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Accelerated recovery of 5-fluorouracil-damaged bone marrow after rosiglitazone treatment

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Abstract

Our preliminary data indicate that rosiglitazone may be myeloprotective. We investigated whether it can modify bone marrow recovery. Five-day pre-treatment with rosiglitazone significantly accelerated recovery of 5-fluorouracil-damaged bone marrow in mice. Frequency and femoral content of granulocyte–macrophage progenitors reached mean baseline faster in pre-treated groups than in 5-fluorouracil-treated controls. Consequently, neutropenia was milder. Five-day insulin pre-treatment had similar effects in vivo. Insulin supports in vitro hematopoiesis. The observed myeloprotection demonstrated the importance of insulin in vivo. Clinical use of insulin to moderate myelotoxicity is impractical but rosiglitazone, an insulin sensitizer, could offer hope. Although rosiglitazone tends to increase plasma insulin levels, the significant myeloprotection was partly due to direct effects on progenitors. In vitro rosiglitazone enhanced the survival of both murine progenitor and human mobilized blood stem cells in the presence of 5-fluorouracil, the effect of which was neutralized by a peroxisome-proliferator-activated receptor-γ antagonist.

Keywords: Rosiglitazone; Myeloprotection; Stem cell; CFU-GM (Granulocyte–macrophage progenitor cell); Neutropenia; Insulin

1. Introduction

Antineoplastic therapy-associated hematopoietic toxicity often results in neutropenia, anemia and thrombocytopenia. Neutropenia specifically has been shown to force dose reductions, cause treatment delays in subsequent chemotherapy cycles, lead to increased opportunistic infections and ultimately reduce survival. The risk of infection and complications is related to both the severity and duration of neutropenia (Kuhn, 2002). In clinical experience, these serious infections will not improve, despite modern antibiotic and antifungal therapy, until the patient's absolute neutrophil count is normalized (Bodey et al., 1994). Chemotherapy-induced hematopoietic toxicity is a multifactorial challenge in the treatment of oncology patients. Hematopoietic growth factors are often used to moderate chemotherapy-induced myelotoxicity. Insulin is an early-acting growth factor in hematopoiesis, but its use to support bone marrow recovery is impractical.

Rosiglitazone, a thiazolidinedione insulin sensitizer drug, was investigated in mice as a possible protector against 5-fluorouracil-induced myelotoxicity (Benkő et al., 2003). Such protective effects offer significant clinical application only if a considerable enhancement in restoration of stem cells and facilitation of the recovery phase after injury can be demonstrated. The present study therefore focuses on evaluating the degree of bone marrow regeneration in rosiglitazone pre-treated groups after 5-fluorouracil administration over time. Rosiglitazone is an insulin sensitizer drug developed to treat non-insulin dependent diabetes mellitus (Vamecq and Latruffe, 1999). Although insulin is widely used to support colony formation in cultures of hematopoietic progenitor cells, its

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effects regarding hematopoiesis in vivo have not been documented. Our aim was to study whether insulin itself could have a similar myeloprotective effect against cytostatic agents in vivo, and whether rosiglitazone has a direct influence on bone marrow cells, improving survival following 5-fluorouracil administration.

2. Materials and methods

2.1. Animals

Ten- to 11-week-old female (C57bl × DBA2)F_1 mice from the National Institute of Oncology (Budapest, Hungary) were used. Animals were housed in an animal room with 3–5 animals per pen, fed commercial laboratory chow and water ad libitum. The present experiments conform to the European Community's guiding principles for the care and use of laboratory animals. The experimental protocol has been approved by the Ethics Committee for Animal Research, University of Debrecen (11/2003 DEMAB).

2.2. Patients

The management of patients conformed to the Helsinki Declaration. Informed consent was obtained and study design was approved by the Regional Human Ethics Committee, University of Debrecen (55/2003). The three patients with hematological malignancies were waiting on autologous peripheral stem cell transplantation in the Second Department of Internal Medicine, University of Debrecen. During clinical remission progenitor cells were mobilized from their bone marrow. Samples were obtained from the leftover separated frozen stem cell suspensions at the time of transplantation.

2.3. Study design

The influence of rosiglitazone (Avandia, GlaxoSmithKlein, Brentford, United Kingdom) on the recovery of 5-fluorouracil-damaged bone marrow was studied as a function of time. Mice were randomly assigned to five groups. The mice in groups 3, 4 and 5 were injected intraperitoneally with 100 mg/kg of 5-fluorouracil while those in groups 1 and 2 received physiological NaCl. Each mouse was pre-treated orally by gavage for 5 days with rosiglitazone (3 mg/kg in group 4, and 6 mg/kg in groups 2 and 5) or vehicle (groups 1 and 3). Blood was obtained from the retroorbital plexus of the animal. Bone marrow was obtained from femoral bone after sacrifice by cervical dislocation on days 3, 4, 5, 6, 8, and 10 after the 5-fluorouracil injection. The above-mentioned experiments were performed separately.

