Original Article

Time– and dose–dependent vascular changes induced by the novel vascular disrupting drug NPI–2358 in a murine cancer model monitored with DCE–MRI

Yuanyuan Shen[1], JingWu Sun[2] , Kai Cheng[3], Chengxia Liu[1],

1 From Department of Gastroenterology and Hepatology internal medicine , Affiliated Hospital of Binzhou Medical University, Binzhou 256603, China

2 From Department of Cardiology, The Affiliated Hospital of Binzhou Medical University ,Binzhou 256603,China

3 From Department of Breast and Thyroid surgery, Affiliated Hospital of Binzhou Medical University, Binzhou 256603, China

ABSTRACT **Objective:** To monitor the time- and dose-dependent vascular changes induced by the novel vascular disrupting drug NPI-2358 with DCE-MRI parameter IAUC. **Methods:** Mice bearing C3H mammary carcinomas were injected intraperitoneally with NPI-2358 and with the control solvent at a volume of 0.02 ml/g. Repeated dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) measurements, with gadolinium-DTPA performed on 7-Tesla spectroscopy/imaging system before and up to 24 h after drug injection. The data was analyzed by Matlab 2008 software.**Results:** In the control group, there was no significant change of IAUC between pre- and 1 h, 3 h, 6 h, 24 h after injection. In the 7.5mg/kg NPI-2358 treated group, the IAUC of post-treatment at 1 h (P=0.012), 3 h (P=0.001) and 6 h (P=0.007) decreased significantly (P<0.05). Using the optimal time of 3 h a linear relationship was obtained between drug dose and IAUC change at 2.5, 5.0, 7.5, 10.0 mg/kg, the effects at 7.5 and 10 mg/kg were significantly different from control values.

Conclusion: NPI-2358 can decrease the blood perfusion in tumour in early time. And the anti-tumour effect is related to the doses of NPI-2358 at the optimal 3 h interval.

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Key Words: Tumour therapy, NPI-2358, murine cancer

NPI-2358 is a novel vascular disrupting agent[1]. It has cytotoxic and antiproliferative effects against dividing endothelial cells and thereby induces vascular damage in various tumor types with varying degrees of efficacy. Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) can provide in vivo information about the vasculature of NPI-2358 with IAUC[2][3]. The aim of this study is to utilize DCE-MRI to monitor the time and dose dependent vascular changes induced by NPI-2358 in C3H murine carcinoma model.

Materials and Methods

Animal and tumour model

5-10 μL C3H mammary carcinomas material grown in female CDF1 mice was used for all experiments[4]. All tumours were implanted into the right rear foot of the animals which were 15-25 weeks old and 20-30 g weight. After 2-3 weeks, experiments were performed when tumours reached 200 mm3, calculated from the formula D1×D2×D3×∏/6, where D values represent the three

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Corresponding author:Dr JingWu Sun, From Department of Cardiology, The Affiliated Hospital of Binzhou Medical University ,Binzhou 256603,China e-mail: xiaoshen8307@163.com, tel:18763040586

orthogonal diameters.

Drug preparation

The stock solution of NPI-2358 (Nereus Pharmaceuticals, San Diego, USA), dissolved in polyethylene glycol/solutol solution, was diluted to the required concentration with 5% dextrose, which was the control solvent, immediately before each experiment. The doses range from 2.5 to 10 mg/kg mouse body weight at a constant volume of 0.02 ml/g. It was kept cold and protected from light.

Dynamic contrast enhanced magnetic resonance imaging

A 7 Tesla spectroscopy/imaging system (Varian Medical Systems, Palo Alto,CA) was used for DCE-MRI. The tail of the mice was secured by tape and a cannula with a syring primed with contrast agent Gd-DTPA (0.02 mmol/ml, Magnevist, Schering, UK) was applied intravenously in the tail vein. An intraperitonealy line connected with a syring was primed with NPI-2358 solution (2.5 mg/kg, 5.0 mg/kg,7.5 mg/kg and 10 mg/kg) and control solvent. The jig was positioned in a cradle with 11 mm surface coil and tuning box. The mice were restrained with the tumour-bearing leg exposed with tumour under the coil. A warm water tube was wrapped around the jig.

A single slice along the foot through the tumor center was chosen for imaging. The imaging protocol included an inversion recovery sequence for T mapping (FOV 25×25mm, slice thickness 2mm, matrix size 128×64 reconstructed to 128×128, repetition time 2430 ms, inversion times 100,400,800,1600 and 2400 ms, and echo time 13 ms), followed by dynamic image acquisition using a fast spoiled gradient echo sequence (FOV 25×25 mm, slice thickness 2mm, matrix size 128×100 reconstructed to 128×128, flip angle 70°, repetition time 60 ms, and echo time 3 ms). This gave a time resolution of 6 s for the dynamic images. During the initial 4 s of the sixth image acquisition, Gd-DTPA was administered intravenously at a dosage of 0.1 mmol/kg and a concentration of 0.02 mmol/ml. At 1h after Gd-DTPA administration, NPI-2358 or control solution was administered intraperitoneally. The mice were kept in place and the imaging protocol was repeated, with the dynamic imaging acquisition starting 3 h after NPI-2358 administration (at 2.5 mg/kg, 5.0 mg/kg and 10 mg/kg), 3 h,6 h,24 h after NPI-2358 (7.5 mg/kg) and contrast solution.

