# CHAPTER 1

# General Introduction

## 1.1. Motivation for the study

Haemangiomas are the most common tumours of infancy. [1-3] Although the incidence of haemangioma development has not been well documented, it is estimated that one in every ten children develops a haemangioma, most of which are on the head or neck. [1,2] The incidence of tumour development is increased to 22.9% for premature infants with a birth weight below 1 Kg. [2,3]

The pathophysiology of these vascular tumours is not well known. According to Pepper (1995), haemangioma development is associated with an imbalance of negative and positive regulators of angiogenesis. [4] Previously, light microscopic examination of haemangioma tissue demonstrated that the hallmark of the growing haemangioma was proliferating endothelial cells. [5,6] Furthermore, some of the growth factors which mediate the complex stages of angiogenesis, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), have been implicated in the pathogenesis of haemangiomas. [6,7] The recognition that excessive angiogenesis underlies haemangioma development offers an opportunity for the development of therapeutic strategies based on the inhibition of angiogenesis.

At present, the first-line treatment for haemangiomas is high-dose corticosteroids. [8,9] Steroid therapy for haemangiomas produces variable results. [1,2] Indeed according to Enjolras (1997), steroids dramatically regress haemangiomas in 30% of the patients; have little effect in 40%, and fail completely in the remaining 30%, with patients showing signs of worsening while on treatment. [1] Life-threatening haemangiomas that do not respond to corticosteroids are treated with interferon α. [8,9] Although success rates of approximately 80% have been reported with interferon α, there is a risk of developing irreversible neurotoxicity in haemangioma patients treated with the drug. [1]

Another pharmacological agent, bleomycin, has been cited as simple and adequate for the treatment of complicated cutaneous and massive symptomatic inoperable haemangiomas, without any severe complications. [10,11,12]

In studies undertaken at the University of Pretoria, South Africa, no major side effects were observed in haemangioma patients treated with intralesional bleomycin (IB), however, ulceration and flagellate pigmentation were observed in a small percentage of these patients (unpublished data).

The major complication of bleomycin treatment, pulmonary fibrosis, has been observed in cancer patients treated systemically with the drug. [13,14] It is not yet known whether IB therapy for haemangiomas carries the same degree of risk to the pulmonary vasculature as it does with intravenous administration for cancer chemotherapy. Determination of bleomycin spill-over levels is thus imperative to establish safety of use.

On the other hand, the mechanism by which bleomycin induces haemangioma regression is unknown, and there are concerns about the use of this chemotherapeutic drug to treat benign tumours. Kullendorf (1999) has attributed bleomycin's induction of haemangioma regression to the drug's possible sclerosing effect on vascular endothelium. [9] However, since haemangioma has been reported to be an angiogenic disease, it is plausible that bleomycin inhibits haemangioma growth by inhibiting angiogenesis. The elucidation of bleomycin's mechanism of action, and identification of other drugs with potential in the treatment of haemangiomas, represent important therapeutic objectives.

## 1. 2. Purpose of investigation

The initial aim of this study was to determine the degree of systemic circulatory spill-over of bleomycin in haemangioma patients who underwent IB therapy. Also, this study aimed to determine the effects of bleomycin on angiogenesis *in vitro* (in order to elucidate its mechanism of action in inducing haemangioma regression) and to compare bleomycin's effects on endothelial cells with those of drugs previously reported to inhibit aspects of angiogenesis, namely, mitomycin C, and cytoskeletal-disrupting drugs (2-methoxyestradiol, taxol, vincristine, vinblastine, colchicine, nocodazole, and cytochalasin D). Lastly, the aim of this study was to test the effectiveness of bleomycin, mitomycin C, and various cytoskeletal-disrupting drugs *in vivo* in an animal model of haemangioma.

## 1. 3. Objectives

I. To develop a high performance liquid chromatographic method for the measurement of bleomycin in human plasma, and to use this method to determine bleomycin levels in haemangioma patients treated with intralesional bleomycin.

II. To determine the effects of bleomycin, mitomycin C, 2-methoxyestradiol, taxol, vincristine, vinblastine, colchicine, nocodazole, and cytochalasin D on two of the key cell functions in the angiogenesis process, namely, endothelial cell migration and endothelial cell growth.

III. To determine the effects of test drugs (bleomycin, mitomycin C, 2-methoxyestradiol, taxol, vincristine, vinblastine, colchicine, nocodazole and cytochalasin D) on endothelial cell apoptosis.

IV. To determine the effects of bleomycin and other test drugs on *in vitro* angiogenesis in a three-dimensional collagen gel model.

V. To induce vascular tumour development in a syngeneic mouse strain using pym T-immortalized endothelial cells and to determine the effects of bleomycin, mitomycin C, 2-methoxyestradiol, taxol, colchicine, and vinblastine on tumour development in this model.

## References

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