Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 477 (2003) 179–182
www.elsevier.com/locate/ejphar

Short communication

Rosiglitazone-induced protection against myelotoxicity produced by 5-fluorouracil

Ilona Benkő[a,*], Katayoun Djazayeri[a], Csongor Ábrahám[b], Judit Zsuga[a], Zoltán Szilvássy[a]

[a] *Department of Pharmacology and Pharmacotherapy, Medical and Health Science Center, University of Debrecen, P.O. Box 12, H-4012 Debrecen, Hungary*
[b] *N-Gene Research Laboratories Incorporation, Csillagvirág u. 8, H-1106 Budapest, Hungary*

Received 16 June 2003; received in revised form 8 August 2003; accepted 12 August 2003

Abstract

Insulin promotes survival of haemopoietic progenitors. We investigated if rosiglitazone, an insulin sensitizer, could confer protection against 5-fluorouracil (5-FU)-induced myelotoxicity in mice. The decrease in bone marrow cellularity, frequency and content of granulocyte–macrophage progenitors (CFU-GM) characterized myelotoxicity in mice, while insulin sensitivity was determined by hyperinsulinaemic euglycaemic glucose clamping. CFU-GM colony numbers increased in groups pre-treated with rosiglitazone (1.5–6 mg/kg, 5 days), compared to that in mice treated with 5-fluorouracil alone. Since rosiglitazone pre-treatment significantly promoted the clonal expansion of CFU-GM when given in the insulin sensitizing dose, we conclude that rosiglitazone had myeloprotective effects possibly by amplifying endogenous insulin action.

Keywords: Thiazolidinedione; CFU-GM; Bone marrow toxicity; Insulin sensitizer

1. Introduction

Myelosuppression is the most common dose-limiting toxicity of anti-tumour agents as they inhibit stem and progenitor cell proliferation. Progenitor cells have shorter duplication times than stem cells, therefore, these are more sensitive to chemotherapy-induced damage. As neutrophil leukocytes have the shortest half-life in circulation, their continuous renewal is essential to prevent the sequel of chemotherapeutic agent-induced neutropenia, infection and death.

Insulin is an early-acting growth factor for stem and progenitor cells. It enhanced survival, significantly increased the number of all progenitors and doubled both the granulocyte–macrophage colony-forming units (CFU-GM) and erythroid burst-forming units (BFU-E) recovered from CD34+ early progenitor haemopoietic cells ex vivo when compared to their insulin-free counterparts (Ratajczak et al., 1998). Conversely, it is widely used for haemopoietic cell cultures often in the absence of other colony stimulating factors (Echarti and Maurer, 1989). However, the use of insulin in vivo is hindered by insulin's numerous metabolic effects and its ability to promote tumour growth (Argiles and Lopez-Soriano, 2001). Since in vivo bone marrow protection is more advantageous, we tested if rosiglitazone, a thiazolidinedione insulin sensitizer, by increasing the host's insulin sensitivity could prevent anticancer drug induced myelotoxicity. Therefore, the present work was concerned with the possibility that rosiglitazone alleviates myelotoxicity induced by 5-fluorouracil (5-FU), an anticancer drug, in mice.

2. Materials and methods

The present experiments conform to the European Community 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 (11/2002 DEMAB).

2.1. Animals

Ten- to eleven-week old BDF_1 female mice from the National Institute of Oncology (Budapest, Hungary) were used. Animals were housed in an animal room with three to five animals per pen, fed commercial laboratory chow and water ad libitum.

* Corresponding author. Tel./fax: +36-52-427899.
E-mail address: benko@king.pharmacol.dote.hu (I. Benkő).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.08.021

2.2. Determination of insulin sensitivity

The animals were anaesthetized with an initial intraperitoneal dose of 50 mg/kg thiopenthal-sodium that was repeated as needed. For hyperinsulinaemic euglycaemic glucose clamping, two venous catheters and one arterial cannula were placed in the two external jugular veins, and the right carotid artery, respectively.

Human regular insulin was infused at a constant rate (20 mU/kg, NOVO, Nordisk, Copenhagen) via one of the venous catheters over 120 min to yield steady state plasma insulin immunreactivity of 100 ± 5 μU/ml. Blood samples of 0.2 ml were taken from the arterial cannula for blood glucose concentration measurements at 10-min intervals. Blood glucose concentration was maintained constant (5.5 ± 0.5 mmol/l) by variable rate of glucose infusion via the second venous cannula. Stable blood glucose level of 30-min duration was defined as steady state. The glucose infusion rate (mg/kg/min) during the steady state characterized insulin sensitivity (DeFronzo et al., 1979). In the steady state, additional blood samples were taken for plasma insulin determination three times at 10-min intervals.

