Clinical study on the recombinant human endostatin regarding improving the blood perfusion and hypoxia of non-small-cell lung cancer

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Abstract
Objective To observe the dynamic changes of blood perfusion and hypoxic status with CT perfusion imaging and hypoxia imaging in patients of non-small-cell lung cancer (NSCLC) who were treated with recombinant human endostatin (RHES).
Methods Fifteen previously untreated patients with histologically or cytologically confirmed NSCLC were enrolled. They were randomly divided into research group (n=10) and negative control group (n=5). The patients of the research group continuously used RHES for ten days, and simultaneously had CT perfusion imaging and hypoxia imaging performed on days 1, 5 and 10, respectively. The remaining 5(control) only had CT perfusion imaging and hypoxia imaging, without using RHES, on days 1, 5 and 10, respectively. According to the above results, we could obtain a “time window” during which RHES improves blood perfusion and hypoxia of lung cancer.
Results In the research group, after using RHES, capillary permeability surface (PS) and tumour to normal tissue (T/N) decreased at first, and then increased. Their lowest points occurred on about the fifth day with statistical significance compared with the first day (T/N, \(p<0.01\); PS, \(p<0.01\)). Blood flow (BF) was first increased and then decreased. Its highest point occurred on about the fifth day with statistical significance compared with the first and tenth day (all \(p<0.01\)). The PS, BF and T/N peaked on the fifth day in the research group with statistical significance compared with the negative control group as well (all \(p<0.01\)). The above results suggested that RHES’s “time window” was within about one week after administration.
Conclusion RHES’s “time window” is within about one week after administration, which provides an important experimental basis for combining RHES with radiotherapy in human tumours.

Keywords Recombinant human endostatin · Hypoxia imaging · Non-small-cell lung cancer · CT perfusion imaging

Introduction
Lung cancer is the most common cancer in the world in terms of incidence and mortality. Radiation therapy is considered a standard treatment for unresectable locally advanced non-small-cell lung cancer (NSCLC). However, patients treated with radiotherapy alone have a median survival of less than 1 year and the 5-year survival rate is approximately 5–10%. This is mainly due to the tumour-specific microenvironment, hypoxia. A large body of clinical data has been published demonstrating that hypoxia is a leading reason to induce tumour cells’ resistance to chemoradiation. Therefore, a new generation of strategies has come into focus increasing radiosensitivity. At present, recombinant human endostatin (RHES), an antiangiogenesis drug, has been extensively studied. On the one hand, many investigators are exploring whether it could be used as a radiosensitiser. Some of them presumed that the reduction of vascularisation with RHES would decrease the blood flow (BF) volume and the oxygen supply, which resulted in radiation resistance. However, antiangiogenic therapies...
have been shown to improve radiosensitivity in more and more basic research. Numerous preclinical studies have indicated that endostatin (ES) could improve the effects of radiotherapy on a variety of malignant tumours [1], but their mechanisms of action are not yet completely defined. Some studies have confirmed that its mechanism of action may be related to normalisation of the vasculature within tumours [2] and animal experiments have found that angiogenesis inhibitors could normalize vasculature in a “time window”, alleviating tumour hypoxia [3]. However, it has not been reported whether RHES has a “time window” in human tumours. Therefore, we performed this study to confirm that RHES has a “time window” of vascular normalisation also in human tumours.

Patients and methods

Eligibility

From January 2008 to June 2010, 15 hypoxia-positive patients with NSCLC were deemed eligible. Additional eligibility requirements included age 18 years or older, measurable disease, an ECOG performance status (PS) of 0–2, life expectancy of greater than 12 weeks, and adequate haematologic, hepatic and renal functions. All patients gave written informed consent prior to registration. Exclusion criteria were prior radiotherapy; unmeasurable disease; pregnancy and breastfeeding; CNS metastases; active infection and unhealing wounds; hepatic and renal function deficiency; serious cardio-cerebrovascular disease such as coronary heart disease, unstable angina pectoris, myocardial infarction, cardiac arrhythmias, cerebral infarction and haemorrhage; or psychiatric illness that would have affected compliance. The study was approved by the ethics committee of Lianyungang First People’s Hospital and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All persons gave their informed consent prior to their inclusion in the study. Ethics No. is 20080105.

Of the 15 cases, 9 were men and 6 women; 8 had squamous cell carcinoma and 7 adenocarcinoma; mean age was 63 years. According to the TNM staging standard of the International Union Against Cancer (UICC) in 1997 for NSCLC: 5 were stage II and 10 stage III. The research and control groups were similar and comparable (p>0.05).

CT perfusion imaging and analysis

Non-ionic iodine contrast agent (40 ml) was injected through the antecubital vein with a high-pressure syringe at a flow rate of 4 ml/s. The dynamic scan began 5 s after administration with a GE Light Speed16 scanner unit at 120 kV and 50–80 mAs and lasted one minute. Slice thickness was 5 mm, exposure time was 0.5 or one second (an exposure every 2 s), and an exposure could obtain 4 images. We obtained a total of 120 images. The axial images were selected to be analyzed with Perfusion software in a GE ADW4.0 workstation. The tumour BF, blood volume (BV), mean transit time (MTT) and permeability surface (PS) maps were reconstructed with the thoracic aorta as the input artery and with the vertebral vein or brachiocephalic vein as the output vein. Based on these maps, BF, BV, MTT and PS values were calculated. Parameters were statistically analysed with SPSS13.0 software.

99mTc HL91 hypoxia imaging

A HL91 kit was provided by Sinkostar Company (Beijing, China). Na 99mTcO4 (1–2 ml) was injected into a HL91 kit at room temperature and 10 min later the dissolved solution was kept for future use. A DTPA kit containing 1 mg of SnCl₂:2H₂O was dissolved with 4 ml of physiological saline, then 40 μl of dissolved solution containing 10 μg of SnCl₂:2H₂O was immediately removed into the above kit followed by mixing. A Millennium VG5 HawkeyeSPECT/CT system with LEHR collimators (GE Company, USA) was used to collect data. Thoracic planar imaging was performed 1, 2 and 4 h after intravenous injection of 99mTc-HL91 and SPECT/CT tomography imaging was performed 4 h after intravenous injection of 99mTc-HL91. Images was analysed independently by two experienced nuclear physicians who were blind to the clinical data and information pertaining to the corresponding scan. Hypoxia positivity was identified as lesions of focally increased uptake, exceeding that in the lung background. In contrast, if the uptake of the lesions was equal to or lower than that in lung background, this was regarded as negative hypoxia. In positive patients, the radioactivity in tumour (T) and normal tissue (N) was determined to calculate the radioactivity ratio (T/N).

Design and methods

First, 15 patients were randomly divided into a research group (n=10) and negative control group (n=5). Second, 10 hypoxia-positive patients with NSCLC were intravenously given RHES for 10 days. The dose of RHES was 7.5 mg/m², which was combined with 500 ml of 0.9% normal saline (NS) and intravenously injected within 3–4 h. 99mTc-HL91 hypoxia imaging and CT perfusion imaging were performed on days 1, 5 and 10. Third, at the same time as performing successive scans for the selected levels within the CT perfusion images, the tumour BF, blood volume (BV), mean transit time (MTT) and PS maps were obtained. Fourth, regions of interest (ROIs) were drawn in the tumour and the contralateral normal lung tissue, the radioactivity ratio of T/N was calculated and a fitting curve was drawn to evaluate the dynamic changes in tumour blood perfusion. Last but not least, all the parameters and the obtained “time window” in which RHES improves hypoxia were compared between the research and control groups.