European Journal of Pharmacology 696 (2012) 172–178

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and inflammation

Toxicity of cytotoxic agents to granulocyte–macrophage progenitors is increased in obese Zucker and non-obese but insulin resistant Goto-Kakizaki rats

Krisztina Géresi [a], Klára Benkő [b], Boglárka Szabó [c], Attila Megyeri [a], Barna Peitl [a], Zoltán Szilvássy [a], Ilona Benkő [a,*]

[a] Medical and Health Science Center, University of Debrecen, Department of Pharmacology and Pharmacotherapy, Nagyerdei krt. 98, Debrecen 4032, Hungary
[b] Euromedic Diagnostics Ltd., Debrecen, Hungary
[c] Heart Center, Semmelweis University, Budapest, Hungary

ARTICLE INFO

Article history:
Received 10 November 2011
Received in revised form
3 September 2012
Accepted 17 September 2012
Available online 26 September 2012

Keywords:
Granulocyte–macrophage progenitor cells (CFU-GM)
Obesity
Myelotoxicity
Carboplatin
Doxorubicin
5-fluorouracil

ABSTRACT

Increased risk of anticancer chemotherapy in seriously obese patients is known. Obesity may be among factors that predict treatment-related toxicity during chemotherapy. We investigated whether functional changes in granulopoiesis may also contribute to increased myelotoxicity in addition to the known alterations of pharmacokinetic parameters in obesity. Hemopoiesis – as measured by cellularity, frequency of granulocyte–macrophage progenitors (CFU-GM) and total CFU-GM content of the femoral bone marrow – did not differ in obese, insulin resistant Zucker rats compared with Wistar rats. Nevertheless increased sensitivity of their CFU-GM progenitor cells to cytotoxic drugs was found by culturing them *in vitro* in the presence of carboplatin, doxorubicin and 5-fluorouracil. All drugs were more toxic on CFU-GM progenitor cells of insulin resistant Zucker rats than on CFU-GM cells of the control strain. This might be based on metabolic disorders, at least in part, because we could demonstrate a similar increase in toxicity of the studied anticancer drugs to the CFU-GM progenitors originated from the non-obese but insulin resistant Goto-Kakizaki rats in the same dose ranges. After *in vivo* administration of rosiglitazone, an insulin sensitizer, the anticancer drug sensitivity of CFU-GM progenitors of Goto-Kakizaki rats was decreased concurrently with improvement of insulin resistance. Although the increased treatment-related myelotoxicity and mortality are well-known among obese patients with malignant diseases, only the altered half lives, volumes of distribution and clearances of cytotoxic drugs are thought to be the underlying reasons. According to our knowledge the results presented here, are the first observations about an impaired granulopoiesis in obese animals.

1. Introduction

In our days obesity has become a worldwide epidemic, the incidence of excess body weight and obesity significantly increased during the last two decades both in children and adults. Obesity leads to many health problems, such as diabetes mellitus, cardiovascular, pulmonary, orthopaedic and other surgical problems (Pi-Sunyer, 2002). Although obesity itself is not defined as a disease, there are numerous alterations in cellular metabolism in many organs. Insulin resistance in muscle, liver and fat cells is well-known and it was recognized as a link between obesity and diabetes mellitus type 2 (Gupta et al., 2012).

Our previous work was the first demonstration of the myeloprotective effect of rosiglitazone, an insulin-sensitizer drug (Benkő et al., 2003) and we also found that rosiglitazone accelerated recovery of bone marrow damaged by single or repeated doses of 5-fluorouracil possibly by amplifying endogenous insulin action (Djazayeri et al., 2005, 2006). These observations turned our attention to the possible influence of the insulin resistance on bone marrow functions. Especially because insulin resistance is most frequently associated with obesity which affects a large and still growing population.

Moreover, evidence shows that obesity is related to increased risk of several types of tumours including colorectal (Anderson et al., 2007), breast (Kroenke et al., 2005), endometrial (von Gruenigen et al., 2006), renal (Chow et al., 2000), pancreatic (Gumbs, 2008), oesophageal and gastric cancers (Abnet et al., 2008).

* Corresponding author. Tel.: +36 52 427899; fax: +36 52 427899; Mob.: +36 20 5463838.
E-mail address:
benko@king.pharmacol.dote.hu (I. Benkő).

0014-2999/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2012.09.018

It is not clear what influence obesity has on anticancer chemotherapy. Pathophysiological changes in obese patients may affect pharmacokinetic parameters such as volume of distribution and drug clearance. The pharmacokinetic parameters of a drug are highly unpredictable in obese patients which means that calculating the effective dose range of chemotherapeutic drugs is difficult. The standard method of drug dose individualization uses body surface area (BSA). However use of BSA for dose calculation was adopted without adequate investigation of the relationship between dose, BSA, and other parameters of body size (Gurney, 1996). In particular, there are no specific dosage recommendations for obese patients undergoing chemotherapy (Abdah-Bortnyak et al., 2003).

It is reasonable to assume that, in addition to changes in pharmacokinetics, several other factors may influence the severity of side-effects of chemotherapy in obese patients. One of the most frequent adverse effects of cytotoxic therapy is myelotoxicity, i.e., damage to hemopoiesis in bone marrow, which is mainly due to the high proliferation rate of progenitor cells. Depletion of granulocyte–macrophage progenitor cells (CFU-GM), the common ancestors of granulocytes and macrophages, can cause serious neutropenia. Neutropenia, the most feared risk of chemotherapy, is associated with infections necessitating the use of intravenous antibiotics and often even can result in death.

