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Current Status Review

Therapeutic potential of the anti-angiogenesis drug TNP-470

P. GERVAZ* AND C. FONTOLLIET†
*Departments of *Surgical Research and †Pathology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland*

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Summary. Neovessel formation is a pre-requisite for tumour growth and dissemination. Inhibition of angiogenesis is a promising approach for the treatment of neoplasms. In recent years, antiangiogenic drugs, such as TNP-470, have entered clinical trials. In this paper, we review the key experimental and clinical data on the role of TNP-470 in anti-tumour treatment.

Keywords: angiogenesis, applications, cancer, clinical testing

Angiogenesis, the process leading to the formation of new blood vessels from pre-existing ones, is characterized by proliferation and migration of the endothelial cells. In the healthy adult this process is restricted to female reproductive tissues (Folkman 1971). Neovascularization is critical for the growth and dissemination of tumours and is a dominant feature in a variety of angiogenic disorders such as diabetic retinopathy, arthritis and psoriasis (Folkman 1995). Inhibitors of angiogenesis have been recently developed which offer the hope of new treatment options for these diseases. The identification of new drugs that target the endothelial compartment of tumours carries potential advantages: low toxicity, since normal endothelial cells are relatively quiescent in the body and exhibit an extremely long turn-over; lack of resistance to treatment, since endothelial cells do not exhibit the same degree of genomic instability as tumour cells; and the potentiation of cytotoxic drugs in combination therapy (Kakeji & Teicher 1997). It is not surprising, therefore, that experimental and clinical research with anti-angiogenic drugs has focused on cancer therapy.

TNP-470 (AGM-1470, O-chloroacetylcarbamoyl-fumagillol) is a semisynthetic derivative of fumagillin, a naturally secreted antibiotic of *Aspergillus fumigatus*, that exhibits a strong inhibitory effect on endothelial cells growth and migration *in vitro* and *in vivo* (Ingber *et al*. 1990).

TNP-470 is currently under investigation in phase I, II and III anti-tumour clinical trials, and is considered to be a leading anti-angiogenic compound. Its molecular mode of action has not been fully elucidated; covalent binding with the metalloprotease methionine aminopeptidase (Sin *et al*. 1997) and suppression of mRNA expression of cyclin A in endothelial cells (Abe *et al*. 1994) have been demonstrated. *In vitro*, TNP-470 inhibits growth factor-stimulated proliferation of endothelial cells with an IC_{50} of pg/ml. For most tumour cell lines (except glioblastoma) cytotoxicity is not seen until concentrations in the range of μg/ml (Kusaka *et al*. 1994).

A search in the bio medical literature published since 1990 provides the basis for this short review, which aims to review the key experimental and clinical data on the role of TNP-470 in anti-tumour treatment.

Experimental studies

TNP-470 has demonstrated activity against several types of tumours xenotransplanted into nude mice: subcutaneous growth of renal (Morita *et al*. 1994), glioblastoma (Takamyia *et al*. 1994), breast, prostate (Yamaoka

Correspondence: Dr Pascal Gervaz, Department of Surgical Research, Centre Hospitalier Universitaire Vaudois (CHUV), Pavillon 4, Rue du Bugnon, 1000 Lausanne, Switzerland.

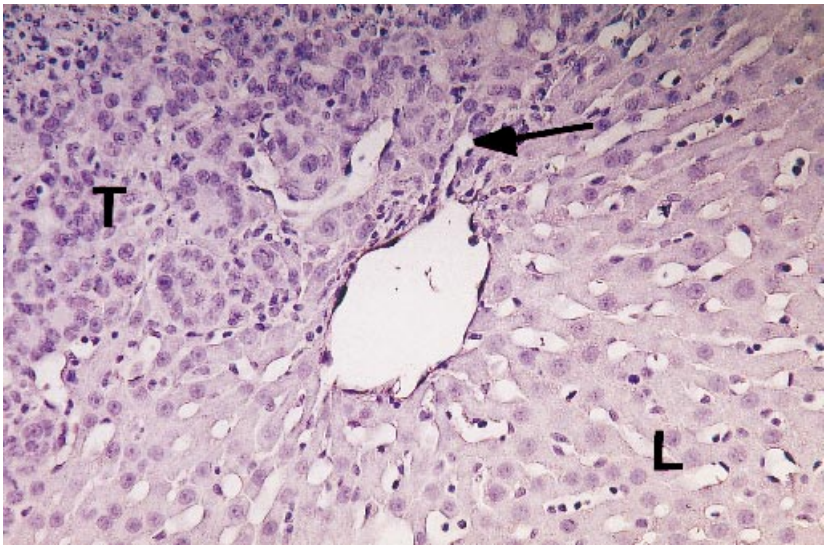

Figure 1. Angiogenesis of liver metastases from colorectal cancer (syngeneic rodent model). The ischaemic areas of tumour (T) produce growth factors that stimulate sinusoid endothelial cells proliferation and migration in the adjacent liver (L). Tumour neovessels are visible (arrow) sprouting from large sinusoid at the tumour-liver interface.

et al. 1993a), ovarian (Yanase *et al*. 1993), colon (Tanaka *et al*. 1995) and hepatocellular (Xia *et al*. 1997) carcinomas were inhibited following TNP-470 administration subcutaneously at the dose of 30 mg/kg daily or every second day. However, it should be emphasized that the skin is rarely involved in the metastatic process of such tumours, and the cancer cells may be more dependent on neovascularization in such a location than in the liver or the lung, where the secondary tumour may benefit from a large pre-existing vascular network. Angiogenesis is dependent not only on the tumour but also on the characteristics of the host tissue. Moreover, direct toxicity of TNP-470 against tumour cells is well documented and can occur at concentrations in the range of μg/ml. Although endothelial cells are much more sensitive (in the range of pg/ml), it is debatable whether the antitumour effect realized is not partly due to a direct cytotoxic action.