Changes in the bone marrow function were characterized by measuring total cellularity, frequency of granulocyte–macrophage colony forming units (CFU-GM) and total femoral CFU-GM content. Damage to granulocyte–macrophage colony forming units, the common progenitors of granulocytes and macrophages, precipitating neutropenia, infections and death has the greatest importance in myelotoxicity. Neutropenia-associated infections are the leading causes of mortality of patients with malignant diseases. Cellularity of femoral bone marrow was calculated using bone marrow cell counts and volumes of the samples. Frequency of CFU-GM progenitors was established from soft agar cultures and total CFU-GM content of the femur was calculated by multiplying cellularity times frequency of CFU-GM.

The effects of insulin on colony formation of granulocyte–macrophage progenitor cells were studied. Mice were randomly assigned to eight groups. Groups 1–3 and group 6 served as separate controls. Each mouse was injected subcutaneously for 5 consecutive days with physiological NaCl in groups 1, 3 and 6 and 4 U/kg of insulin (Insulin Monotard HMge, Novo Nordisk, Bagsvaerd, Denmark) in groups 4 and 7. A dose of 6 U/kg of insulin was administered to groups 2, 5 and 8. On day 5 mice were injected intraperitoneally with physiological NaCl in groups 1 and 2 and 100 mg/kg of 5-fluorouracil (Fluorouracil-TEVA, Pharmachemie, Haarlem, Netherlands) in the other groups. Two days later bone marrow samples were obtained from the femora of the mice to determine the colony formation of CFU-GM cells.

To evaluate the effect of rosiglitazone on plasma insulin and blood sugar levels six groups of mice were randomly formed. In the first control group mice were treated with vehicle. The mice in groups 2 and 5 were treated orally with 3 mg/kg rosiglitazone for 5 days and in groups 3 and 6 with 6 mg/kg doses. The mice in groups 4, 5 and 6 were injected with 5-fluorouracil (100 mg/kg) intraperitoneally on the 5th day 1 h after the last oral dose of rosiglitazone. Blood samples were collected after a 12-h fast at 1 h after the 5-fluorouracil administration.

Direct effects of rosiglitazone on human progenitor cells were studied in vitro in soft gel cultures. Mobilized peripheral blood stem cells of the patients were divided into four portions. Cells were grown in the presence of rosiglitazone (a gift from Rich Heyman, X-Ceptor Therapeautics Inc., San Diego, CA, USA) in two series using 0.5 and 1.0 μM concentrations. On the 5th day of the culturing period, 5-fluorouracil was added to the previous cultures at a 1 mg/l final concentration. In the two series of control cultures, cells were treated with vehicle or 5-fluorouracil in the same manner.

In vitro effects of rosiglitazone on murine CFU-GM cells were studied in methylcellulose cultures. The femoral bone marrow cell suspension was divided into six portions and cells were grown in the presence of rosiglitazone at a concentration of 1 μM in the 2nd, 5th and 6th series. On the 5th day of the culturing period 5-fluorouracil was added to the 4th, 5th and 6th series cultures in a 1 mg/l final concentration. In the 3rd and 6th series of cultures GW9662, a peroxisome-proliferator-activated receptor-gamma (PPARγ) antagonist (gift from T. M. Willson, GlaxoSmithKline, Research Triangle Park, NC) was also present from the beginning of the culturing period in a 5 μM concentration. Cells in the control cultures (1st series) were treated with vehicle in the same manner as the 1st series.

2.4. CFU-GM assay and blood cell counts

Soft agar cultures were prepared as described earlier (Benkő et al., 1999). Briefly, bone marrow cells were washed out from

the aseptically removed femora of mice following cervical dislocation and single cell suspensions were prepared. Inocula of 10^5/ml bone marrow cells were used in petri dishes (Greiner, Nürtingen, Germany) and the murine bone marrow cells were grown in McCoy's 5A modified medium (GIBCO Grand Island, NY, USA) supplemented with amino acids, Na pyruvate, $NaHCO_3$, antibiotics (streptomycin, penicillin), 0.3% agar (Oxoid, London, Great Britain), and with 25% horse serum. The conditioned medium of WEHI-3B cells was also added as a source of colony stimulating factors. Cultures were grown in triplicates for 7 days in a CO_2 incubator (Jouan Co., France) containing humidified atmosphere with 5% CO_2. Following this the colonies were counted under a dissection microscope (Olympus, Hamburg, Germany). Colonies were defined as groups of at least 50 cells, consisting of granulocytes and monocytes, verified by smears or cytospin preparations.

Total white blood cell count was measured by hemocytometer and the frequency of neutrophil granulocytes was determined by differential count of 200 cells from blood smears stained with May-Grünwald-Giemsa.

