In: ACE Inhibitors. Volume 2
Editor: Macaulay Amechi Onuigbo
ISBN: 978-1-62948-422-8

Chapter 20

TREATMENT OF INFANTILE HEMANGIOMA WITH AN ACE INHIBITOR: A PARADIGM SHIFT

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ABSTRACT

Traditionally the use of angiotensin converting enzyme inhibitors (ACEi) has focused on their role in blocking angiotensin converting enzyme (ACE), and the downstream production of the vasoactive peptide, angiotensin II, typically in the setting of hypertension treatment. Earlier studies have demonstrated the lungs as the major site of ACE production, and therefore the predominant site of ACE inhibition. More recently the expression of ACE has been shown on hemangiongioblasts, the stem cell precursors of both endothelial and hematopoietic cells. The *in vivo* equivalent of hemangioblasts has been proposed to be the hemogenic endothelium, a developmentally primitive phenotypic endothelium, with the capacity for hematopoietic differentiation. This hemogenic endothelium constitutively expresses both primitive endothelial (CD34 and VEGFR-2) and hematopoietic (Tal-1, GATA-2, and Runx-1) associated markers. The novel finding of the expression of ACE on a phenotypic hemogenic endothelium has recently been reported on the endothelium of the microvessels of proliferating infantile hemangioma (IH), the most common tumour of infancy. IH which predominantly affects females, premature and white infants typically undergoes an initial rapid proliferation followed by spontaneous involution, often leaving a fibrofatty residuum. β-blockers, particularly propranolol, are now the preferred treatment for proliferating IH. The expression of ACE and the role of the angiotensin II peptide in regulating cellular proliferation via the angiotensin II receptor 2 has been proposed to account for the programmed biologic behavior of IH and its accelerated involution induced by β-blockers. Our recent clinical trial showing the efficacy of captopril, an ACEi, on IH confirms the crucial role of the renin-angiotensin system in the regulation of the hemogenic endothelium. This concept

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underscores the paradigm shift in the understanding and implications of ACE inhibition in human development, IH and other tumors.

INTRODUCTION

Angiotensin converting enzyme (ACE) was discovered by Skeggs and his colleagues at the Cleveland Veterans Administration Hospital in the 1950s [1]. This discovery of an enzyme that converts angiotensin I (ATI) to the vasoactive peptide, angiotensin II (ATII), later led to its isolation and purification [2]. Further work by Erdos *et al* demonstrated that ACE also inactivates bradykinin [3,4]. However, it was not until the pioneering work of Laragh and his team in the 1970s that suppression of ATII by the inhibition of ACE forms the basis of effective treatment for hypertension and cardiac failure [5,6]. This led to the discovery of captopril, the first orally active ACE inhibitor (ACEi) [7], followed by the pro-drugs such as enalapril and ramipril [8].

ACE inhibition now forms an important cornerstone in the management of hypertension with recent data showing a potential reduction in all-cause mortality by ACE inhibition [9].

ANGIOTENSIN CONVERTING ENZYME INHIBITORS

Since the discoveries of the mid to late 1950s, ACE has been the enzyme critically associated with the maintenance of the concentration of ATII, by the cleavage of the ATI, with its predominant effects on the cardiovascular system. The most profound of these is its effect on vasoconstriction of blood vessels and on cardiac muscle hypertrophy [10]. Since the introduction of ACE inhibitor (ACEi) in 1981 there have been multiple clinical indications for ACEi in reducing mortality from hypertension, congestive cardiac failure, myocardial infarction, diabetes mellitus, chronic renal insufficiency and atherosclerotic cardiovascular disease [11].

