REVIEW ARTICLE

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Propranolol for infantile haemangiomas: insights into the molecular mechanisms of action

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Summary

Infantile haemangiomas (IH) are the most common benign tumours of infancy. Although most IH are innocuous and 85–90% regress spontaneously, some may become life- or function-threatening and require immediate treatment. Previous standard therapeutic options include physical measures (laser surgery, cryosurgery) and systemic corticosteroids, in severe cases also vincristine, α-interferon or cyclophosphamide, all bearing the risk of serious side-effects. Oral propranolol is a very recent therapeutic option for complicated IH with impressive efficacy and generally good tolerance. The effects of propranolol on IH were discovered by chance, and very little is known about its mechanisms of action in IH. Here we present a summary of current knowledge of how propranolol interferes with endothelial cells, vascular tone, angiogenesis and apoptosis. Early, intermediate and long-term effects of propranolol on IH can be attributed to three different pharmacological targets. Early effects (brightening of the haemangioma surface within 1–3 days after start of therapy) are attributable to vasoconstriction due to decreased release of nitric oxide. Intermediate effects are due to the blocking of proangiogenic signals (vascular endothelial growth factor, basic fibroblast growth factor, matrix metalloproteinase 2/9) and result in growth arrest. Long-term effects of propranolol are characterized by induction of apoptosis in proliferating endothelial cells, and result in tumour regression.

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Haemangiomas are the most common benign tumours of infancy, affecting 5–10% of all infants, and up to 30% of premature babies.[1] They can be differentiated from vascular malformations by their dynamic growth and spontaneous regression (Fig. 1a). Although 85–90% of all infantile haemangiomas (IH) eventually undergo spontaneous involution, they can still cause disfigurement and serious complications depending on their location (obstruction of airways and vision), size (cardiac insufficiency, hypothyroidism), and speed of regression, which can be associated with painful ulcerations and haemorrhage. Standard treatment options for complicated haemangiomas include oral steroids, laser surgery, cryosurgery, or vincristine, interferon or cyclophosphamide in life-threatening cases. Each of these options has its restrictions and/or side-effects. In 2008, Léauté-Labrèze *et al.*[2] described their serendipitous observation of an antiproliferative effect of propranolol on IH. Propranolol has since become the first choice of therapy for complicated IH, even though randomized controlled studies have not been finished yet and a generally accepted concept of how propranolol actually works in IH does not exist.

Here we present a summary of current knowledge of how propranolol interferes with endothelial cells, vascular tone, angiogenesis and apoptosis. Propranolol is a nonselective beta-adrenergic antagonist, which competitively inhibits β_1- and β_2-adrenoceptors with the same affinity. Propranolol is a pure antagonist without partial agonistic effects. On account of its lipophilic properties propranolol also exhibits certain membrane-stabilizing characteristics. α_1-adrenoceptors are not antagonized by propranolol.[3]

The following pharmacodynamic characteristics are discussed in this review as possible mechanisms of action of propranolol in IH: vasoconstriction, inhibition of angiogenesis and induction of apoptosis.

Vasoconstriction

The vascular tone is controlled by a complex interplay of various endogenous factors. The autonomic nervous system plays a key role in the control of the vascular tone: adrenaline can cause vasoconstriction (by activating α_1-receptors) as well as

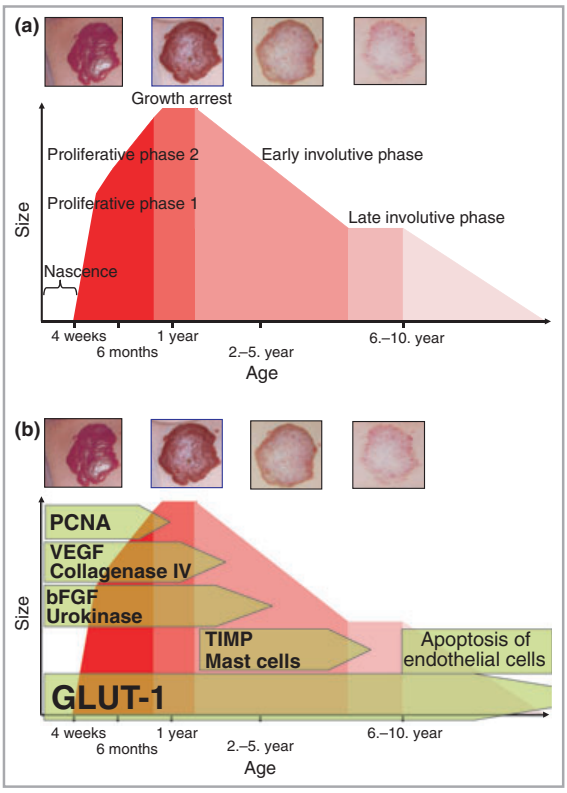

Fig 1. (a) Growth and regression of infantile haemangiomas (IH). (b) Molecular markers characterizing individual phases of IH. PCNA, proliferating cell nuclear antigen; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; TIMP, tissue inhibitor of metalloproteinases; GLUT-1, glucose transporter type 1.