2.5. Measurement of plasma insulin and glucose levels

Plasma glucose concentrations were determined in blood samples taken from the retroorbital plexus of the mice using Accu-Chek (Roche Diagnostics, Mannheim, Germany). Plasma insulin level was measured by means of radioimmunoassay (RIA) using commercially available kits (RK 400 M, Institute of Isotopes, Budapest, Hungary). Both intra- and inter-assay variations were lower than 5%.

2.6. Mobilization of bone marrow stem cells and leukapheresis

Bone marrow stem cells were mobilized to the blood with a single 4 g/m^2 dose of Cytoxan (Brystol Myers Squibb Co., Princeton, New Jersey USA) and 48 million units granulocyte colony stimulating factor (G-CSF, Neupogen, Hoffmann-La Roche Ltd., Basel, Switzerland) was administered two times daily from the 3rd through the 10–11th day. Mobilized peripheral blood stem cells were obtained by leukapheresis using a Fresenius Com.Tec system (Fresenius Com.Tec GmbH, Hamburg, Germany). Apheresis was initiated on the 10–11th day in the recovery phase after chemotherapy if the CD34+ cell count was higher than 20/μl in the blood. Two–3×10^8/kg mononuclear cells with $3–4\times10^6$/kg CD34+ cells were obtained from the patients. Cells were resuspended in 100 ml Iscove's Modified Dulbecco's Medium (IMDM) with 1% human serum albumin and mixed slowly with equal volume of freezing solution containing 5% dimethyl sulfoxide (DMSO) in final concentration. The samples were then frozen by a computer-controlled cryopreservation system (Cryomed Freezer, Thermo Forma, Marietta, Ohio, USA) at −190 °C using liquid nitrogen at the Cell Therapy Laboratory, University of Debrecen. Cells were thawed rapidly in a water bath maintained at 37 °C. The total volume of the separated and subsequently thawed cells was used for the autologous transplantation and the rest of the cells were cultured in our experiments.

2.7. Colony formation of human mobilized peripheral blood stem cells

Methylcellulose (Methocel, 3000–5000 cP; FLUKA, Buchs, Switzerland) at 1.2% was used as the support matrix for semisolid cultures. McCoy's 5A modified medium was supplemented with amino acids, vitamins, Na pyruvate, $NaHCO_3$, penicillin and streptomycin as well as with 5×10^{-5} M 2-mercaptoethanol (LOBA, Fischamend, Germany) and 20% fetal bovine serum (Benkő et al., 2000). Mobilized peripheral blood stem cells were separated by Ficoll-Iodamide (Pharmacia, Uppsala, Sweden) gradient centrifugation at 1000 *g* for 15 min (specific gravity, 1.077 g/ml). Mononuclear cells from the interphase were washed twice with McCoy's 5A medium containing 5% fetal bovine serum (GIBCO, Grand Island, NY, USA). Using 35 mm plastic petri dishes (Greiner, Nürtingen, Germany), 10^5 cells were plated in 1 ml volume of this medium and were incubated for 14 days at 37 °C in a humidified atmosphere containing 5% (v/v) CO_2. Cultures were seeded in triplicates or quadruplicates. Cytokines were added to the soft gel cultures just before plating in final concentrations of 300 mg/l for granulocyte colony stimulating factor (G-CSF, Genzyme, Cambridge, England) and of 100 mg/l for granulocyte–macrophage colony stimulating factor (GM-CSF, Genzyme, Cambridge, England). Rosiglitazone was mixed into the medium and 5-fluorouracil was added to the cultures on the 5th day of the 14-day culturing period. Colonies were counted under a dissection microscope (Olympus, Hamburg, Germany). Colonies were defined as groups of at least 20 cells. The morphology of the cells in the colonies was evaluated with in situ touch and cytospin preparations. Slides were stained by May-Grünwald-Giemsa or by conventional cytochemical reactions.

2.8. Statistical analysis

Data obtained from individual mice were used for statistical analysis. Each hematologic variable was evaluated using one-way analysis of variance, followed by Bonferroni's post test for multiple comparisons. Differences were regarded as statistically significant at $P<0.05$.

