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Note

The enhancement of interstitial transport of a doxorubicin–lactosaminated albumin conjugate by imatinib: In rat hepatocellular carcinoma it is not preferentially higher than that in liver and bone marrow

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ABSTRACT

The finding that imatinib enhances the drug transport from bloodstream to neoplastic cells suggested a possible role of this drug as an adjuvant to the chemotherapeutics given in the treatment of solid malignancies. The present experiments aimed to verify whether imatinib can selectively increase the penetration of a doxorubicin–lactosaminated human albumin conjugate (L-HSA-DOXO) in chemically induced rat hepatocellular carcinomas (HCCs). We observed that imatinib increased the uptake of L-HSA-DOXO by HCCs but at the same time caused a similar enhanced penetration of the conjugate in liver and bone marrow. To our knowledge, this is the first demonstration that the enhancing effect of imatinib on interstitial drug transport is not restricted to the tumors, but can be also displayed in normal tissues. This observation casts some doubts about the possibility that the value of anticancer agents with toxic side effects on liver and bone marrow can be improved by imatinib.

1. Introduction

By inhibiting PDGFβ-receptor tyrosine kinase, imatinib reduces tumor interstitial fluid pressure [1] and consequently increases the transport of drugs from bloodstream to neoplastic cells [1–6]. Since this enhanced transport was not observed in normal tissues such as mouse liver, kidney and intestine [3], it was suggested that imatinib might increase the chemotherapeutic index of the drugs used in the treatment of solid tumors [3–6]. The experiments reported here aimed at verifying whether imatinib selectively increases the penetration of a conjugate of doxorubicin (DOXO) with lactosaminated human albumin (L-HSA) in the HCCs induced in rats by diethylnitrosamine (DENA). L-HSA-DOXO is a hepatocellular carcinoma-targeted conjugate which in rats with HCCs displays an anticancer efficacy and a tolerability higher than those of unconjugated DOXO [7]. We administered L-HSA-DOXO to rats with HCCs and in animals treated with imatinib or with saline measured the concentrations of DOXO raised by the conjugate in the tumors, liver and bone marrow.

2. Materials and methods

2.1. Synthesis of L-HSA-DOXO

L-HSA-DOXO conjugate was prepared according to [8] using the 6(maleimidocaproyl) hydrazone derivative of DOXO, which contains an acid sensitive hydrazone bond that is stable at the neutral pH of plasma but allows doxorubicin to be rapidly released intracellularly in the acidic endosomal or lysosomal compartments after cellular uptake of the conjugate. The molar ratio DOXO/L-HSA was 5.2 and was calculated as described in [8].

2.2. Animals

Male Wistar rats were used. Their weight was 125–150 g when the experiment was started and 350–400 g when it was completed. They were obtained from Harlan Italy (Udine, Italy) and were maintained in an animal facility at the Department of Experimental Pathology (Bologna), receiving humane care in accordance with the European Legislation. The protocols of the experiments were approved by the Ethical Committee of the University of Bologna. Animals were maintained at a temperature of 20–22 °C and were fed with a standard pellet diet *ad libitum*.

2.3. Experimental procedure

HCCs were induced in 20 rats which received DENA given in their drinking water (100 mg/l) for 8 weeks. Five weeks after the

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last day of DENA administration, HCCs were detected by ultrasonography [7] in 17 animals, which were randomly assigned to two groups. Rats of one group (nine animals) received intraperitoneally 50 μg/g imatinib mesylate (provided by Novartis Pharma AG, Basel, Switzerland), dissolved in saline (NaCl 0.9%) and given in a volume of 10 μl/10 g. Administration was repeated for four consecutive days. The animals of the other group were similarly treated only with saline. Thirty minutes after the fourth injection of imatinib (or saline alone) rats received in the dorsal vein of penis under isoflurane anaesthesia 24 μg of L-HSA-DOXO/g (corresponding to 1 μg/g DOXO) administered in saline in 10 μl/10 g. Four hours later, animals were killed under isoflurane anaesthesia. From each animal, a specimen of liver surrounding HCCs, bone marrow (ejected from two femurs into a pre-weighed test tube by blowing air with a syringe) and 2–4 liver tumor nodules were collected and frozen for subsequent determination of DOXO concentration. Care was taken to collect only neoplastic nodules without macroscopic signs of necrosis; they were not larger than 4–7 mm in diameter and had solid consistence. DOXO was measured according to [7] with modifications.

3. Results and discussion

The Fig. 1 shows the DOXO concentrations in HCCs, liver and bone marrow 4 h after i.v. injection of L-HSA-DOXO (24 μg/g) when in rats with HCCs all the conjugate was removed from bloodstream [9] and imatinib could not further affect its penetration in tissues and tumors. Compared to the values measured in saline-injected rats, in animals treated with imatinib, DOXO concentrations in HCCs, liver and bone marrow were increased by 51%, 40% and 70%, respectively. In contrast to the results of Pietras et al. [3], who did not observe an increased transport of drugs from bloodstream to the cells of liver, kidney and small intestine of mice treated with imatinib, in the present experiments imatinib enhanced the DOXO concentrations in HCCs as well as in rat liver and bone marrow.

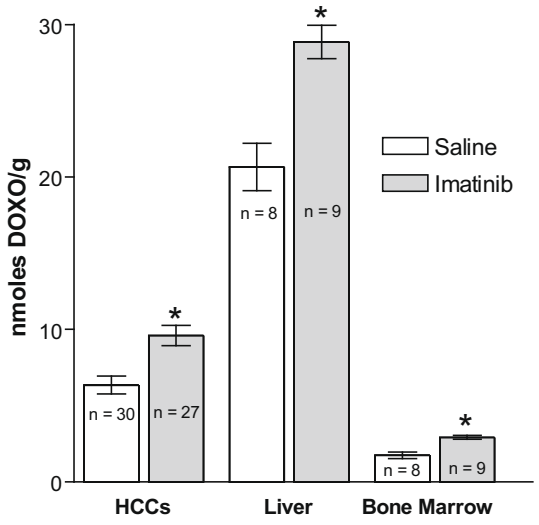

Fig. 1. Effect of imatinib on DOXO concentrations in HCCs, liver and bone marrow of rats injected with L-HSA-DOXO conjugate. Animals were i.p. administered with imatinib (50 μg/g × 4 days) or with saline. Thirty minutes after the fourth injection, the animals received 24 μg/g of L-HSA-DOXO and 4 h later they were killed and HCCs, liver and bone marrow were collected for DOXO determinations. The number of tumors and of organs examined is indicated inside the bars. Data are given as mean values ± SE. The results were evaluated by Student's *t*-test. *Statistically significant difference ($p = 0.000$) from DOXO concentrations measured in saline-injected animals.

This result suggests that imatinib reduces the interstitial fluid pressure not only in tumors, but also in some organs and is in agreement with the finding that oedema and fluid retention are characteristic side effects of the drug in its clinical use [10–11]. In conclusion, our result casts doubts about a possible improvement by imatinib of the therapeutic index of drugs with toxic effects on liver or bone marrow and fits with the finding that in patients with solid tumors imatinib intensifies the myelotoxicity of DOXO and gemcitabine [12].

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