THE RENIN-ANGIOTENSIN SYSTEM

The renin-angiotensin system (RAS) is the endocrine system in which ATII is derived. Physiologically, renin is predominantly secreted from the juxtaglomerular cells of the kidney and this is primarily regulated by the macula densa in response to urine osmolality [12], and also by the stimulation of β-adrenergic receptors on the juxtaglomerular cells through the formation of the second messenger, cAMP [13]. Renin which is responsible for cleaving circulating angiotensinogen, is produced primarily in the liver and white adipose fat [14], to form the decapeptide, ATI, which is subsequently cleaved by ACE to form the vasoactive octapeptide, ATII [15]. The actions of ATII are predominantly mediated via its two receptors, angiotensin II receptor1 (ATIIR1) and angiotensin II receptor 2 (ATIIR2) [15].

HEMANGIOBLASTS AND HEMOGENIC ENDOTHELIUM

Hemangioblasts, primitive cells derived from the extra-embryonic mesoderm [16], which are regarded as the common precursor cells for both endothelial and hematopoietic cells were first demonstrated *in vitro* by Eichmann *et al*, in 1997 [17]. This was based on the expression of vascular endothelial growth factor receptor 2 (VEGFR-2) on cells derived from chicken embryos, and their differential response to the administration of VEGF [17]. This revealed the dual plasticity for a VEGFR-2 cellular signature that enabled the cells to undergo either hematopoietic or endothelial differentiation. Using cells derived from mice embryos, Sugiyama *et al*, subsequently generated hematopoietic cells through an endothelial intermediate [18]. However, it was not until 2009, using live cell imaging that two independent research groups confirmed the ability for hematopoietic differentiation from hemangioblasts to occur through a hemogenic endothelium intermediate (Figure 1) [19,20]. Although these studies have, for the most part, managed to generate hemangioblasts, the identification of an *in vivo* equivalent of the hemogenic endothelium currently remains elusive [21].

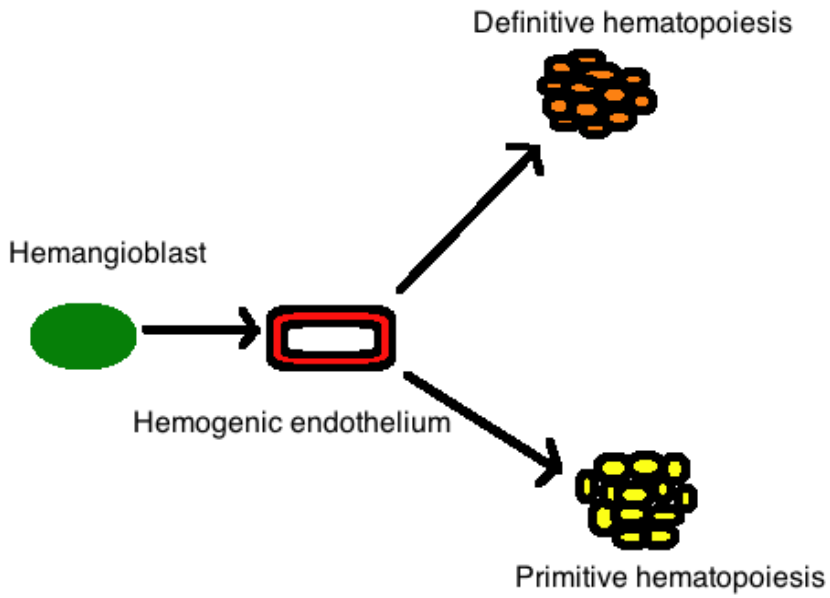

Figure 1. The plasticity for dual primitive and definitive hematopoietic differentiation of hemangioblasts through a hemogenic endothelium stage (*Adapted from* Lancrin C, *et al*, 2009) [20].

HEMANGIOBLASTS, ANGIOTENSIN CONVERTING ENZYME AND ANGIOTENSIN II

Significant further progress in the field occurred following the identification of ACE (also known as CD143) as a cellular marker for primitive hematopoietic stem cells during human hematopoietic ontogeny [22]. Further work revealed ACE as a novel marker for hemangioblasts and the vital role of the RAS through ATII, in regulating the proliferation and

differentiation of hemangioblasts [23]. Although the effect of ATII in promoting proliferation of hematopoietic progenitors had been known since the turn of the 21st century [24], it was not until the discovery by Zambidis *et al*, that the extent of both ACE in generating ATII and the differential effects of ATII on its two receptors was better appreciated [23]. They showed that ATII cause preferential effect on endothelial or hematopoietic differentiation in hemangioblasts via activation of ATIIR1 or ATIIR2 respectively (Figure 2) [23].