3. Results

3.1. Effects of rosiglitazone on recovery of 5-fluorouracil-damaged bone marrow

Characterizing bone marrow function by total cellularity, CFU-GM colony formation and CFU-GM content of femoral bone marrow demonstrated that damage to bone marrow function was serious even by the 3rd day following a single dose of 100 mg/kg of 5-fluorouracil. Total cellularity and frequency of CFU-GM cells were 30% and the femoral content of CFU-GM was only 10% of the control value. Pre-

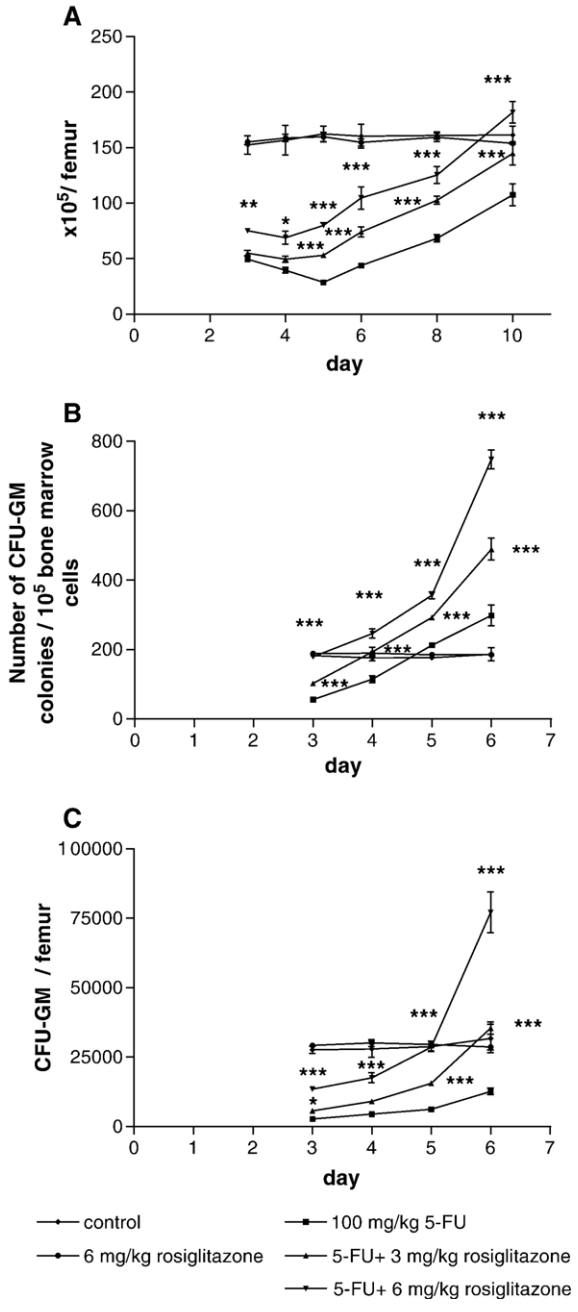

Fig. 1. Influence of 5-day-long oral rosiglitazone pre-treatment in doses of 3 and 6 mg/kg on recovery of the 5-fluorouracil-damaged bone marrow indicated by total cellularity of the bone marrow (PANEL A), CFU-GM colony numbers (PANEL B) and femoral CFU-GM content (PANEL C). Days are numbered from the single intraperitoneal injection of 5-fluorouracil. Cultures were grown in triplicates. Values are means±S.E.M., $n=13$ in each group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to mice treated with 5-fluorouracil alone. Mice in control group were treated with vehicle.

treatment with 6 mg/kg rosiglitazone for 5 days resulted in significant improvement of these variables. The total cellularity and the CFU-GM pool increased to 50% of the control values and the frequency of CFU-GM cells was normal even on the 3rd day of regeneration. Recovery of hematopoiesis was very slow after bone marrow damage caused by 5-fluorouracil. By the 6th day cellularity stood at 30% after a nadir of 18% and CFU-GM content came up to only 40% of the control value despite the elevated intensive proliferation of the CFU-GM cells (Fig. 1).

Rosiglitazone had no effect on healthy bone marrow function but recovery of damaged bone marrow was accelerated by rosiglitazone pre-treatment (Fig. 1). Higher intensity of proliferation in CFU-GM cells resulted in normal colony numbers as early as day 4 using 3 mg/kg doses and normalization was achieved by day 3 with 6 mg/kg doses. The enhanced proliferation of these groups is highlighted when compared to the group treated by 5-fluorouracil alone where standard colony numbers were reached by day 5 (Fig. 1B). The CFU-GM pool was replenished earlier in rosiglitazone pre-treated groups and was normalized on day 6 using 3 mg/kg doses. In addition, a 2.5-fold higher than normal expansion was observed using 6 mg/kg doses. In the same time frame the group treated with 5-fluorouracil alone reached only 40% of the normal CFU-GM pool (Fig. 1C). The nadir of the cellularity was reached one day earlier in the pre-treated groups as opposed to the group treated with 5-fluorouracil alone. These values in pre-treated groups were dose-dependently higher by significant margins (Fig. 1A).

