Pathol. Oncol. Res. (2011) 17:887–892
DOI 10.1007/s12253-011-9399-2

RESEARCH

Metabolic Effects of Pioglitazone in Chemically-Induced Mammary Carcinogenesis in Rats

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Received: 27 January 2011 / Accepted: 30 March 2011 / Published online: 7 June 2011

Abstract In this paper, the effect of peroral antidiabetic pioglitazone, a thiazolidinedione derivate, on selected parameters of carbohydrate and lipid metabolism in N-methyl-N-nitrosourea-induced mammary carcinogenesis in female Sprague–Dawley rats was evaluated. Pioglitazone was administered in the diet at two concentrations (10 ppm and 100 ppm), the chemoprevention was initiated 12 days before carcinogenesis induction and lasted until the termination of the experiment. The experiment was terminated 17 weeks after carcinogenesis induction, selected organs and tissues were removed and weighed and basic metabolic and hormonal parameters were determined. Pioglitazone increased glycemia (without exceeding normal values) and glycogen concentration in both liver and heart muscle without altering insulinemia and increased triacylglycerol concentration in liver, these changes were more prominent in group with higher dose. Pioglitazone also reduced corticosterone serum concentration and attenuated lipid peroxidation. Pioglitazone and other glitazones may be useful in alleviation of unfavourable metabolic changes in cancer patients.

Keywords Chemoprevention · Mammary carcinogenesis · Metabolism · Pioglitazone · Rat

Abbreviations

CH	cholesterol
CTS	corticosterone
GLU	glucose
GLY	glycogen
IGF-1	insulin-like growth factor 1
INS	insulin
MDA	malondialdehyde
NMU	N-methyl-N-nitrosourea
PIO	pioglitazone
PL	phospholipids
TAG	triacylglycerols

Introduction

Breast cancer is the most common neoplasm among women in developed countries and often occurs together with diabetes mellitus type 2. As two of the major type 2 diabetes risk factors, older age and obesity, are associated with breast cancer (and other cancers too), these pathways are probably interconnected, most likely through activation

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of the insulin and insulin-like growth factor pathway and altered regulation of endogenous sex hormones and adipocytokines [1]. Therefore an effective treatment of impaired insulin signalling should reduce cancer risk too.

There is a growing evidence that peroral antidiabetics biguanides and thiazolidinediones possess antitumour activity. Biguanides (buformin, phenformin, metformin) had been used in type 2 diabetes treatment for several decades, but buformin and phenformin have been withdrawn from most countries because of lactic acidosis risk and nowadays only metformin is used. Thiazolidinediones, or glitazones (pioglitazone, rosiglitazone), have been used in type 2 diabetes treatment only from the late 1990s. However, due to increased cardiovascular risk the European Medicines Agency in September 2010 recommended to suspend rosiglitazone from the market. A new glitazone analogue rivoglitazone is undergoing research for use.

Metformin activates AMP-activated protein kinase (AMPK) and subsequently inhibits mammalian target of rapamycin (mTOR) pathway which leads to decrease of protein synthesis and cell proliferation [2, 3]. Thiazolidinediones (synthetic ligands of peroxisome proliferator-activated receptors γ) are transcription factors which are involved in cell proliferation, lipid transport and accumulation, and immune system modulation. PPARγ ligands may modulate malignant transformation through cell cycle arrest and apoptosis induction, angiogenesis suppression, and anti-inflammatory actions [4]. Thiazolidinediones were reported to activate AMPK pathway too [5, 6]. Numerous reports showed inhibitory effects of thiazolidinediones in various cancer cell lines including breast cancer cells [7–10]. Thiazolidinediones inhibited mammary carcinogenesis in vivo too [7, 11–13]. Our group found significant tumour growth inhibition in chemically-induced mammary carcinogenesis in Sprague–Dawley rats after long-term pioglitazone administration [14].

Cancer incidence in diabetic patients administered with thiazolidinediones did not differ in comparison with other treatment; however, these studies either lacked duration response analysis [15] or the duration did not exceed 52 weeks [16]. Phase II clinical trial of rosiglitazone (stages 0–II, 2–6 week treatment) [17] and troglitazone administration (stage IV, 8-week median treatment) [18] in breast cancer patients showed no anticancer effect. PIO in combination with cyclooxygenase-2 inhibitors showed some response in patients with metastatic melanoma, soft tissue sarcoma, and glioma [19, 20]. The relationship between thiazolidinedione administration and cancer risk in humans remains to be revealed as available data are insufficient. Nevertheless, as malignant tumour progression is associated with metabolic and hormonal disturbancies, their alleviation may improve prognosis and survival in cancer subjects.