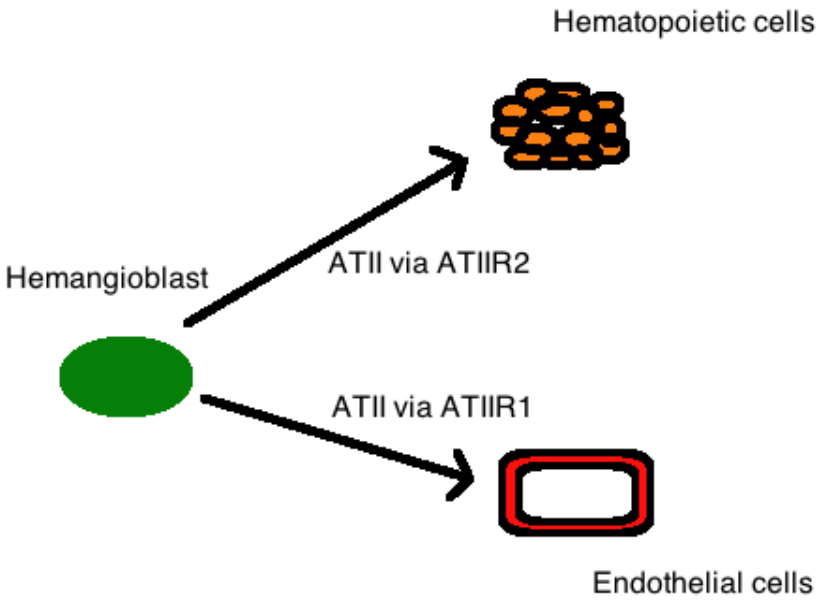

Figure 2. The differential effects of angiotensin II signaling on hemangioblast differentiation, with preferential differentiation towards hematopoietic cells via angiotensin II receptor 2 activation and towards an endothelial lineage via angiotensin II receptor 1 activation (*Adapted from* Zambidis ET, *et al*, 2008) [23].

INFANTILE HEMANGIOMA

Infantile hemangioma (IH) is the most common tumor of infancy affecting up to 10% of the population, with a predilection for Caucasian, female and premature infants [25,26]. It is typified by excessive vasculogenesis during infancy (proliferative phase), followed by spontaneous slow involution in which the cellular elements are gradually replaced by fibro-fatty tissues over the next 1-5 years (involuting phase), with continued improvement up to 10 years, often leaving behind a fibro-fatty residuum (involuted phase) (Figure 3) [27].

During the proliferative phase the excessive vasculogenesis leads to accumulation of immature endothelial cells organized into capillaries composed of an inner luminal layer characterized by the expression of endothelial markers such as CD31, CD34, and von Willebrands factor [28]. This endothelial layer is surrounded by an outer concentric pericyte layer, expressing smooth muscle actin (Figure 4) [28,29].