vasodilation (by activating β_2-receptors). Depending on the partial pressures of oxygen and carbon dioxide, respectively, the vascular tone is either increased or decreased. It is further regulated by transmitters which either lead to vasoconstriction (e.g. endothelin-1, angiotensin II, vasopressin) or to vasodilation [e.g. prostacyclin, nitric oxide (NO), dopamine].

The vasodilatory effect of adrenaline by activating β_2-adrenoceptors is mediated by a cascade of signals[4] (Fig. 2): β-adrenoceptors belong to the group of G_S-protein-coupled receptors[4] and are expressed on endothelial cells.[5] As soon as an agonist binds to the receptor, adenylate cyclase is activated by the α-subunit of the stimulatory G-protein. The adenylate cyclase converts adenosine triphosphate into cyclic adenosine monophosphate (cAMP), which acts as a second messenger and diffuses into the cytosol where it activates the cAMP-dependent protein kinase A (PKA). PKA can in turn phosphorylate intracellular proteins and thus modulate their activity. In endothelial cells the activation of PKA leads to an activation of the endothelial NO synthase, which results in the formation and release of NO. NO diffuses into the vascular smooth muscle cell, where it leads to an activation of the soluble guanylate cyclase resulting in the formation of cyclic guanosine monophosphate (cGMP), which in turn activates protein kinase G (PKG, cGMP-dependent protein kinase). This activation of PKG finally induces relaxation of the vascular smooth muscle and vasodilatation.

Beta blockers without α-antagonistic effects such as propranolol inhibit the vasodilation mediated by adrenaline via β-receptors and thus lead to vasoconstriction.[3] In haemangiomas, vasoconstriction of supplying capillaries caused by propranolol induces a reduction of blood flow within the haemangioma which is associated with a visible change in colour and a palpable softening of the haemangioma. These effects can be observed within 1–3 days after the onset of therapy[2] (Fig. 3).

Inhibition of angiogenesis

Impact on the expression of proangiogenic growth factors (vascular endothelial growth factor and basic fibroblast growth factor)

In the proliferative phase of IH endothelial cells exhibit increased expression of proliferating cell nuclear antigen, type IV collagenase, and proangiogenic factors, in particular vascular endothelial growth factor (VEGF) more than basic fibroblast growth factor (bFGF)[6–11] (Fig. 1b). VEGF could be detected in various cell types both on the protein level (immunohistochemistry) and at messenger RNA level (by reverse transcription–polymerase chain reaction): endothelial cells (so-called hemangioma-derived endothelial cells, HemECs), pericytes and connective tissue cells. bFGF was also identified in mast cells.[10] Serum levels of VEGF are elevated in infants during the proliferative phase of IH.[9,12,13] Conversely, expression of VEGF and bFGF is significantly reduced during the involution phase as well as in completely regressed haemangiomas (involuted phase).[7,12,13] Transforming growth factor-β, an inhibitor of endothelial growth,[14] is expressed only at a relatively low level both in the proliferation phase and the involution phase,[7] and tissue inhibitor of metalloproteinases (TIMP), also an inhibitor of angiogenesis,[14] is expressed only during the involution phase.[11] These and other findings are evidence that IH result from a dysregulation of angiogenesis, characterized by an imbalance between pro- and antiangiogenic factors.[7] The expression of VEGF is regulated by the transcription rate and changes in the stability of the mRNA.[15] Physiologically, hypoxaemia leads to increased expression of VEGF. This effect is mediated by the transcription factor hypoxia-inducible factor (HIF)-1α: oxygen deficiency leads to an increase of the intracellular concentration of HIF-1α in its active form. HIF-1α induces the transcription of the VEGF gene. As a result, VEGF is secreted from the cell, diffuses into the surrounding tissue and induces proliferation of adjacent endothelial cells, which in turn leads to secretion of proteases necessary for reorganization of the extracellular matrix [e.g. matrix metalloproteinases (MMPs); see below], and the coordinated differentiation of vascular cells (endothelial cells, smooth muscle cells, pericytes) into functional