The numbers of mature cells originating from hematopoiesis decreased after a latent period in peripheral blood, as the

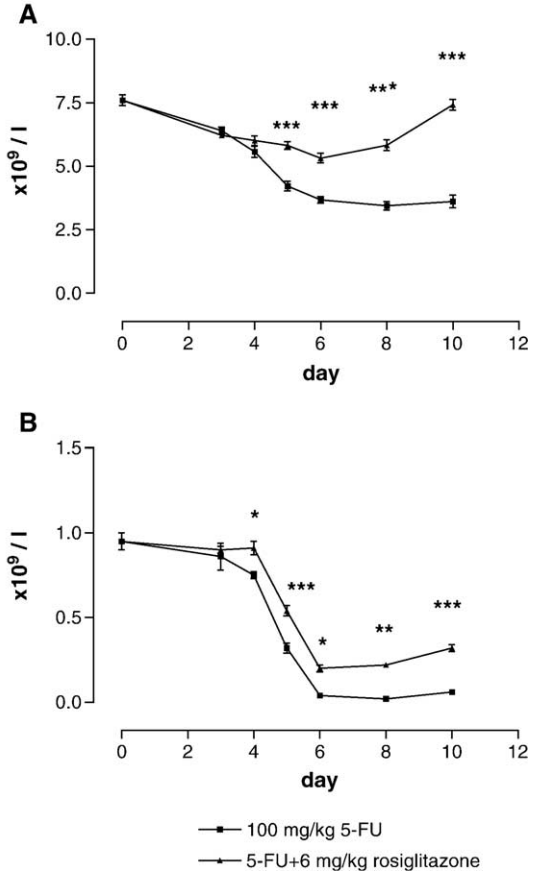

Fig. 2. Influence of 5-day oral 6 mg/kg of rosiglitazone pre-treatment on recovery of white blood cell counts (PANEL A) and absolute neutrophil counts (PANEL B) in peripheral blood. Days are numbered from the single intraperitoneal injection of 5-fluorouracil. Values are means±S.E.M., $n=13$ in each group, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to mice treated with 5-fluorouracil alone.

previously formed cells were present. White blood cell counts and absolute neutrophil counts decreased from the 4th day onward. An especially marked slope in levels of absolute neutrophil counts was seen along with a slow recovery phase in the remaining days of the observed period. Mice pre-treated with rosiglitazone had a milder decrease in absolute neutrophil counts, which was also less extensive ($P<0.05$–0.001). Up through the 10th day following 5-fluorouracil administration, the absolute neutrophil counts remained significantly higher than those of the non-pre-treated mice ($P<0.001$) (Fig. 2). Rosiglitazone in 6 mg/kg doses had no effect on blood cell counts (data not shown).

3.2. In vivo effects of insulin on 5-fluorouracil-induced myelotoxicity

Serious bone marrow damage resulted from 5-fluorouracil in 70 and 100 mg/kg doses. The dose-dependent decrease in colony formation of CFU-GM progenitor cells is evident 2 days after 5-fluorouracil administration. When 6 U/kg of long-acting insulin was administered once a day for 5 days before the single 5-fluorouracil dose, the CFU-GM colony numbers grown from 10^5 mononucleated cells were significantly higher than in vehicle-pre-treated groups (Fig. 3).

3.3. Effect of rosiglitazone on plasma insulin and glucose levels

Insulin levels tend to increase after a 5-day rosiglitazone treatment. In the group pre-treated with 6 mg/kg of rosiglitazone before the 5-fluorouracil dose, a slightly increased plasma insulin was observed together with a significant decrease in plasma glucose compared with the mice treated with 5-fluorouracil alone (Fig. 4).

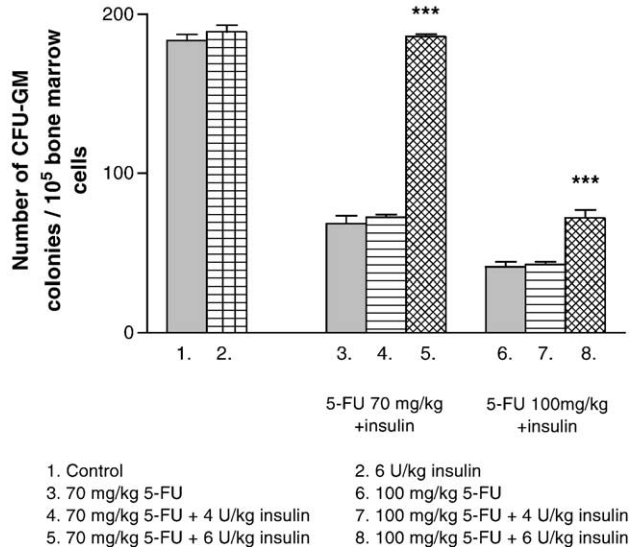

Fig. 3. Influence of 5-day subcutaneous insulin pre-treatment on 5-fluorouracil-induced myelotoxicity indicated by CFU-GM colony formation. Cultures were grown in triplicates. Values are means±S.E.M., $n=13$ in each group, ***$P<0.001$ compared to mice treated with 5-fluorouracil alone. Mice in control group were treated with vehicle.

