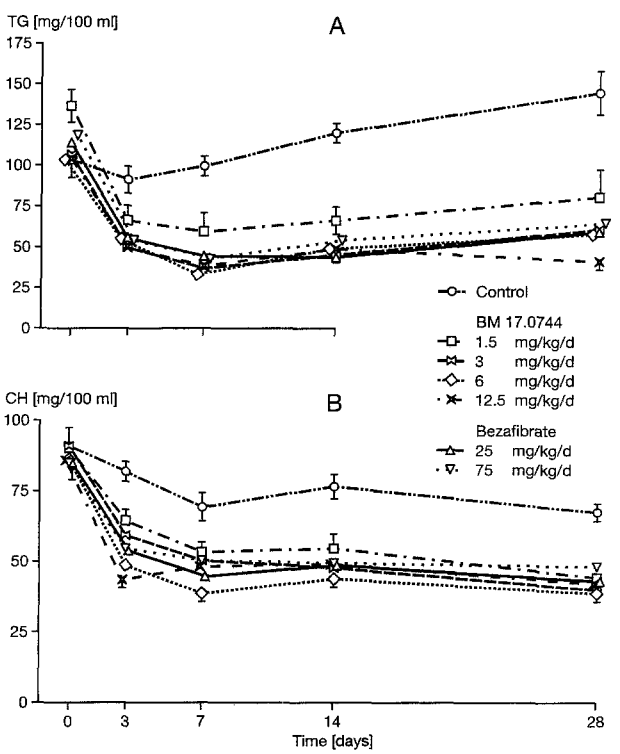


Fig 6. Serum TG and cholesterol (CH) in male Sprague-Dawley rats after oral administration of BM 17.0744 and bezafibrate in aqueous sodium carboxymethylcellulose for 28 days (mean ± SEM, n = 6-8 per group).

Table 2. Effects of Drug Treatment in Sprague-Dawley Rats

Substance		Body Weight Gain 0-28 d (g)	Liver Weight (g)	Kidney Weight (g)	CAT in Liver (mU/mg protein)
Control		142 ± 9	13.3 ± 0.37	2.36 ± 0.05	2.0 ± 0.3
BM 17.0744	1.5 mg/kg	140 ± 4	15.0 ± 0.57	2.57 ± 0.07	49 ± 13†
BM 17.0744	3 mg/kg	115 ± 8	14.7 ± 0.50	2.44 ± 0.08	110 ± 14†
BM 17.0744	6 mg/kg	120 ± 4	17.9 ± 0.76†	2.58 ± 0.08	188 ± 15†
BM 17.0744	12.5 mg/kg	99 ± 10*	19.4 ± 1.25†	2.66 ± 0.13	202 ± 36†
Bezafibrate	25 mg/kg	138 ± 7	15.1 ± 0.74	2.53 ± 0.07	84 ± 9†
Bezafibrate	75 mg/kg	149 ± 7	18.4 ± 0.89†	2.76 ± 0.11	110 ± 12†

NOTE. Body weight gain, liver and kidney weight, and carnitine acetyltransferase (CAT) activity were measured in liver homogenate of male Sprague-Dawley rats after oral administration of BM 17.0744 and bezafibrate in aqueous sodium carboxymethylcellulose for 28 days (mean ± SEM, n = 6-8 per group).

Mann-Whitney test: *$P \leq .05$, †$P \leq .01$.

in insulin secretion associated with unaffected insulin sensitivity leads to an increase in blood glucose, as described repeatedly with diazoxide.[27,28] The effects can be observed at a much lower dose range than described for troglitazone.[6] The antidiabetic effect of BM 17.0744 is independent of gender and the genetic background of the disease, as shown by the data in ob/ob, db/db, and yellow KK mice.

In euglycemic glucose clamp studies in obese fa/fa rats (an animal model with high-grade insulin resistance), hyperinsulinemia and endogenous glucose production were comparable in drug- and vehicle-treated animals in phases II and III of the experiment. Under these conditions, the enhanced glucose infusion rate strongly supports the conclusion of enhancement of insulin action by BM 17.0744 drawn from the data in diabetic mice and in vitro experiments. The higher overall glucose metabolism in BM 17.0744–treated fa/fa rats in all phases of the experiment, and the comparability of this parameter between phase III controls and the phase I treatment group, and taking the more than threefold higher serum insulin levels in controls into account, strongly indicate an improvement of insulin action by BM 17.0744. Furthermore, the enhanced glucose infusion rate by BM 17.0744 and simultaneously constant blood glucose levels indicate increased cellular glucose uptake,[29] which supports the finding in adipocytes. The significantly higher blood glucose values in drug-treated animals in phase I are paralleled by lower serum insulin, and may be caused by higher endogenous glucose production. Enhancement of precursors for gluconeogenesis can be expected from an increase in hepatic β-oxidation of fatty acids[30] indicated by the dramatic increase in carnitine acetyl transferase activity in the liver of BM 17.0744–treated Sprague-Dawley rats. The extent to which enlargement of the liver contributes to higher glucose production under basal conditions needs further clarification. Both the extent of enhancement of carnitine acetyltransferase and liver weight are specific for small rodents.[21,31] From these data, a worsening of the diabetic status in humans is not to be expected, because amelioration of the disturbed metabolic situation is observed under treatment with fibrates.[32]

There should be no risk of hypoglycemia, because comparable dosages that led to amelioration of insulin action in fa/fa rats did not show any effect on blood glucose in Sprague-Dawley rats.

The serum lipid-lowering effect of BM 17.0744 in diabetic and metabolically healthy animals cannot be explained by amelioration of the diabetic status and is therefore a further property of the drug. The increase in fatty acid transport capacity shown by carnitine acetyltransferase activity in the liver suggests enhancement of fatty acid catabolism due to increased β-oxidation.[33] This would explain why NEFA in serum is also reduced in db/db mice. Both a lipid decrease and an elevation of carnitine acetyltransferase are described for fibrates, and were also demonstrated here using bezafibrate.[11] Both effects are a part of a modulation of lipid metabolism at the cellular level by interference of xenobiotics with a nuclear receptor known as peroxisome proliferator–activated receptor (PPAR) subtype α,[33] which needs further study to be clarified with regard to BM 17.0744 also. The increase in kidney and liver weight could also be interpreted as a consequence of BM 17.0744 binding to PPARα.[34] This increase in organ weight is species-specific for small rodents like mice, rats, and hamsters, and is not applicable to other animal species and man.[21,31]

An elevation of enzymes responsible for thermogenesis and calorigenesis by the interference of agents with effects on lipid metabolism at the transcriptional level is described,[35] and could explain the reduced body weight gain with BM 17.0744 in rats. A reduction in adipose mass and not a general retardation of the development of body weight can be expected because organ weights, except for the liver and kidney, were inconspicuous. This is in contrast to insulin sensitizers of the thiazolidinedione family, which induce adipogenesis, an increase in body weight gain as a consequence of PPARγ activation,[36,37] and an increase in heart weight.[9]

In conclusion, BM 17.0744 is a structurally new insulin sensitizer with intense antihyperglycemic and antihyperinsulinemic potency in animal models of type II diabetes. The mode of action of the agent's additional lipid-lowering properties seems to be comparable to that of fibrates, and can be assumed to be mediated by PPARα activation. Because of its antidiabetic and lipid-lowering potency, the agent is of great interest in combating the metabolic syndrome.

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