2.3. CFU-GM assay and blood cell counts

Soft agar cultures were prepared as described earlier (Benkő et al., 1999). Briefly, the animals were exterminated by cervical dislocation, the femoral bones were aseptically removed. Bone marrow cells were washed out, and single cell suspensions were prepared by suspending them in McCoy's 5A medium (GIBCO, Grand Island, NY, USA) through thin needle syringe. 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 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 containing colony stimulating factors was also added. Cultures were grown in triplicates for 7 days, in a CO_2 incubator (Jouan, France) containing humidified atmosphere with 5% CO_2. Following this, the colonies were counted under a dissecting microscope (Olympus, Hamburg, Germany). Colonies were defined as groups of at least 50 cells, consisting of granulocytes and/or monocytes, verified by smears or cytospin preparations.

Total white blood cell count was taken in haemocytometer, the frequency of neutrophil granulocytes was determined by differential count of 200 cells from blood smears stained with Wright-Giemsa.

2.4. Study design

Mice were randomly assigned to six groups. Groups 1–3 served as different controls. Vehicles of rosiglitazone and 5-FU, or 6 mg/kg of rosiglitazone or 100 mg/kg of 5-FU were administered to mice in groups 1, 2 and 3, respectively. Animals in groups 4–6 received 1.5 or 3 or 6 mg/kg rosiglitazone (Avandia, GlaxoSmithKlein, Brentford, United Kingdom) followed by a single dose of 5-FU. Rosiglitazone diluted in distilled water was delivered by oral gavage for 5 days, 96, 72, 48, 24 and 1 h before the single intraperitoneal dose of 100 mg/kg 5-FU (Fluorouracil-TEVA, Pharmachemie, Haarlem, Netherlands) dissolved in 0.9% NaCl.

Bone marrow function and insulin sensitivity were evaluated on the 2nd day following the injection of 5-FU in separate sets of animals. The peripheral leukocyte count and absolute neutrophil count (ANC) were determined from samples of retro-orbital sinus blood. Cellularity of femoral bone marrow was calculated from bone marrow cell counts and volumes of the samples, the frequency of CFU-GM progenitors was established from the soft agar cultures. Total CFU-GM content of the femur was calculated (cellularity × frequency of CFU-GM). Insulin sensitivity was determined by hyperinsulinaemic euglycaemic glucose clamp.

2.5. Statistical analysis

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

3. Results

3.1. Effect of rosiglitazone on insulin sensitivity

Rosiglitazone applied at oral doses of 6 mg/kg resulted in a significant increase of the insulin sensitivity. The lower doses (1.5 and 3 mg/kg) and the vehicle were without effect (Fig. 1).

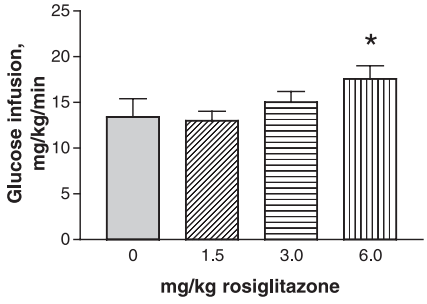

Fig. 1. The influence of rosiglitazone administered orally at doses of 1.5, 3 and 6 mg/kg over the course of 5 days on insulin sensitivity determined by hyperinsulinaemic euglycaemic glucose clamping. Values are means ± S.E.M., $n = 6$ in each group, $^*P < 0.05$ compared to vehicle-treated mice.

3.2. Effect of rosiglitazone pre-treatment on 5-fluorouracil-induced myelosuppression

5-FU significantly reduced cellularity, CFU-GM colony number and content of the femoral bone marrow ($P<0.001$) (Fig. 2). The cellularity of the bone marrow did not differ in the rosiglitazone pre-treated groups and the 5-FU-treated group (Fig. 2A). Furthermore, the frequency of CFU-GM progenitors, indicated by the colony numbers grown from 10^5 bone marrow cell inoculates, was higher in samples obtained from mice treated with rosiglitazone and 5-FU than in mice treated with 5-FU alone. This effect of rosiglitazone was dose-dependent. Rosiglitazone in a dose of 1.5 mg/kg had no influence on colony numbers. Three and six milligrams per kilogram of rosiglitazone increased colony numbers by 2- ($P<0.01$) and 2.5-fold ($P<0.001$), respectively (Fig. 2B). Proliferation of the increased number of progenitors results in an expansion of the CFU-GM pool and 6 mg/kg of rosiglitazone could significantly increase the CFU-GM content as early as the 2nd day after the bone marrow damage ($P<0.05$) (Fig. 2C).

