



The mechanism of bleomycin in inducing haemangioma regression

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To the Editor: Haemangiomas are neoplasms of the vasculature frequently encountered in paediatrics which, although benign, may present with serious complications.¹⁻³ The potential beneficial effects of intralesional bleomycin injection (IBI) in the treatment of haemangiomas were initially reported by Kullendorf⁴ and Sarihan *et al.*⁵ More recently, the effectiveness of IBI was evaluated in 37 patients with haemangiomas in a study conducted by the Pretoria Vascular Malformation Study Group. Complete resolution or significant improvement was seen in 87% of the patients.⁶

In another study undertaken at the Cape Town Red Cross Children's Hospital, following the treatment of 30 haemangioma patients with IBI, a response rate of 75 - 100% was attained in 73% of the patients; a response rate of 50 - 75% was reported for the rest of the patients.⁷

Despite the promising results observed following IBI treatment of haemangiomas, bleomycin's mechanism of action in haemangiomas remains unknown. As an initial study into how bleomycin may cause haemangioma regression, we investigated the drug's effect on human haemangioma biopsies.

Materials and methods

Fresh operative human haemangioma tissue biopsies were cultured in fibrin gel and MCDB 131 medium and exposed to bleomycin (0 - 100 µg/ml). Biopsies were monitored daily using an inverted light microscope fitted with a Pixera digital image camera. For quantitative analysis, the Image 1.62b7 software program was used. Ethical clearance for the use of patient samples was granted by the Wellington Review Board, New Zealand.

Results

Cultured haemangioma tissue biopsies gave rise to an array of microvessels (Fig. 1, A and B). However, fewer neovessels were observed in tissue fragments treated with varying doses of bleomycin (Fig. 1, B).² Quantitative analysis showed that bleomycin inhibited neovessel growth in a dose-dependent and time-dependent manner.

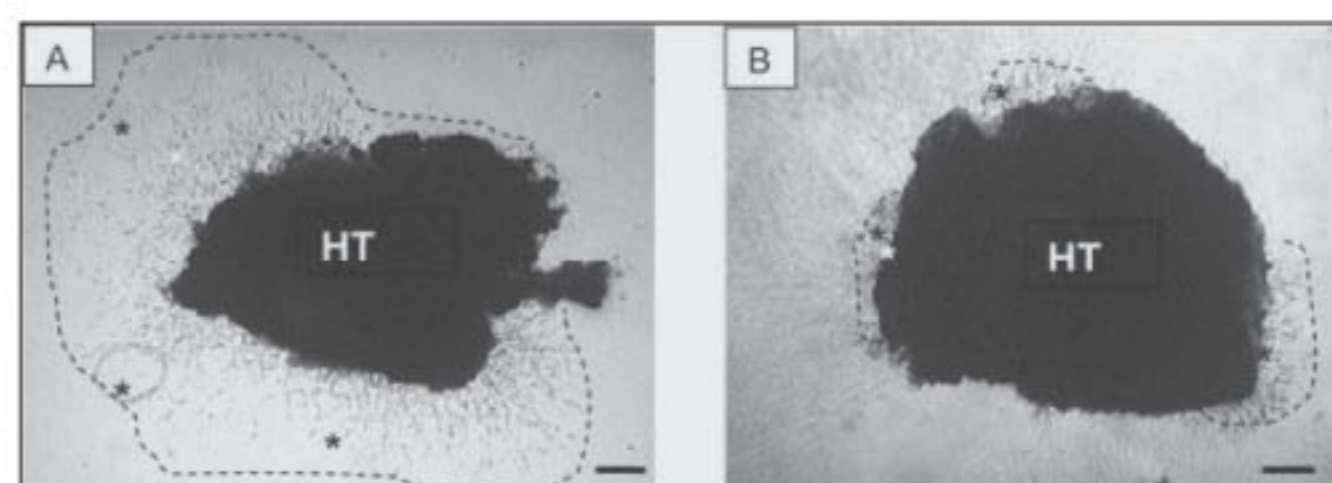


Fig. 1. Haemangioma tissue (HT) cultured in fibrin gel. A: control, B: bleomycin-treated tissue. The asterisks indicate microvessels emanating from the surface of haemangioma tissue (extent shown by dotted line). Bar = 250 µm.