For each tumor, a region of interest (ROI) containing the whole tumor was drawn manually for the data analysis. T maps were calculated from the pre- and post- NPI-2358 inversion recovery images. The voxel concentration time curves were used to calculate map of the vascular parameters. The semi-quantitative parameter of initial are a under the curve (IAUC) was calculated by trape gration of the Gd-DTPA concentration during the first 90 s after administration. All data were analyzed by Matlab 2008 (Math Works, Inc, Natick, MA, USA) and statistical analysis was performed with SigmaStat3.1 (Systat Software, Richmond, CA). Student's t-test was subsequently used to perform group-by-group test. A statistical significance level of 0.05 was used. All data were given as means±SD.

Results

The time dependent between NPI–2358 and control solvent treatment

In 7.5 mg/kg NPI-2358 group, the IAUC(10-3) after treatment at 1h, 3 h and 6 h, 24 h were separately 9.87±1.26 (P=0.012), 7.72±1.25 (P=0.001), 9.58±1.12 (P=0.007), 13.50±1.75 (P=0.415), compared with 15.40±1.53 at pre-treatment. There is significantly decreasing at 1 h, 3 h, 6 h and 3 h is the lowest point.

In control group, there is no significant change between pre- and post- treatment, IAUC (10-3) before and after 1 h, 3 h, 6 h, 24 h treatment was seperately 12.4±1.60, 12.1±0.97 (P=0.859), 10.8±1.17 (P=0.407), 9.3±0.92 (P=0.103) and 14.8±1.75 (P=0.327).

The dosage dependent of NPI–2358 solvent

Using the optimal time of 3 h, at NPI-2358 dose of 2.5, 5.0, 7.5 and 10.0 mg/kg the respective IAUC values were 80% (P=0.132), 56% (P=0.074), 50% (P=0.001) and 32% (P=0.037) compared to the pre-treatment. And the effects at 7.5 and 10 mg/kg were significantly different from control values.

Discussion

Solid tumors rely on a functional tumor vasculature for their survival and continuous growth[5]. NPI-2358 is a novel vascular disrupting agents (VDAs) acting on tubulin dimerization that destabilizes tumour vascular endothelial cells and has cytotoxic activity. It can selectively induce tumor vascular collapse and tumor regression in murine tumor models and potentiates other oncology agents[6]. Utilizing DCE-MRI technique to trace small molecular paramagnetic contrast agent Gd-DTPA and depending on the relative T1-weighted signal increase has a linear dependence on tissue concentration, so the proportional change in signal intensity over time is equivalent to proportional change in contrast agent tissue concentration[7]. Initial area under the contrast agent concentration-time curve (IAUC) after Matlab

software analysis is the lead currently parameter[8].

In the study, compared pre- and post-treatment at 1 h, 3 h, 6 h and 24 h in control group, there is no significantly changes of IAUC. We chose the dose of NPI-2358 at 7.5 mg/kg (22.5 mg/m2) to study the time course within 24 h because this dose is similar to those doses which demonstrated decreases in ktrans in tumours in oncology patients (13.5 – 30 mg/m2)[9]. In 7.5 mg/kg NPI-2358 group, after 1 h of treatment, the IAUC in tumour area has significantly decreased, and it reaches the peak at 3 h after treatment and then it has gone back until 24 h. There are no significantly changes at 24 h after NPI-2358 was injected into mice body. As IAUC can show the information of blood flow in the tumour vascularization, we think that NPI-2358 can induce the blood flow in tumour vessels significantly decrease and the best time is at 3 h after treatment. There is no significant inhibition of blood flow in tumour region.

We chose the best time of treatment as 3 h to study the dose dependent with 2.5, 5.0, 7.5 and 10.0 mg/kg NPI-2358 between pre- and post-treatment. There is a linear relationship as decreasing of IAUC in these different doses of NPI-2358 and has significantly changes at 7.5 and 10 mg/kg. This shows that the blood flow inhibition in tumour area can been magnified by increasing the dose of NPI-2358 and there will be significantly changes when the dose reaches at 7.5 and 10 mg/kg.

Above all, NPI-2358 is a vascular agent which can rapidly inhibit the tumour blood flow, the effect starts at 1h after NPI-2358 intraperitoneally injection and peak time is at 3 h and no significantly changes till 24 h. So NPI-2358 is a novel VDAs which effect and recovery both rapidly and it has anti-vascular activity for tumour in early time. Besides, its inhibition of blood flow for tumour depends on the dose of NPI-2358.

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