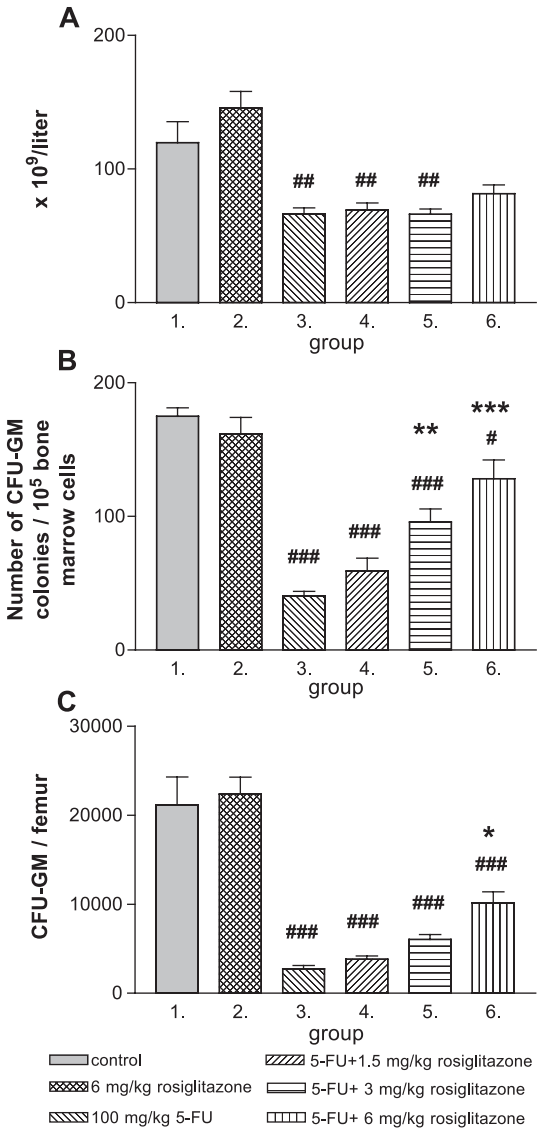

Fig. 2. Influence of 5-day-long oral rosiglitazone pre-treatment on 5-FU-induced myelotoxicity determined by cellularity (Panel A), the frequency of colonies grown in soft agar cultures (Panel B) and CFU-GM content of femoral bone marrow (Panel C). Cultures were grown in triplicates. Values are means ± S.E.M., $n=13$ in each group, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ compared to vehicle-treated mice, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to mice treated with 5-FU alone.

The toxic effect of 5-FU was also investigated in peripheral blood samples. Cytopenia originated from the lack of progenitors appears later in blood than it does in bone marrow. The absolute neutrophil counts (ANC) were somewhat lower in the 5-FU-treated group than in the control group, however, this difference was not significant. Furthermore, these values were all within the normal range in each group (not shown).

4. Discussion

The major finding of the present study is that rosiglitazone pre-treatment is able to confer protection on bone marrow cells against 5-FU-induced myelotoxicity. To the best of our knowledge, this is the first report concerning the chemoprotective effect of thiazolidinediones. The increase of the frequency of CFU-GM progenitors without change in bone marrow cellularity in the rosiglitazone pre-treated murine groups following 5-FU indicates intensive proliferation of these progenitor cells. The expansion of the CFU-GM pool is required to compensate for the 5-FU-induced loss and allows faster recovery. Granulocyte colony-stimulating factor, which is recently used for facilitating recovery from neutropenia, increases CFU-GM content to twofold in the 5-FU-damaged bone marrow in mice (Gilmore et al., 1995). Rosiglitazone pre-treatment could enhance the CFU-GM content to 3.7-fold compared to that in mice treated by 5-FU alone.

This was probably the extension of its insulin sensitizing effects since the dose range, which increased insulin sensitivity, was similar to that resulting in increased CFU-GM colony numbers. Additionally, we have found the expansion of CFU-GM pool a characteristic effect of insulin, in rosiglitazone pre-treated mice. Ex vivo insulin is added to the culture media when growing and expanding human CD34+ cells (Mobest et al., 1998). Insulin, if added alone to human CD34+ cells cultured under serum-free conditions, does not stimulate their proliferation, rather it augments the viability of haemopoietic progenitors by preventing them from undergoing apoptosis (Ratajczak et al., 1998).