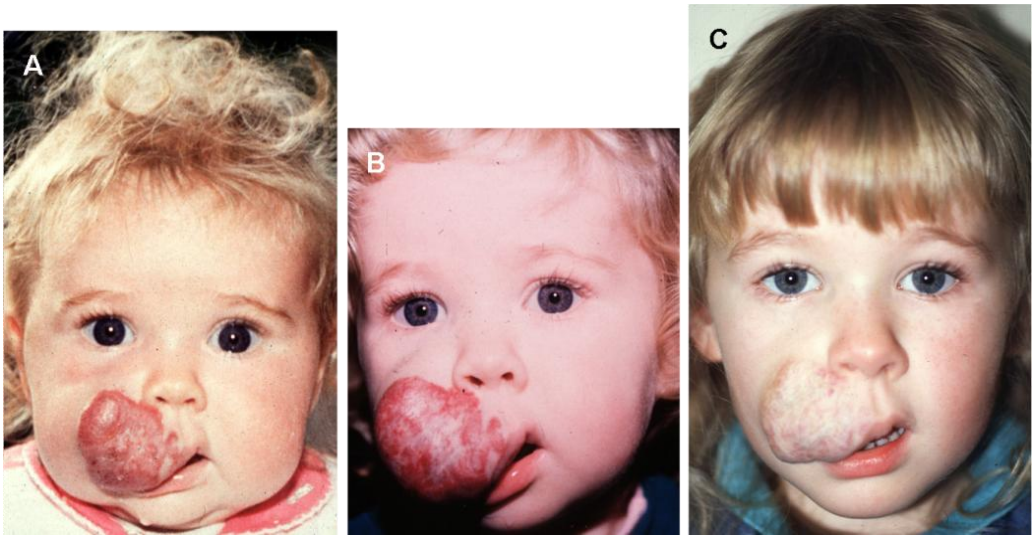

Figure 3. Serial photographs showing a typical infantile hemangioma progression; at 8 months of age (proliferative phase) (A); at 3 years of age (involuting phase) (B); and at 8 years of age (involuted phase) (C).

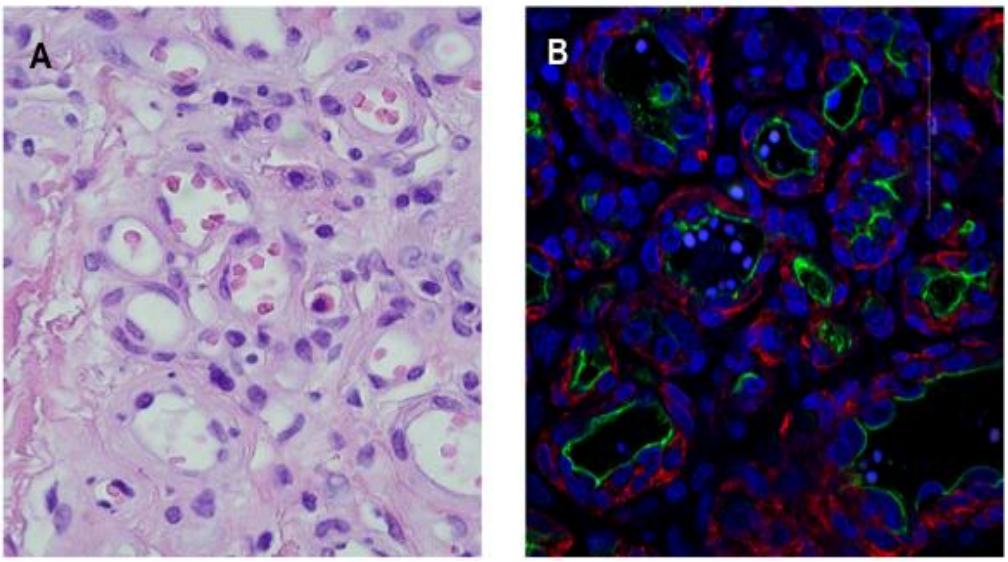

Figure 4. Hematoxylin & eosin staining of a proliferating infantile hemangioma showing an abundance of micro-vessels (A) consisting of an inner endothelial layer expressing CD34 (green) with an outer concentric pericyte layer expressing smooth muscle actin (red) (B). Original magnification: 100X.

