Biochimie 101 (2014) 256–259

Contents lists available at ScienceDirect

Biochimie

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

Short communication

Ribonuclease binase decreases destructive changes of the liver and restores its regeneration potential in mouse lung carcinoma model

A.V. Sen'kova [a,1], N.L. Mironova [a,*,1], O.A. Patutina [a,1], V.A. Mitkevich [b], O.V. Markov [a], I.Y. Petrushanko [b], K.M. Burnysheva [b], M.A. Zenkova [a], A.A. Makarov [b]

[a] Institute of Chemical Biology and Fundamental Medicine SB RAS, 8, Lavrentiev Ave., Novosibirsk, Russia
[b] Engelhardt Institute of Molecular Biology RAS, Vavilov Str. 32, 119991 Moscow, Russia

ARTICLE INFO

Article history:
Received 24 December 2013
Accepted 13 February 2014
Available online 22 February 2014

Keywords:
RNase
Destructive changes of the liver
Murine tumor model
Regeneration potential of the liver
Cancer

ABSTRACT

The successful application of exogenous ribonucleases of different origin to suppress tumor growth allows one to consider them as perspective therapeutics for treatment of oncological diseases. An important aspect of the success of an anti-cancer drug is low hepatotoxicity, which will reduce, if not eliminate entirely the undesirable side effects of treatment. Previously we have shown that ribonuclease from *Bacillus intermedius* (binase) exhibits high antitumor and antimetastatic activity in tumor models of different histological origin. In this work we studied hepatotoxic action of binase using mouse tumor model of Lewis lung carcinoma. Binase at doses of 0.1–1 mg/kg which produced effective suppression of tumor growth and metastasis, showed positive effect on the liver of tumor-bearing mice expressed in a significant reduction in the volume of destructive changes in the liver parenchyma and return to the normal level of the liver regenerative potential impaired due to endogenous intoxication and tumor burden.

1. Introduction

Most anticancer drugs undergo biotransformation in the liver by microsomal monooxygenase system resulting in the formation of toxic metabolites that cause hepatocyte damage [1,2]. In addition, a significant contribution to liver damage is made by endogenous intoxication syndrome that develops in most malignancies. In clinical practice special attention is given to the hepatotoxicity of drugs used, because together with the damage of bone marrow cells and the myocardium, liver damage is one of the limiting factors for application of high-dose chemotherapy schemes necessary for effective treatment. Therefore, when studying the properties of new anticancer drugs it is necessary to investigate morphological changes in the liver and its compensatory capacity during tumor progression. Of greatest interest therefore are the compounds with antitumor activity, which are void of severe toxicity, and also have the protective effect.

Exogenous cytotoxic ribonucleases (RNase) cause disruption of RNA-dependent regulatory processes in tumor cells and are considered as promising chemotherapeutic agents. The antitumor activity was shown for Onconase from oocytes of *Rana pipiens* [3], bovine seminal RNase (BS-RNase) [4], bovine pancreatic RNase A [5], a number of microbial RNases and their mutants [6–10]. Despite the high antitumor capacity of some RNases identified in pre-clinical and clinical trials, these drugs produce side effects in mammals. Application of BS-RNase to treat animals with malignant diseases results in the degradation of testicular tissue in males, affects the embryogenic tissue and oocyte maturation in females and has a general immunosuppressive effect [11]. A major shortcoming of therapy using Onconase revealed in clinical trials is the occurrence of severe renal failure, inhibition of lymphocyte proliferation, bone marrow toxicity, ataxia, muscular stiffness and tremor, leading to death [12].

Bacterial RNases are an alternative platform for novel anticancer therapy [13,14]. Previously we have shown that the RNase from *Bacillus intermedius* (binase) is an effective agent for inhibition of tumor cell growth *in vivo* and *in vitro* [15–18]. It produces cytotoxic effect on a number of transformed cells including human leukemic K562 and Kasumi-1 cells [15,17]. The ability of binase to retard primary tumor and metastasis growth was demonstrated on the three mouse tumor models that differed from each other by

Abbreviations: LLC, Lewis lung carcinoma; RNase, ribonuclease; binase, *Bacillus intermedius* RNase.

* Corresponding author. Tel.: +7 383 363 51 60; fax: +7 383 363 51 53.
E-mail addresses: mironova@niboch.nsc.ru, arstanova@ngs.ru (N.L. Mironova).

[1] Equally contributing authors.

http://dx.doi.org/10.1016/j.biochi.2014.02.006
0300-9084/

histological type and had strong relevance to human tumors: Lewis lung carcinoma (LLC); lymphosarcoma RLS_{40} and melanoma B-16 [18], indicating binase as a prospective antitumor drug.

In this study, the effect of binase on the liver of mice with Lewis lung carcinoma was investigated. It was found that in addition to anti-tumor activity binase has positive effect on the liver of tumor-bearing animals, reducing the volume of destructive changes in the liver parenchyma, and increasing the amount of binuclear hepatocytes, which reflects the regeneration capacity of the liver.

2. Material and methods

2.1. Animal experiment

All animal procedures were performed in compliance with the approved protocols and recommendations for proper use and care of laboratory animals [ECC Directive 86/609/EEC]. Ten- to 12-wk-old female C57Bl/6J mice were used in the experiments. Solid tumors LLC were induced by intramuscular injection of LLC cells (10^6, 0.1 ml) into the right thighs of C57Bl/6J mice. Starting from day 4 after tumor transplantation LLC-bearing mice were injected intramuscularly or intraperitoneally with saline buffer or binase at doses of 0.1, 0.5 and 1 mg/kg, in total, 8 injections were administered within two weeks. Healthy C57Bl/6J mice were injected intraperitoneally with binase at the dose of 1 mg/kg thrice a week during two weeks. Material for histological study (livers) was collected on day 15 after LLC transplantation.