There appears to be a difference between the direct measurements of insulin sensitivity (Fig. 1) and the improved indices of viability of progenitors (Fig. 2). Among the possible explanations, probably the most important, is that insulin sensitivity was determined in otherwise healthy animals, whereas the myeloprotective response occurred in diseased mice due to the preceding toxic 5-FU-dose admin-

istration. Nevertheless, it is known that cancer 'per se' may induce insulin resistance, which is then accentuated when anticancer drug is given (Yoshikawa et al., 2001). Therefore, it is possible that the insulin sensitizing effect of rosiglitazone occurs at lower doses in 5-FU-treated mice than in healthy animals. This possibility will be a subject of our forthcoming research.

Whatever the precise mechanism of the chemoprotective action of thiazolidinediones is, it is probable, that they do not have direct stimulating effects on the proliferation of progenitor cells. On the contrary, they can suppress clonal growth of myeloid leukemia cell lines and they can induce differentiation of monocytes and HL-60 promyelocytic cells (Fujimura et al., 1998; Tontonoz et al., 1998). Furthermore, they are beneficial for patients with liposarcoma (Demetri et al., 1999), prostata (Mueller et al., 2000) or breast cancer (Stoll, 2002).

Summarizing, we found that rosiglitazone, an oral insulin sensitizer, is able to promote the clonal expansion of CFU-GM when given in the dose producing insulin sensitization, possibly by enhancing the insulin sensitivity of progenitor cells. Thiazolidinediones may be new chemoprotective drugs for preventing infections associated with neutropenia caused by cytostatic agents.

Acknowledgements

This work was supported by the grants of the Hungarian government (NKFP 26-3900/2001), Committee of Innovative Pharmacologists and National Research and Development Fund (ALK-00055/2002).

References

Argiles, J.M., Lopez-Soriano, F.J., 2001. Insulin and cancer. Int. J. Oncol. 18, 683–687.

Benkő, I., Hernádi, F., Megyeri, A., Kiss, A., Somogyi, G., Tegyey, Z., Kraicsovits, F., Kovács, P., 1999. Comparison of the toxicity of fluconazole and other azole antifungal drugs to murine and human granulocyte–macrophage progenitor cells in vitro. J. Antimicrob. Chemother. 43, 675–681.

DeFronzo, R.A., Tobin, J.D., Andres, R., 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am. J. Physiol. 237, E214–E223.

Demetri, G.D., Fletcher, C.D., Mueller, E., Sarraf, P., Naujoks, R., Campbell, N., Spiegelman, B.M., Singer, S., 1999. Induction of solid tumor differentiation by the PPAR-gamma ligand troglitazone in patients with liposarcoma. Proc. Natl. Acad. Sci. U. S. A. 96, 3951–3956.

Echarti, C., Maurer, H.P., 1989. Defined, serum-free culture conditions for the GM-micro-clonogenic assay using agar-capillaries. Blut 59, 171–176.

Fujimura, S., Suzumiya, J., Nakamura, K., Ono, J., 1998. Effects of troglitazone on the growth and differentiation of hemopoietic cell lines. Int. J. Oncol. 13, 1263–1267.

Gilmore, G.L., DePasquale, D.K., Fischer, B.C., Shadduck, R.K., 1995. Enhancement of monocytopoiesis by granulocyte colony-stimulating factor. Exp. Hematol. 23, 1319–1323.

Mobest, D., Mertelsmann, R., Henschler, R., 1998. Serum-free ex vivo expansion of CD34+ hematopoietic progenitor cells. Biotechnol. Bioeng. 60, 341–347.

Mueller, E., Smith, M., Sarraf, P., Kroll, T., Aiyer, A., Kauffman, D.S., Oh, W., Demetri, G., Figg, W.D., Zhou, X.P., Eng, C., Spiegelman, B.M., Kantoff, P.W., 2000. Effects of ligand activation of PPAR-gamma in human prostate cancer. Proc. Natl. Acad. Sci. U. S. A. 97, 10990–10995.

Ratajczak, J., Zhang, Q., Pertusini, E., Wojczyk, B.S., Wasik, M.A., Ratajczak, M.Z., 1998. The role of insulin and insulin-like growth factor-I in regulating human erythropoiesis. Leukemia 12, 371–381.

Stoll, B.A., 2002. Linkage between retinoid and fatty acid receptors: implications for breast cancer prevention. Eur. J. Cancer Prev. 11, 319–325.

Tontonoz, P., Nagy, L., Alvarez, J.G.A., Thomazy, V.A., Evans, R.M., 1998. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 93, 241–252.

Yoshikawa, T., Noguchi, Y., Doi, C., Makino, T., Nomura, K., 2001. Insulin resistance in patients with cancer: relationships with tumor site, tumor stage, body-weight loss, acute-phase response, and energy expenditure. Nutrition 17, 590–593.