INFANTILE HEMANGIOMA AND HEMOGENIC ENDOTHELIUM

The endothelial layer of proliferating IH has been shown to possess a primitive phenotype typically associated with an embryonic-like expression pattern [30]. The exact origin of the micro-vessels within proliferating IH, has previously been suggested as arising *de novo* through a primitive process of vasculogenesis [31], with later studies hypothesizing 'a more downstream' bone marrow derived endothelial progenitor cell (EPC) origin [32]. It was not until 2010, that we first revealed the unique co-expression of primitive hematopoietic-associated markers (brachyury, GATA-2, Tal-1 and CD133), as well the neural crest stem cell markers (p75, Sox 9, and Sox-10) on the phenotypic endothelial layer of proliferating IH [33,34]. This novel finding and the appreciation of the hemogenic endothelial nature of the micro-vessels of proliferating IH led to the better understanding of these enigmatic tumors. We also provided evidence of the first trimester placental chorionic villous mesenchymal core origin of IH [35], supporting the findings of Bree *et al*, [36] on the null involvement of placental trophoblasts in IH. This chorionic villous mesenchymal core cell origin of IH concept supports previous theories of placental embolization as a cause for IH [37], based on the unique co-expression of certain proteins in the endothelium of IH and placenta [29,37].

INFANTILE HEMANGIOMA, ANGIOTENSIN CONVERTING ENZYME AND ANGIOTENSIN II

We have also demonstrated the expression of ACE on the same phenotypic hemogenic endothelium of IH and that cells derived from IH form blast colonies *in vitro* [38,39]. This coupled with the expression of the ATIIR2 isoform (Figure 5), and the proliferative effect of exogenous ATII on the IH derived blast colonies *in vitro*, point to the crucial involvement of the RAS in the regulation of these tumors [38].

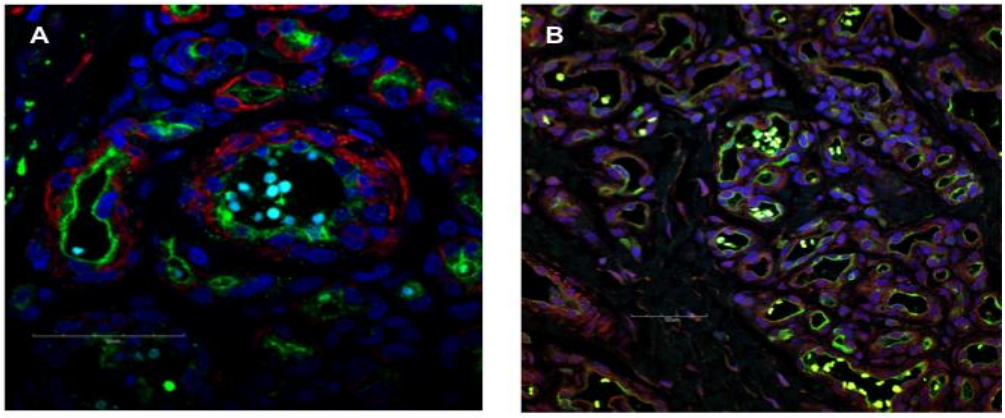

Figure 5. Immunohistochmical staining of proliferating infantile hemangioma showing expression of ACE (green) on the inner endothelial layer with the outer pericyte layer expressing smooth muscle actin (red) (A); and the expression of ATIIR2 (red) also localizing to the hemogenic endothelial layer highlighted by ACE expression (green) (B). Original magnifications: (A) 100X, (B) 40X.

INFANTILE HEMANGIOMA AND ANGIOTENSIN CONVERTING ENZYME INHIBITORS

Our discovery of the involvement of the RAS in IH underscores the basis of the treatment of this condition with β-blockers [38] and led to a prospective observational clinical trial demonstrating the clinical effect of captopril on IH [40] (Figure 6). Accelerated involution of IH was observed in seven out of the eight subjects, with a more gradual response in the remaining patient [40]. Although the clinical response was not as dramatic for one-third of the patients treated with captopril compared to those treated with propranolol [41,42], it is worth noting that the upper limit of the dosage of captopril of 1.5mg/kg/day used in this study was low compared with the maximal cardiovascular dosage of 2mg/kg thrice daily [43]. For safety reasons a low maximum dosage of captopril was used in that pilot study [40]. A retrospective study by Christou *et al* [44], based on a review of IH patients they had been treated with captopril for steroid-induced cardiomyopathy showed no effect of captopril in inducing involution. However, there were flaws in that study as there was no documentation of the dosage and the duration of captopril treatment [44].