The aim of this work was to evaluate the effect of long-term pioglitazone administration in well-established model of chemically-induced mammary carcinogenesis in female Sprague–Dawley rats on selected metabolic and hormonal parameters.

Materials and Methods

In the experiment female Sprague–Dawley rats (AnLab Prague, Czech Republic) aged 31 days were used. The animals were adapted to standard vivarium conditions with the temperature of 23±2°C, relative humidity 50–60%, artificial regimen light:dark 12:12 h, lights on from 7:00 a.m. (light source: fluorescent lamps Tesla – 40 W, light intensity 150 lux per cage). During the experiment the animals (four per cage) were fed the Ssniff diet (Soest, Germany) and drank tap water ad libitum. Mammary carcinogenesis was induced by N-methyl-N-nitrosourea (NMU) (Sigma, Deisenhofen, Germany). NMU was administered in two intraperitoneal doses (50 mg/kg b.w.) on the 43rd and 50th postnatal days. NMU solution was freshly prepared by dissolving NMU in isotonic saline solution (the average volume dose per animal was 0.5 ml).

Pioglitazone (PIO) (Actos TM, Lilly, Alcobendas, Madrid, Spain) was administered in the diet at two concentrations: 10 ppm and 100 ppm. Chemoprevention with PIO began 12 days before the first carcinogen administration and lasted until the end of the experiment (17 weeks after the first NMU administration). Animals were divided into four groups: (1) NMU, control group without chemoprevention, (2) NMU+PIO10, chemoprevention with PIO at lower concentration, (3) NMU+PIO100, chemoprevention with PIO at higher concentration, (4) INT, intact group. Each group consisted of 16 animals except the intact group (12 animals). Animals were weekly weighed and palpated to register the presence, location, and size of each palpable tumour.. Food and water intake during 24 h was monitored in 5th, 10th, and 15th week of the experiment (dated from the first carcinogen administration), overall in 12 measurements (four times in a given week). In the 17th week of the experiment the animals were quickly decapitated, selected organs and tissues (heart muscle, thymus, liver, and periovarial fat) were removed and weighed and samples were taken for further analysis. Basic metabolic and hormonal parameters were determined in serum and selected organs: serum concentration of glucose (GLU); serum and liver concentration of triacylglycerols (TAG), cholesterol (CH), and phospholipids (PL); liver and heart muscle glycogen (GLY) concentration; liver and thymus malondialdehyde (MDA) concentration; serum corticosterone (CTS), insulin (INS), and IGF-1 concentration. GLU and TAG were measured using commercial sets

(Lachema, Brno, Czech Republic), INS and IGF-1 were determined using commercial RIA sets (Linco Research, St Charles, MO, USA and DRG Instruments GmbH, Germany, respectively), PL were measured from lipid phosphorus according to Bartlett [21], CH according to Zlatkis et al. [22], GLY according to Roe and Dailey [23], MDA was measured in reaction with thiobarbituric acid according to Satoh [24], CTS was measured using fluorimetry according to Guillemin et al. [25]. Results were evaluated by one-way analysis of variance or Kruskal-Wallis test, respectively, the criterion for the choice of the relevant test was the Bartlett´s number value. The experiment was carried out from January to June.

Results

This experiment is a follow-up to our previous work where the effect of pioglitazone in NMU-induced mammary tumour growth in Sprague–Dawley rats was evaluated [14]. Body mass gain in NMU was decreased when compared to intacts during the whole experiment. Lower PIO dose increased body mass gain to the level of intacts from the second half of the experiment. Higher PIO dose decreased body mass gain in comparison with control group and lower dose in first four weeks of experiment (Fig. 1). Periovarial fat tissue weight was decreased in control group when compared to intacts, PIO administration increased it (significantly at lower dose). Food intake in 5th and 15th week in control group was decreased in comparison with intact group. Both PIO doses increased food intake in 5th week when compared to NMU. Lower