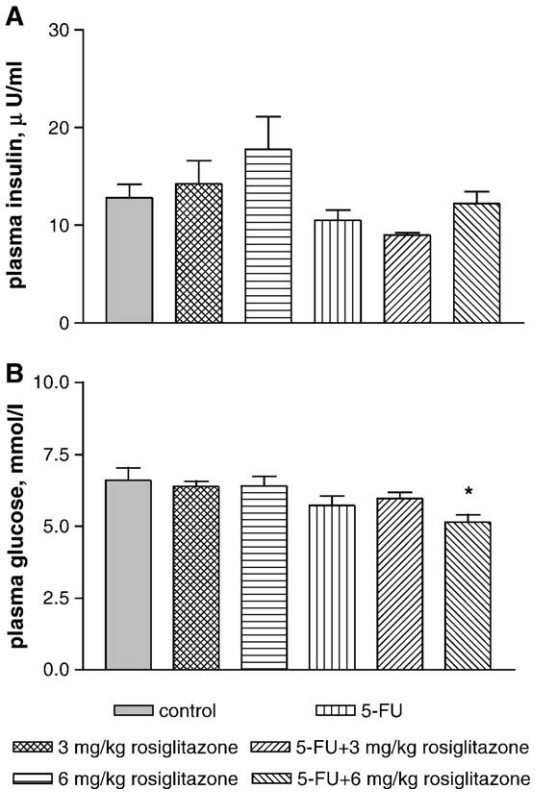

Fig. 4. Influence of 5-day-long oral rosiglitazone treatment alone and in combination with a single 5-fluorouracil dose on the 5th day on plasma insulin (PANEL A) and glucose levels measured 2 h after the 5-fluorouracil i.p. injection (PANEL B). Values are means±S.E.M., $n=7$ in each group *$P<0.05$ compared to the control group treated with vehicle.

3.4. Effects of rosiglitazone on colony formation of human mobilized peripheral blood stem cells

To detect whether previous beneficial effects of rosiglitazone had a direct or an indirect influence on the hematopoietic cells,

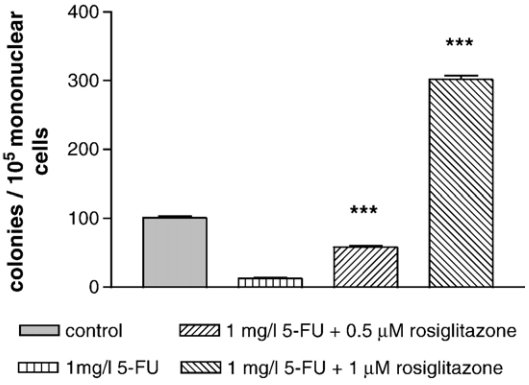

Fig. 5. Influence of rosiglitazone application at concentrations of 0.5 or 1 μM on colony formation of human mobilized peripheral blood stem cells after administration of 5-fluorouracil on the 5th day of the cultural period in 1 mg/l final concentration, versus the cultures treated with 5-fluorouracil alone in the same manner. Cultures were grown in triplicates. Stem cells were originated from the peripheral blood of three patients. Values are means±S.E.M., ***$P<0.001$ compared to cultures treated with 5-fluorouracil alone. In control cultures vehicle was used in the same manner.

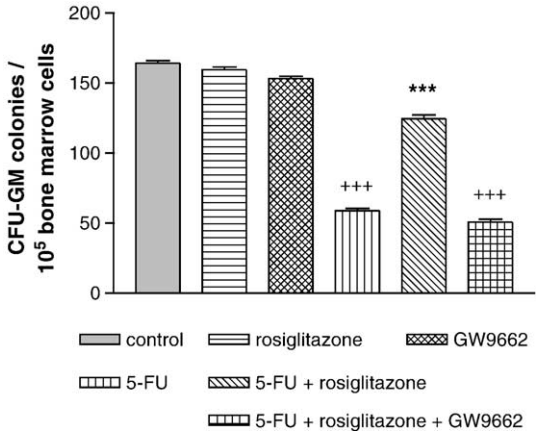

Fig. 6. Influence of rosiglitazone (1 μM) and a PPARγ antagonist (GW9662, 5 μM) application on damage caused by 5-fluorouracil administered in 1 mg/l final concentration on the 5th day of the cultural period indicated by the colony formation of murine CFU-GM progenitor cells.

in vitro cultures were used. The chosen human mobilized peripheral blood stem cell suspension contained many types of hematopoietic progenitor and stem cells. In our experimental system their differentiation into granulocyte–macrophage cells could be studied, as the grown colonies were selectively matured granulocytes and monocytes. Cells were cultured both in the presence and absence of rosiglitazone. 5-fluorouracil was added to cultures later on the 5th day. In 1 mg/l concentration 5-fluorouracil suppressed colony formation in 87% of the samples. Rosiglitazone dose-dependently protected progenitor and stem cells against 5-fluorouracil damage. Colony formation was inhibited by only 42% in the presence of 0.5 μM rosiglitazone. Rosiglitazone at 1 μM concentration enhanced differentiation of stem and progenitor cells towards granulocytes and macrophages to 300% of the control values (Fig. 5).