Discussion

The treatment of infantile haemangiomas has remained unsatisfactory. More recently, in South Africa and parts of Asia, intralesional bleomycin has been employed to treat these tumours, with very good results. However, concerns remain about the use of chemotherapy to treat benign tumours and the possible development of bleomycin-induced pulmonary fibrosis in such patients. Therefore, elucidation of bleomycin's mechanism of action in haemangiomas is of clinical relevance.

In this study, neovascularisation was observed in all tumour biopsies. Previously, using this model, endothelial cell markers Von Willebrand factor (vWF) and CD31 were localised to the neovessels, confirming that the outgrowths were indeed blood vessels.²

Furthermore, comparison of the number, localisation and phenotype of endothelial and mast cells and the distribution of basement membrane constituents (type IV collagen and laminins) in the biopsy tissue before and after culture showed that many of the characteristics of the original haemangioma tissue were retained in culture.²

Excessive angiogenesis is considered a central event underlying haemangioma development.³ In addition, pro-angiogenic growth factors VEGF and bFGF were previously detected in proliferating lesions.² The development of neovessels observed in cultured haemangiomas in the present study may therefore be reminiscent of angiogenesis occurring in paediatric tumours.

Interestingly, fewer neovessels were observed in tissue fragments treated with bleomycin than in untreated fragments. Studies conducted in our laboratory have also shown that bleomycin possesses anti-angiogenic activity (manuscript in preparation).

Our findings therefore indicate that bleomycin inhibits haemangioma growth by inhibiting angiogenesis. The evidence that bleomycin inhibits haemangioma growth by inhibiting



neovascularisation offers an opportunity for the use of therapeutic strategies based on the inhibition of angiogenesis.

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Bleomycin plasma spill-over levels in paediatric patients undergoing intralesional injection for the treatment of haemangiomas

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To the Editor: Haemangiomas are the most common tumours of infancy.¹ Although most are symptomless, some cause serious complications related to their anatomical location or biological behaviour and therefore require treatment.² The beneficial effect of intralesional bleomycin infiltration (IBI) in the treatment of haemangiomas has been reported.^{3,4}

The Pretoria Vascular Malformation Study Group evaluated the effectiveness of IBI treatment in 37 patients with haemangiomas. Complete resolution or significant improvement occurred in 87% of patients with an extremely low side-effect profile, reported complications mainly including local pain and transient flu-like symptoms.⁴ Ulceration and

flagellate pigmentation were observed in a small percentage of patients (unpublished data).

The major complication seen in cancer patients treated with systemic bleomycin is the development of pulmonary fibrosis, which is considered to be dose-dependent.⁵ It is not known whether IBI treatment for haemangiomas carries the same degree of risk to the pulmonary vasculature as intravenous administration for cancer chemotherapy.

Materials and methods

IBI was used for the treatment of 4 paediatric patients with haemangiomas at dosages of 0.2 - 0.9 mg/kg/treatment (Table I). The bleomycin sulphate formulation used was Blenoxane (Bristol-Myers Squibb, Bedfordview, South Africa), which is a mixture of glycopeptides containing approximately 69% bleomycin A₂ and 29% bleomycin B₂.⁶ The diagnosis of haemangioma was based on medical history and physical examination. Magnetic resonance imaging provided further useful information on the location and extent of the haemangioma, and involvement of deeper structures in the more complex lesions. Intralesional injections were followed by local application of pressure for 10 minutes after which, where possible, a pressure dressing was applied. Baseline blood samples were obtained from each patient before treatment and at 10 minutes, 30 minutes, 1 hour, 6 hours and 24 hours after IBI. As controls, blood samples were taken at the same time intervals as for the haemangioma patients from 4 paediatric cancer patients who received bleomycin intravenously. A Waters LC Module 1 HPLC fitted with a model 486 UV

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