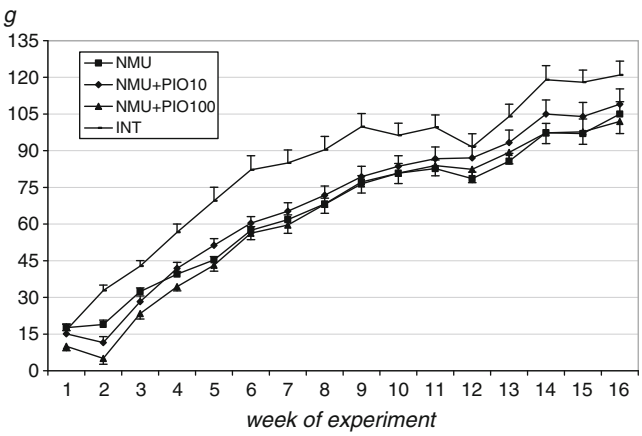

Fig. 1 The effect of pioglitazone in NMU-induced mammary carcinogenesis in Sprague–Dawley rats on body mass gain. Data are expressed as means ± S.E.M. For statistical significant differences between groups see the Results section. **Abbreviations:** *NMU* control group without chemoprevention, *NMU+PIO10* chemoprevention with pioglitazone (10 ppm), *NMU+PIO100* chemoprevention with pioglitazone (100 ppm), *INT* intact group

PIO dose decreased water intake in comparison with other groups during the whole experiment (Table 1). Daily intake of PIO ranged from 0.134 to 0.176 mg/rat/day in NMU+PIO10 and 1.44 to 1.88 mg/rat/day in NMU+PIO100, respectively.

Serum GLU and INS concentration in NMU group was lower when compared to intacts, PIO administration increased glycemia (proportionally to dose, within normal range) without changing insulinemia. PIO administration increased GLY concentration in both liver and heart muscle (significant changes were recorded after lower dose administration in heart muscle and higher dose in liver). Serum TAG concentration was higher in control group in comparison with intacts, PIO administration had no effect. Higher PIO dose increased TAG concentration in liver when compared to controls and lower PIO dose. Serum CH was not changed, liver CH concentration was increased in control group in comparison with intacts. No changes were recorded in PL concentration. Liver MDA concentration was higher in control group when compared to intacts, lower PIO dose decreased it to the level of intacts. Thymus MDA concentration in control group was increased in comparison with intacts, with no change after PIO administration.

PIO administration decreased serum CTS in comparison with control group. No significant changes were recorded in serum IGF-1 (Table 2).

Discussion

Tumour burden is connected with metabolic disturbances which are similar to those observed in diabetes. Cancer patients show total lipid body content decrease due to enhanced lipid mobilisation and oxidation. Elevated fat oxidation rates probably arise from reduced lipogenesis rather than increased lipolysis [26]. The progression of cancer disease leads to food intake reduction, increased energy expenditure, or a combination of the two and results in cachexia. In our experiment a correlation among food intake, body mass gain, and periovarial fat weight was found, with decrease of these parameters in experimental groups in comparison with intacts.

The hallmark of carbohydrate metabolism in tumour bearing objects is the increased rate of gluconeogenesis which also occurs in type 2 diabetes. Glucose clearance from the plasma compartment increases due to large consumption of glucose in tumours in the process of anaerobic glycolysis. The plasma insulin level decreases in cancer patients [27]. This corresponds to our results where decreased glycemia and insulinemia in all experimental groups in comparison with intacts was recorded. In diabetic patients PIO increases insulin stimulated glucose uptake and therefore stimulates peripheral glucose disposal without hypoglycemia. In our experiment PIO increased

Table 1 The effect of pioglitazone in N-methyl-N-nitrosourea-induced mammary carcinogenesis in Sprague–Dawley rats on food and water intake, periovarial fat weight, and final body mass gain

		INT	NMU	NMU+PIO10	NMU+PIO100
5th week	Food intake (g/rat/day)	19.4±0.704	16.0±0.336 **aaa**	17.6±0.448 ↑↑	18.8±0.486 ↑↑↑
	Water intake (g/rat/day)	33.6±1.75	30.0±0.798	26.7±1.07 ↓	30.6±0.584 **bb**
10th week	Food intake (g/rat/day)	13.5±1.36	13.6±1.03	13.9±0.735	15.3±0.932
	Water intake (g/rat/day)	30.1±1.18	27.7±1.46	23.4±0.544 ↓↓	28.3±0.832 **bbb**
15th week	Food intake (g/rat/day)	17.3±0.460	13.5±0.803 **aa**	13.4±0.950	14.4±1.01
	Water intake (g/rat/day)	30.3±0.496	26.4±1.40	20.6±1.52 ↓↓	25.4±1.20 **b**
Periovarial fat weight (g)		4.12±0.749	1.94±0.234 **aa**	2.85±0.258 ↑	2.39±0.476
Final body mass gain (g)		121±5.65	105±5.01 **a**	109±6.29	102±4.96