3.5. Effects of rosiglitazone and a PPARγ antagonist on colony formation of murine granulocyte–macrophage progenitor cells

Rosiglitazone is a partial agonist on PPARγ receptors. We investigated whether it has a direct effect on CFU-GM progenitors and whether PPARγ receptor effects are involved in protection. Neither rosiglitazone nor a PPARγ receptor antagonist drug (GW9662) affected colony formation of CFU-GM progenitor cells but they influenced their sensitivity to 5-fluorouracil. Colony formation of CFU-GM progenitors after 5-fluorouracil application was increased if they were grown in the presence of rosiglitazone compared with cells cultured without rosiglitazone. This beneficial effect was neutralized by the presence of the PPARγ antagonist (Fig. 6).

4. Discussion

Technical developments and new drugs against malignant cells, as well as the pharmacological modulation of bone marrow toxicity are expected to influence modern chemotherapy for cancer patients. This will hopefully lead to an increase in cure rates with better quality of life. Our preliminary data were the first demonstration of a myeloprotective effect of rosiglitazone, an insulin-sensitizing drug (Benkő et al., 2003). Based on present results we could state that the non-pre-treated groups reached their intensified regeneration phase following bone marrow damage induced by a cytostatic agent at a later time than groups pre-treated with rosiglitazone. Significant dose-dependent differences were demonstrated by comparing the degree of bone marrow regeneration following 5-fluorouracil injections, as indicated by CFU-GM content and frequency. The high intensity of proliferation in turn resulted in earlier recovery of the CFU-GM pool. Therefore it was logical to conclude that healing started earlier in groups receiving rosiglitazone. The same effect was reflected by the absolute neutrophil counts in peripheral circulation. The nadir of absolute neutrophil count was less deep in pre-treated mice and remained significantly higher during the observed period.

The myeloprotective property of rosiglitazone may be due to a range of direct and indirect effects. Some insulin-like effects could be participating in the observed myeloprotection, as subcutaneous insulin used in similar treatment protocols also increased the frequency and femoral content of CFU-GM in 5-fluorouracil-damaged bone marrow in mice. Although the effects of insulin on myelopoiesis have not been investigated in vivo, the stimulating effects of insulin-like growth factor-I (IGF-I) have been observed on myelopoiesis in mice treated with azidothimidine (Tsarfaty et al., 1994; Difalco et al., 1998).

Insulin and rosiglitazone had no effect on CFU-GM colony numbers in healthy bone marrow (Benkő et al., 2003 and Figs. 1and 3), but they could preserve more progenitor cells in damaged marrow. Hematopoiesis is controlled by a network of cytokine interactions. Many parallel and combined effects converge in hematopoiesis. It is not surprising that neither insulin nor rosiglitazone was able to enhance the optimal colony numbers in balanced normal hematopoiesis in our experiments. But in damaged hematopoiesis they were able to help restore the normal size and ratio of the cell compartments in bone marrow more quickly. Insulin itself has no stimulative effect on progenitor cells in vitro, but it can increase their survival (Ratajczak et al., 1998). Equilibrium between survival and apoptosis can be shifted towards survival by insulin (Iida et al., 2002). In the very flexible hematopoietic system some growth factors are able to substitute for each other and many of them can potentiate each other's effects by increasing sensitivity to the subsequent factor during differentiation. By increasing survival and amplifying the effects of the other colony stimulating factors insulin or rosiglitazone can enlarge the damaged CFU-GM pool and accelerate bone marrow regeneration. Insulin showed a stronger protective effect at lower doses of 5-fluorouracil. A more serious degree of damage could moderate this protection, as was seen in our experiments.

Although hyperinsulinemia is not expected with rosiglitazone treatment (Zawalich et al., 2003; Seda et al., 2002), a tendency toward increased plasma insulin levels was observed in groups treated with 6 mg/kg of rosiglitazone. The decrease in plasma glucose level was significant in mice treated with 6 mg/kg of rosiglitazone and 5-fluorouracil in combination, but it

remained within normal range. Some authors published that rosiglitazone can improve insulin secretory responses of pancreatic beta cells to oscillations in plasma glucose levels (Walter and Lubben, 2005). The effect of this small-scale release of insulin on hematopoietic cells cannot be excluded but the more significant myeloprotection suggests the possibility of other influences. Rosiglitazone may act on progenitor cells indirectly through insulin release or directly by increasing the insulin sensitivity of these cells. A number of other indirect and direct effects could also alter hematopoiesis. Rosiglitazone influences the production of adipocyte-derived factors. These changes may also increase the insulin sensitivity of cells, but some adipokines may also directly regulate myelopoiesis (Bennett et al., 1996).