In our present investigations we studied whether the function of femoral CFU-GM progenitor cells is modified in obesity or insulin resistance, and whether this may influence the effects of cytotoxic drugs on bone marrow.

2. Materials and methods

2.1. Animals

Eleven- to 12-week-old Wistar (362 ± 19 g), Zucker (428 ± 24 g) and Goto-Kakizaki (305 ± 13 g) male rats were purchased from Charles River Laboratories (Budapest, Hungary) and were used throughout the experiments. Animals were housed in an animal room with 12-h light and dark periods a day with 3 animals per pen. They were fed commercial laboratory chow and tap 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 (1/2009 UDCAR).

2.2. Chemicals

Freshly prepared solutions of the following drugs were used: carboplatin (Paraplatin, Bristol Myers-Squibb, Sermoneta, Italy), doxorubicin (Adriblastina, Pharmacia & Upjohn SPA, Milan, Italy), 5-fluorouracil (Fluorouracil-TEVA, Pharmachemie, Haarlem, Netherlands) and rosiglitazone (Avandia, GlaxoSmithKline, Brentford, United Kingdom).

2.3. Study design

First in 10 obese Zucker rats, 10 Wistar rats and 10 Goto-Kakizaki rats we determined the cellularity, the total nucleated cell number of the femoral bone marrow, which mirrors hemopoietic activity. Then the common progenitors of the phagocytic cells, the granulocyte–macrophage colony forming units (CFU-GM) were studied and compared with the hemopoiesis of control rats. The CFU-GM progenitors were cultured in a specific soft gel colony assay. The frequency of CFU-GM progenitors was established from these soft gel cultures. Total CFU-GM content of the femur was calculated from cellularity and frequency of CFU-GM progenitor cells.

Colony assays were used not only as described in the literature but also with some modifications. To test the functional state of these cells they were cultured in the presence of cytotoxic drugs and their sensitivity was determined against carboplatin, doxorubicin and 5-fluorouracil. We chose three anticancer drugs with different mode of actions to see whether there are some drug-relating alterations or a nonspecific disorder which might influence sensitivity of these cells (CFU-GM) to the studied cytotoxic drugs. Dose-response curves were determined by using increasing concentrations (0.001–10 mg/L) of drugs in the cell cultures.

Henceforward we pre-treated further 10-10 Goto-Kakizaki rats and 10-10 Wistar rats with vehicle or rosiglitazone, an insulin sensitizer drug, using 3 mg/kg daily doses *in vivo*. Rosiglitazone or its vehicle was administered orally to animals on fourteen consecutive days. At the end of the pre-treatment period femoral cellularity, frequency of CFU-GM progenitor cells and total femoral CFU-GM content were determined in soft-gel cultures containing the same drugs in the same dose ranges as in our previous experiments.

2.4. Bone marrow samples

Animals were exterminated by intravenous overdose of thiopental sodium (100 mg/kg body weight dose; Sandoz GmbH, Austria). Femoral bones of rats were prepared under sterile conditions and bone marrow was completely washed out. Single cell suspensions were obtained by suspending bone marrow samples in McCoy's 5A medium (Sigma-Aldrich, Budapest, Hungary) and forcing them several times through a thin needle with a syringe. We separated the mononuclear cell fractions from bone marrow cell suspensions by Ficoll-Paque™ Plus (Immunotrin Ltd., Budapest, Hungary) gradient centrifugation (1.077 g/ml).

2.5. CFU-GM assay

Special soft-gel cultures were prepared as described earlier (Benkő et al., 1999). Briefly, mononuclear cell fractions were obtained from bone marrow cell suspensions by Ficoll-Paque™ Plus (Immunotrin Ltd., Budapest, Hungary) gradient centrifugation (1.077 g/ml). Nucleated rat bone marrow cells were plated in petri dishes (Greiner, Nürtingen, Germany) at a density of 10^5 cells per dish and were grown in McCoy's 5A modified medium (Sigma-Aldrich, Budapest, Hungary) supplemented with amino acids, Na pyruvate, $NaHCO_3$, antibiotics (streptomycin, penicillin) and 20% foetal bovine serum. Human recombinant growth factors (SCF, G-CSF, GM-CSF) were tested and used as colony stimulating factors. Methylcellulose (Methocel, 3000–5000 cP; FLUKA, Buchs, Switzerland) at 1.2% was used as the support matrix for semi-solid cultures. Cultures were grown in triplicates for 14 days at 37 °C at 100% relative humidity in an atmosphere containing 5% CO_2. Colonies were counted under stereomicroscope (Olympus, Hamburg, Germany) at the end of the incubation period. Colonies were defined as groups of at least 50 cells.

2.6. Rapid insulin sensitivity test (RIST)

We determined insulin sensitivity in different rat groups. The rapid insulin sensitivity test (RIST) was performed with 50 mU/kg insulin as described by Lautt et al. (1998). In brief, after a 30-min post surgery stabilizing period, arterial blood samples were taken every 5 min for blood glucose determination. The mean blood glucose level of 3 consecutive determinations was referred to as the control value. The total amount of glucose (expressed in milligrams per kilogram of body weight) required to counteract