Data are expressed as means ± S.E.M. Significant differences between groups are designated as follows: NMU vs INT as **a** for $p \leq 0.05$, **aa** for $p \leq 0.01$, **aaa** for $p \leq 0.001$; a decrease of given parameter in groups with chemoprevention (NMU+PIO10, NMU+PIO100) vs NMU as ↓ for $p \leq 0.05$, ↓↓ for $p \leq 0.01$; an increase of given parameter in groups with chemoprevention (NMU+PIO10, NMU+PIO100) vs NMU as ↑ for $p \leq 0.05$, ↑↑ for $p \leq 0.01$, ↑↑↑ for $p \leq 0.001$; NMU+PIO10 vs NMU+PIO100 as **b** for $p \leq 0.05$, **bb** for $p \leq 0.01$, **bbb** for $p \leq 0.001$

INT intact group, *NMU* control group without chemoprevention, *NMU+PIO10* chemoprevention with pioglitazone (10 ppm), *NMU+PIO100* chemoprevention with pioglitazone (100 ppm)

glycemia (not exceeding normal values) without changing insulinemia when compared to controls. PIO seems to stimulate GLY synthesis as we recorded increased GLY concentration both in liver and heart muscle.

Lipid metabolism alterations in cancer include higher serum TAG and lower total and HDL cholesterol concentrations [28, 29]. Increased serum TAG concentration in experimental groups in comparison with intacts was

Table 2 Metabolic effects of pioglitazone in N-methyl-N-nitrosourea-induced mammary carcinogenesis in Sprague–Dawley rats

n	INT 12	NMU 13	NMU+PIO10 13	NMU+PIO100 15
Serum				
GLU (mmol/l)	5.31±0.0974	3.82±0.0872 **aaa**	4.49±0.128 ↑↑↑	4.88±0.0977 ↑↑↑ **b**
TAG (mmol/l)	0.170±0.0189	0.400±0.0530 **aaa**	0.347±0.0470	0.300±0.0362
CH (mmol/l)	2.73±0.200	2.94±0.192	2.74±0.118	2.99±0.170
PL (mmol/l)	1.72±0.132	1.60±0.131	1.59±0.087	1.64±0.114
CTS (ng/ml)	393±67.3	473±31.7	297±38.4 ↓↓	265±16.8 ↓↓↓
INS (ng/ml)	0.230±0.0347	0.0962±0.0182 **aa**	0.100±0.0181	0.105±0.0162
IGF-1 (ng/ml)	783±46.7	795±74.6	651±73.1	683±43.5
Liver				
GLY (μmol/g)	9.25±1.16	7.03±0.772	9.97±1.94	10.8±1.03 ↑↑
TAG (μmol/g)	31.4±3.09	23.8±2.41	26.1±2.16	38.0±2.72 ↑↑↑ **bb**
CH (μmol/g)	16.8±1.53	22.6±1.06 **aa**	19.1±1.57	20.0±1.60
PL (μmol/g)	56.7±1.38	59.3±4.48	56.3±1.13	55.4±1.46
MDA (nmol/g)	34.3±2.54	48.0±3.16 **aa**	38.6±1.96 ↓	40.6±2.60
Heart muscle				
GLY (μmol/g)	5.51±0.718	4.23±0.591	6.28±0.705 ↑	6.91±1.11
Thymus				
MDA (nmol/g)	13.4±1.10	18.8±1.25 **aa**	18.9±1.65	15.6±1.04