Rosiglitazone is not unique in affecting colony formation of some progenitor cells, i.e. granulocyte–macrophage progenitors. It was shown that rosiglitazone could increase colony formation of bone marrow-derived primitive progenitor cells and promote their differentiation towards the endothelial lineage in mice (Wang et al., 2004). The effective dose range was similar to that which we used in our experiments.

To answer the question whether direct effects on progenitor cells are involved, we studied in vitro cultures of these cells. Autologous peripheral blood stem cell transplantation is now routinely used for patients with certain hematologic malignancies. After mobilization, blood is enriched with a variety of hematopoietic stem and progenitor cells, which can be obtained by leukapheresis. The damage caused by 5-fluorouracil decreased in a dose-dependent fashion in cultures containing rosiglitazone. Therefore we concluded that the effects of rosiglitazone on bone marrow progenitor cells were direct.

Rosiglitazone binds to peroxisome-proliferator-activated receptor-gamma (PPARγ), which is a ligand-activated nuclear transcription factor and important in many metabolic pathways including the control of cellular energy supply. An increased number of glucose transporters enhances glucose uptake especially in fat and muscle cells (Vamecq and Latruffe, 1999). PPARγ activation promotes cellular differentiation in many types of cells, including hepatocytes, fibroblasts, myocytes, epithelial cells and fat cells (Vamecq and Latruffe, 1999). Agonists of this receptor, such as thiazolidinediones, may influence proliferation and differentiation (Kersten, 2002). The beneficial effect of rosiglitazone on damage to murine CFU-GM cells was neutralized by a PPARγ antagonist. PPARγ is common in bone marrow-derived cells and found in many hematopoietic cell lines but its role during macrophage differentiation is not known (Green et al., 2000).

There are only a few myeloprotective drugs in clinical practice today. Currently granulocyte colony stimulating factor (G-CSF) is considered to be the most effective agent among the myeloprotective drugs, overshadowing others like granulocyte–macrophage colony stimulating factor (GM-CSF), stem cell factor (SCF) or many other colony stimulating factors in neutropenic patients, mostly because of its low side effect profile. G-CSF is routinely used to reduce acute toxicities in low and high-dose chemotherapy and adjuvant chemoradiation in cancer patients (Esser and Brunner, 2003). The other promising myeloprotective drug is amifostine, an aminothiol compound with antioxidant properties (Hartmann et al., 2001). However they are both administered parenterally, intravenously or subcutaneously, and they are associated with uncomfortable side effects in a large percentage of patients. In the case of amifostine mainly nausea and hypotension have been documented in more than half of the patients (Dunst et al., 2000; Fulda et al., 2001). During chemo- and radiotherapy the least tolerable side effects for the patients are nausea and vomiting, which can even lead to refusal of therapy. Aggravating such effects would not be desirable. G-CSF causes a whole range of symptoms including high incidences of headache, bone/backache and myalgia/arthralgia even among healthy donors during mobilization of neutrophil granulocytes (Heuft et al., 2004). G-CSF also induces apoptosis in T lymphocytes and immune dysfunction (Rutella et al., 2001). In contrast, the activation of PPARγ with 0.5 μM rosiglitazone can promote the survival of T cells and suggests that PPARγ activation may potentially augment the immune response (Wang et al., 2002).

In vitro amifostine increased CFU-GM colony formation from primitive progenitor cells 2-fold (List et al., 1998), and G-CSF in synergism with GM-CSF, SCF, interleukin-3 (IL-3) and IL-6 increased colony formation from cord blood cells 49-fold, (Denning-Kendall et al., 1998). In our experiments rosiglitazone was able to enhance CFU-GM colony formation up to 25-fold in the 5-fluorouracil-damaged human mobilized primitive progenitor cells. The myeloprotective effects of rosiglitazone may also prevent infections associated with neutropenia caused by cytostatic drugs, which is the highest cause of mortality in malignant diseases. Being able to avoid such disturbing side effects of chemotherapy as oral candidiasis and other chronic mycoses obviously helps to improve quality of life for the patient. It may also have an importance in pharmacological ex vivo purging of peripheral blood progenitor cell collections. Pharmacological ex vivo purging has been developed to eliminate tumor cells in the autograft using cytostatic drugs, but preserving the normal progenitor cells has proven difficult. Rosiglitazone may protect normal progenitor cells and probably has inhibiting effects on tumor cells (Ohta et al., 2001; Fujimura et al., 1998; Mossner et al., 2002; Toyoda et al., 2002). Thus rosiglitazone may be a real alternative to the existing myeloprotective drugs in the future due to its easy oral administration and relatively low risk, providing higher life expectancy accompanied by better quality of life for patients with malignant diseases.

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