We and others have focused on the role of TNP-470 in inhibiting growth of liver (Konno *et al*. 1995; Tanaka *et al*. 1996; Moller *et al*. 1997) or lung (Yamaoka *et al*. 1993b; Mori *et al*. 1995) metastases using syngeneic models of disseminated tumours: liver metastases from colorectal cancer represents a common cause of death and currently, although surgical resection may be achieved in a small number of cases, the role of conventional chemotherapy remains controversial. Anti-angiogenic treatment in this situation may prolong latency phase of dormant micrometastases (Holmgren *et al*. 1995) and/or inhibit growth of established ones despite possible recruitment of sinusoid endothelial cells by the tumour in ischaemic areas (Figure 1).

Some authors have concentrated on the association of TNP-470 with different treatment modalities targeting the epithelial component of tumours. TNP-470 in combination with minocycline, another antiangiogenic drug, potentiated the effect of cyclophosphamide on Lewis lung carcinoma and FsallC fibrosarcoma (Teicher *et al*. 1994). TNP-470 in combination with hyperthermia had a synergistic effect on growth of both gastric and oesophageal human tumours xenotransplanted into nude mice (Yano *et al*. 1995). Combination with tamoxifen (McLeskey *et al*. 1996) and radiotherapy (Murata *et al*. 1997) against mammary carcinoma has also proven effective. In summary, there is a large volume of experimental data showing that TNP-470 inhibits growth of primary and metastatic tumours in different experimental models. The anti-tumour effect seems primarily related to an inhibition of neovascularization, although for certain types of cell lines a direct cytotoxicity may partially be responsible. No major side-effects have been described in animals subcutaneously injected at a dose of 30 mg/kg every other day. We and others have observed a deficit of weight gain in rats, but long term treatment has been described without significant complications (Ahmed *et al*. 1996).

Clinical studies

TNP-470 was the first anti-angiogenic compound to enter clinical trials. Phase I and II clinical trials are ongoing in patients with refractory solid tumours and AIDS patients with Kaposi's sarcoma (Pluda *et al*. 1994; Zukiwski *et al*. 1994; Levy *et al*. 1996). More than 200 patients have been entered in these trials. Pharmakokinetic studies have shown that TNP-470 is rapidly cleared from the circulation with a short half life of around one hour, which

was consistent with preclinical data. Peak plasma concentrations of AGM-1883, an active metabolite, ranged between 0.4 and 150 ng/ml (Figg *et al.* 1997). The ongoing protocol of one-hour i.v. infusion every other day has resulted in minimal side-effects, besides nausea and fatigue. Dose-related toxicity included neurological symptoms (nystagmus, gait disturbance) that disappeared after therapy discontinuation. One 49-year old patient with metastatic squamous cell carcinoma of the cervix experienced complete resolution of pulmonary nodules after treatment with TNP-470 at the dose of 71.25 mg/m^2 of body surface area intravenously. She had no recurrent disease 8 months after therapy discontinuation; three other patients in the trial had stable disease, whereas 14 showed no response (Kudelka *et al.* 1998). A phase III trial of TNP-470 versus synchronous radiotherapy and 5-FU for locally advanced, non resectable pancreatic carcinoma is ongoing.

One should mention, however, that the novel mechanism of action of anti-angiogenic drugs presents new difficulties in assessing their clinical activity. Therefore, there is a need for specific trials designed for cytostatic drugs or for the combination of these compounds with conventional cytotoxic modalities. Stabilization of disease represents a failure of cytotoxic treatment; on the contrary, tumour shrinkage, although possible, is not an accurate endpoint for a cytostatic strategy. Two methods are commonly proposed to assess the angiogenesis characteristics of a specific tumour and its response to therapy:

- repeated serum levels of angiogenic peptides (bFGF and/or VEGF)
- contrast-enhanced MRI to detect contrast uptake and washout in tumours.

Finally, it must be emphasized that embryonic vascular development and endometrial maturation are suppressed by this agent, which restricts its use to postmenopausal women (Klauber *et al.* 1997).

Future prospects

Angiogenesis inhibition is a promising approach for cancer therapy. The low systemic toxicity of these compounds, together with the concept that anti-angiogenic drugs may complement chemo- or radiotherapy are particularly attractive features. Clinical trials designed with endpoints that reflect the cystostatic nature of anti-angiogenic drugs are required.

Another challenge for this research will be to determine which tumours are suitable for anti-angiogenic therapy and to elucidate the mechanism of neovascularization in secondary organs such as the lung, bone or liver.

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