Data are expressed as means ± S.E.M. Significant differences between groups are designated as follows: NMU vs INT as **aa** for $p \leq 0.01$, **aaa** for $p \leq 0.001$; a decrease of given parameter in groups with chemoprevention (NMU+PIO10, NMU+PIO100) vs NMU as ↓ for $p \leq 0.05$, ↓↓ for $p \leq 0.01$, ↓↓↓ for $p \leq 0.001$; an increase of given parameter in groups with chemoprevention (NMU+PIO10, NMU+PIO100) vs NMU as ↑ for $p \leq 0.05$, ↑↑ for $p \leq 0.01$, ↑↑↑ for $p \leq 0.001$; NMU+PIO10 vs NMU+PIO100 as **b** for $p \leq 0.05$, **bb** for $p \leq 0.01$

INT intact group, *NMU* control group without chemoprevention, *NMU+PIO10* chemoprevention with pioglitazone (10 ppm), *NMU+PIO100* chemoprevention with pioglitazone (100 ppm), *n* number of animals per group, *GLU* glucose, *TAG* triacylglycerols, *CH* cholesterol, *PL* phospholipids, *CTS* corticosterone, *INS* insulin, *IGF-1* insulin-like growth factor 1, *GLY* glycogen, *MDA* malondialdehyde

confirmed in this experiment as well as in our previous work where another peroral antidiabetic drug, metformin was tested in the same experimental model [30] and neither PIO nor metformin prevented it. Hypocholesterolemia, however, was not found in this as well as in our previous work [30]. No changes were found in serum and liver PL concentration in this and previous work either [30].

In cancer as well as in diabetes of both type 1 and 2, an oxidative damage increase caused by reactive oxygen species (ROS) is observed [31, 32], which may arise from increased ROS production and/or abnormalities in antioxidant defences and, vice versa, available data indicate oxygen radical damage plays a role in both cancer and diabetes etiology (although the impact is still discussed). ROS induce lipid peroxidation and subsequent MDA formation. In our experiment liver MDA concentration was increased in control group and both PIO doses decreased it to the level of intacts. PIO administration decreased MDA levels in rats with hepatic and sciatic nerve ischemia too [33, 34] which supports the protective role of PIO in lipid peroxidation and maintaining biomembrane integrity.

Serum cortisol tends to increase in patients with malignant tumours [35]. In patients with breast cancer and weight loss serum cortisol was higher in comparison with those without weight loss [28]. In our experiment, serum CTS level in the control group increased in comparison with intacts (non-significantly due to large individual variations) and decreased after PIO administration to the intacts level. As glucocorticoids were shown to inhibit apoptosis in mammary epithelial cells [36, 37], this decrease in PIO groups should be regarded as beneficial.

Increased circulating IGF-1 levels are associated with higher cancer risk including breast cancer. IGF-1 acts as a strong mitogen and a potent cell survival factor suppressing apoptosis and has been suggested to increase breast cancer risk by increasing the amount of dense tissue in female breast [38]. Some reports related elevated IGF-1 levels only to premenopausal breast cancer [39–41] but others [42, 43] suggested this association in postmenopausal breast cancer too. However, IGF-1 is downregulated as cancer disease aggravates, which was reported in cancer patients [44] as well as in animal studies [45]. In this experiment no significant changes in serum IGF-1 were found. In our previous work with metformin administration, a decrease of serum IGF-1 in experimental groups (proportional to body mass gain) in comparison with intacts was recorded which suggests a depressive effect of cancer cachexia on IGF-1 level [30].

Proper evaluation of pioglitazone efficacy in cancer risk reduction requires long-term studies. Considering experimental data, our results, and results of clinical trials, we assume pioglitazone is effective when administered in early stages of carcinogenesis or preferably prior to carcinogenesis induction. As pathways regulating metabolic homeostasis and cell proliferation are interconnected, early alleviation of metabolic disturbances may reduce cancer incidence. Therefore, pioglitazone and possibly a new thiazolidinedione derivate rivoglitazone which is under research for use may be a perspective pleiotropic therapy for diabetic patients.

Acknowledgements This study was supported by VEGA Grant Science Agency, Ministry of Education, Slovak Republic, project No. 1/4321/07; Internal grant system for young researchers of Pavol Jozef Šafárik University in Košice, Slovak Republic, project No. 2/2009/B. The experiment was approved by the State Veterinary and Food Administration of Slovak Republic, accreditation No. Ro-930/05-221. The animals were treated in accordance with principles provided by Law No. 289/2003, 489/2003, and 23/2009 of Slovak Republic for the Care and Use of Laboratory Animals. No writing assistance was used during the process of manuscript preparation and submission.

Conflict of interest statement None declared

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