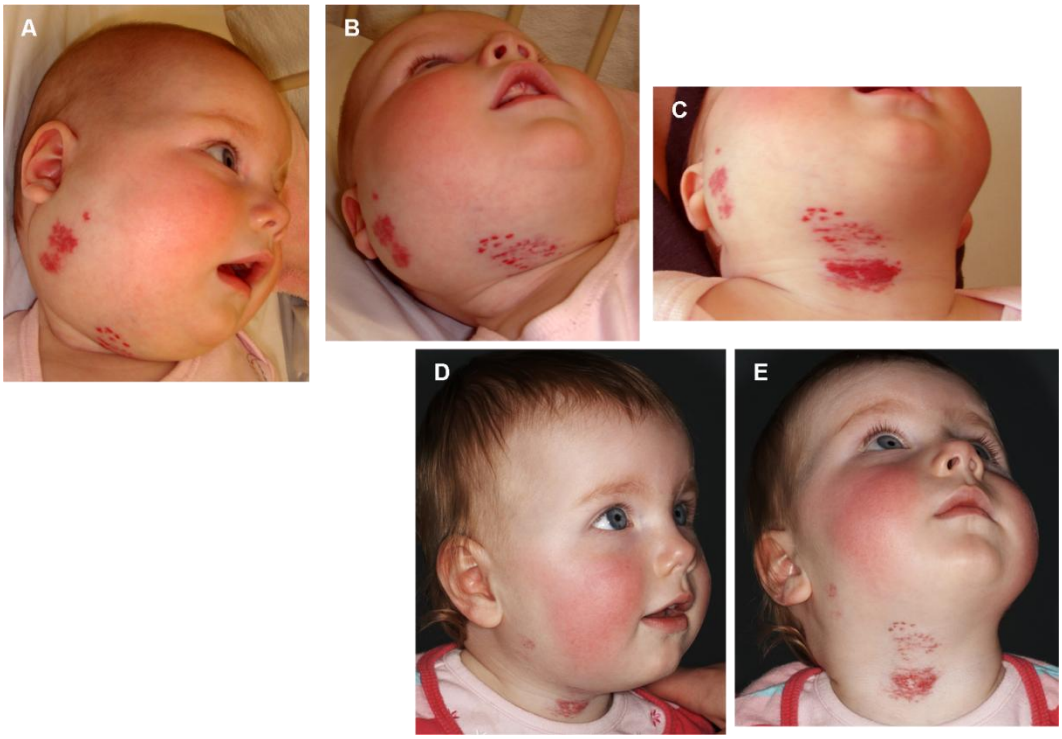

Figure 6. A 22 week-old girl (A,B) with a large right cervico-facial infantile hemangioma 3 weeks (C); and 6 months (D,E) after administration of captopril at a dosage of 1.5mg/kg/day, resulting in accelerated involution (*Reproduced from* Tan ST, *et al*, 2012) [40].

CONCLUSION

Recent studies into the biology of IH reveal the developmental anomalous nature of IH caused by aberrant proliferation and differentiation of a primitive mesoderm-derived hemogenic endothelium with a neural stem phenotype [33]. The involvement of the RAS with expression of ACE [38] on this phenotypic hemogenic endothelium of IH [34] is critical in the modulation of stem cell proliferation in these tumors. These new insights highlight a paradigm shift in the understanding of the biology of IH and underscores the novel treatment of this vascular tumor, through the modulation of RAS using β-blockers [41,45] and ACEi [44]. These recent discoveries have implications in tumor biology in general and the understanding of human development.

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