The effect of angiogenesis inhibitor TNP-470 on the blood vessels of the lungs, kidneys and livers of treated hamsters

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INTRODUCTION

It is well recognised that angiogenesis has essential significance for the growth of solid tumours [4, 5, 10]. Therefore many angiogenesis inhibitors have been examined as potential antitumour drugs. One of the most promising as a future antitumour drug appears to be the synthetic fumagilin analogue TNP-470 [3, 6–8]. In our previous studies on that compound we achieved some effect in the suppression of the growth of the transplantable melanoma in hamsters. The growth rate of this tumour was significantly decreased but the survival time of the tumour-bearing animals was prolonged only slightly [12, 13]. In our last published studies we found that TNP-470 given in the vicinity of a growing tumour can cause complete remission of Bomirski Ab amelanotic melanoma and prevent the development of its metastases [14]. The success of such treatment for a fast-growing tumour that is able to kill a hamster in several weeks indicates that TNP-470 can be one of the most effective antitumour drugs. However, there is the problem of side-effects, which in the case of effective antitumour drugs is usually very serious. In our successful studies TNP-470 was given in the vicinity of a growing tumour, so the dose of this compound applied could be comparatively low. Even so, the systemic action of TNP-470 was inescapable. We therefore decided to test the effect of the strong antiangionesis inhibitor TNP-470 on the blood vessels of the lungs, kidneys and livers of the treated

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hamsters. Since the action of TNP-470 was found to be directed at the endothelial cells [3, 9], our attention was mainly concentrated on the endothelium of the vessels examined.

**MATERIAL AND METHODS**

TNP-470 was the kind gift of Takeda Chemical Industries (Osaka, Japan). The chemical structure of the compound has been presented by Ingbar et al. [8].

Forty random-bred Syrian (golden) male hamsters were used in the study. They were obtained from the animal colony of the Silesian Medical University (Katowice, Poland) and maintained in our colony on a standard diet and water ad libitum. The care and treatment of the animals were in accordance with the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes”. The study was approved by the Local Committee for Animal Experiments, Gdańsk, Poland.

Bomirski melanoma variant Ab was maintained by serial passage in the hamsters using the suspension method of tumour transplantation. The natural history and characteristics of this melanoma are described by Bomirski et al. [2]. The tumour cells were always implanted on the right flank of the animals by subcutaneous (s.c.) injection.

TNP-470 in a suspension vehicle composed of 1% ethanol and 5% Arabic gum in a saline was given s.c. at a dose of 30 mg/kg (about 3.9 mg/animal in 500 μL of vehicle) in the vicinity of the palpable tumour every day for 21 days. The control tumour-bearing animals received injections of vehicle. All animals were sacrificed on the 28th day from the beginning of the experiment.

**RESULTS**

**Lungs**

The structure of blood vessels of various sizes from hamsters, both healthy and tumour-bearing ones, which had been treated with TNP-470 did not differ when examined by light microscope from those of untreated animals (Fig. 1). Endothelial cells did not show any symptoms of histological lesions. Also the ultrastructure of the endothelial cells of the lung vessels from the treated hamsters was normal (Fig. 2).

**Kidneys**

The capillaries of the renal glomerules from animals treated with TNP-470 did not differ from those of the untreated animals, both under a light microscope (Fig. 3, 4) and under an electron microscope (Fig. 5, 6). The larger blood vessels also had a normal structure, both for the treated and the untreated animals.

**Livers**

The structure of the blood vessels of various sizes, lobular and interlobular (Fig. 7), from the livers of the treated animals was similar to those of the untreated animals and no symptoms of histological lesions were noticeable. The ultrastructure of the endothelium of the examined vessels also seemed to be normal (Fig. 8).

The presence of the tumour in animals had no effect on the structure of the vessels of the examined organs.

**DISCUSSION**

The results of our studies indicate that the angiogenesis inhibitor TNP-470, which is able to cause a complete remission of a fast-growing tumour, led neither to changes in the structure, nor probably the function, of the blood vessels of such important organs as the lungs, kidneys and livers. The period of treatment with TNP-470 was 21 days, which is short...
Andrzej Myśliwski et al., Effect of TNP-470 on blood vessels in comparison to the duration of the administration of the antitumour drugs applied in oncology. This was, in any case, sufficient time for recovery for hamsters bearing transplantable melanoma. The good state of the structure of the endothelium and other parts of the blood vessel walls examined by light and electron microscopes did not preclude changes in the function of these vessels. However, if such changes had occurred, they were reversible, since the animals which survived treatment with TNP-470 were examined 5 months after the last injection of that drug and were found to be healthy [14]. The angiogenesis inhibitor TNP-470, therefore, appeared in our studies to be both effective as an antitumour drug and harmless to the animals treated. These results were achieved with an animal model and, at present, it is difficult to make any claims as to whether such a good effect would be possible in humans. TNP-470 is already undergoing pre-clinical investigations [1, 6] but reports about the side-effects of such treatment
in humans are rather scanty and limited to such observations as the occurrence of nausea and fatigue, and the presence of some neurological symptoms which disappeared after therapy was discontinued [6]. Examinations of the effect of TNP-470 on endothelial cells were mainly carried out on endothelial cells cultured in vitro [3]. In our studies TNP-470 was given in the vicinity of a growing tumour, so that the dose applied of that compound could be quite low. The systemic action of TNP-470 was un-
avoidable. We decided, therefore, to test the influence that a strong antiangiogenesis inhibitor could exert on the blood vessels of the lungs, kidneys and liver of the hamsters treated with TNP-470. The problem of the possible side-effects of the treatment with TNP-470 is very important since the only partly effective action of that drug in pre-clinical trails induced some research groups to apply a combined treatment with other antitumour drugs, which themselves sometimes cause severe side-effects [6, 11].

The results of our studies and those of other research groups [3, 6] supply evidence that the side-effects evoked by TNP-470 are fairly limited and reversible after treatment, which indicates that this analogue of fumagilin — TNP-470 is a potentially valuable antitumour drug.

REFERENCES