2.2. Histology and morphometry

The livers were fixed in 10% neutral-buffered formalin, routinely processed, and embedded in paraffin. Paraffin sections (5 μm) were stained with hematoxylin and eosin, microscopically examined and scanned. Stereological quantification of the liver samples was performed by point counting, using closed test-system at a lens magnification ×40. The test-system used had 100 testing points in a testing area equal to 3.2×10^6 μm^2. Ten to fifteen random fields were studied in each specimen, in total forming 100–150 fields for each group of mice.

Morphometric analysis of the liver was performed and the volume densities (Vv) of normal liver parenchyma, hepatocytes with degenerate and necrotic changes were evaluated. The value of Vv representing the volume fraction of tissue occupied by this compartment was determined from the points lying over these structures and calculated using the following formula: $Vv = (P_{structure}/P_{test}) \times 100\%$, where: $P_{structure}$ denotes the number of points over the structure and P_{test} represents the total number of test points, 100 in this case.

The numerical density (Nv) of binuclear hepatocytes reflecting the regeneration capacity of the liver was evaluated. The value of Nv indicating the number of particles in the unit tissue volume was determined by counting the number of structures under study within the test area (3.2×10^6 μm^2).

2.3. Statistics

The data were statistically processed using the Student's *t*-test (two-tailed, unpaired); a *p* value of ≤ 0.05 was considered to indicate a significant difference.

3. Results and discussion

Hepatotoxicity of binase was studied in healthy C57Bl/6J mice and mice with induced LLC, metastasizing to the lungs. Data on the hepatotoxic effect of binase are presented in Fig. 1. In healthy mice histological examination revealed that the liver has tubule structure (Fig. 1A, Left). Hepatocytes forming hepatic tubules have an irregular polygonal shape, some of them containing two or more nuclei. It is known that when exposed to toxic factors the beam-like structure of liver is disrupted, dystrophic changes are developed in hepatocytes (hepatocytes increase in volume, become rounded, their cytoplasm is filled with vacuoles), subsequently followed by necrotic changes (cells are destroyed and replaced by unstructured necrotic foci of homogeneous pink color). Treatment of healthy mice with binase at a dose of 1 mg/kg thrice a week for 2 weeks does not lead to toxic liver damage: no significant differences have been identified between animals receiving intraperitoneal saline buffer and binase in volume density of dystrophies and necrosis in the liver parenchyma (Fig. 1A, Center).

The liver tissue of mice in the control group (LLC-bearing mice without treatment) showed dyscirculatory and destructive changes, specifically, centrilobular plethora, discomplectation of cords, sinusoidal collapse, edematic and balloon protein degeneration of hepatocytes, frequent monocellular and focal necrosis. Upon tumor progression normal liver tissue makes only 22.3%, whereas tissue with degenerative and necrotic changes constitutes 25.5 and 45.6% respectively (Fig. 1A, Right).

Intramuscular administration of binase leads to the decrease in the volume density of destructive changes, and the portion of normal liver parenchyma increases in the dose-dependent manner (Fig. 1B). When binase is administered in a dose of 0.1, 0.5 and 1 mg/kg the normal parenchyma portion increases to 38.8, 49.2 and 64.2% respectively. Upon treatment with binase (dose 1 mg/kg) the significant drop in destructive changes in the liver tissue is observed: 30.8% in comparison with 72.1% in the control group (Fig. 1A, Right and B, Right, respectively). Reduction of destructive changes and the increase in the normal liver parenchyma correlate well with the antitumor and antimetastatic effects of binase [18]: intramuscular administration of binase (0.5–1 mg/kg, thrice a week) caused inhibition of primary tumor growth to 25–40% of control and twofold decrease in the total area occupied by metastases.

In its turn, binase administered intraperitoneally also reduces the destructive changes in the liver (Fig. 1C). However, in this case even at a dose of 0.1 mg/kg the portion of normal liver parenchyma increases from 22.3% (Fig. 1A, Right) to 56.8% (Fig. 1C, Left) and further increase in the binase doses up to 1 mg/kg exhibits only slight effect on the liver: the portion of normal parenchyma increases to 64.2% (Fig. 1C, Right) – to the same level as observed for intramuscular binase administration. It seems likely that in the latter case the destructive changes in the liver decrease more rapidly in comparison with intramuscular administration of binase, due to better absorption and bioavailability of enzyme. The portion of liver parenchymal necrosis is 2.2–2.6 times lower in mice treated with binase (Fig. 1C) in comparison with control without binase treatment (Fig. 1A, Right). Binase administered intraperitoneally (0.1 mg/kg) caused an inhibition of tumor growth by 40% and a twofold reduction in the metastasis area, whereas further increase in the binase dose only slightly changed its tumoricidal effect [18].

The numerical density of binuclear hepatocytes reflecting the liver regenerative capacity is shown in Table 1. Tumor progression in the absence of binase treatment leads to a drop in numerical density of binuclear hepatocytes by 3.3 times, reflecting tumor burden on the liver. Intramuscular administration of binase to mice with LLC does not change the numerical density of binuclear hepatocytes throughout the full range of doses. However, intraperitoneal administration of binase at doses of 0.1, 0.5 and 1 mg/kg results in an increase in the numerical density of binuclear hepatocytes in comparison with the control by 1.8, 2.3 and 3.2 times, respectively, reaching the same level as that in healthy mice