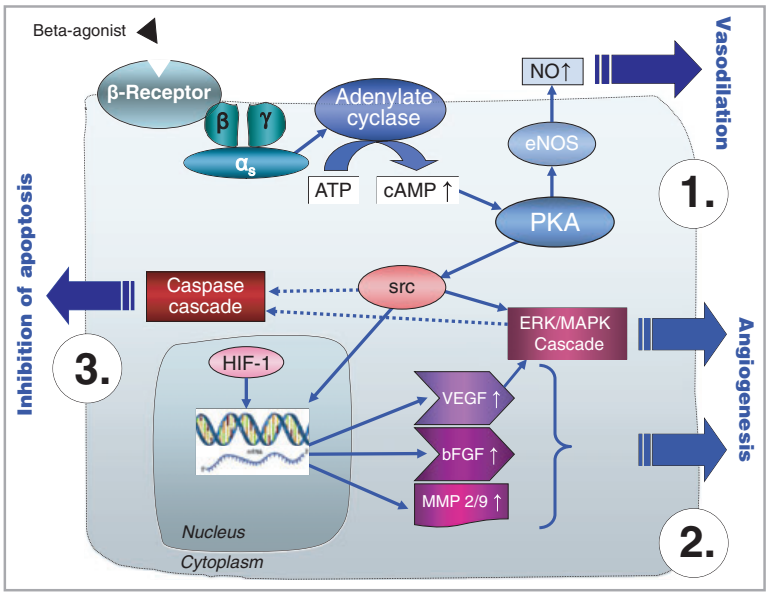

Fig 2. Molecular processes in infantile haemangiomas (IH) that may be affected by propranolol. The figure depicts the pathophysiological situation in IH *in the absence of* a beta-adrenergic antagonist. (1) *Control of vascular tone*. Beta-adrenergic agonists (black triangle) lead to vasodilation via release of NO. Conversely, beta-adrenergic antagonists like propranolol lead to vasoconstriction (through inhibition of NO synthesis and NO release). (2) *Angiogenesis*. Beta-adrenergic agonists (black triangle) stimulate the synthesis of proangiogenic factors [growth factors (VEGF and bFGF) and matrix metalloproteinases (MMP-2 and MMP-9)] and activate proangiogenic cascades (ERK/MAPK cascade) thereby promoting angiogenesis. In contrast, beta blockers like propranolol lead to a downregulation of these proangiogenic proteins and to an inhibition of the ERK/MAPK cascade thus repressing angiogenesis. (3) *Apoptosis*. Beta-adrenergic agonists (black triangle) inhibit apoptosis via src/MAPK. In contrast, beta blockers induce apoptosis. Continuous arrows indicate activation; dashed arrows indicate inhibition; broad arrows indicate pharmacodynamic effects; ↑ indicates upregulation. β, γ, α_s, three subunits of the stimulatory G-protein (heterotrimer); ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A (cAMP-dependent protein kinase); ERK, extracellular signal-related kinases; MAPK, mitogen-activated protein kinases; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; MMP, matrix metalloproteinase; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; HIF-1, hypoxia-inducible factor 1.

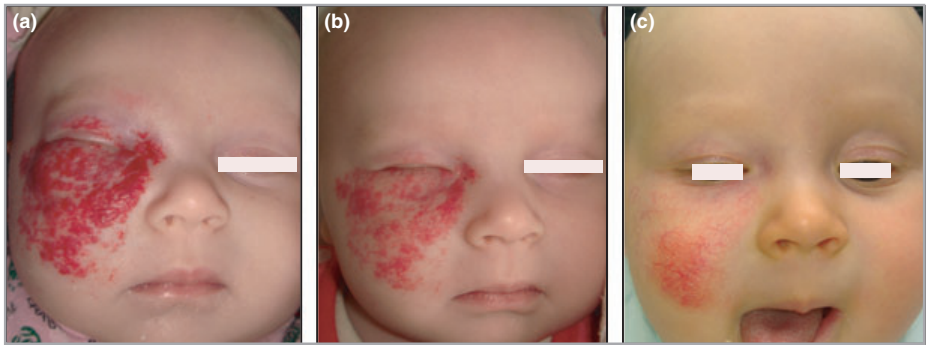

Fig 3. Effect of propranolol on proliferating infantile haemangiomas. (a) Age 8 weeks, before start of therapy; (b) age 9 weeks, 1 week after start of therapy with propranolol 2 mg kg^{-1} daily; (c) age 8 months, after 6 months of treatment.

vessels (angiogenesis). The newly formed vessels improve local oxygen supply, which in turn leads to decreased intracellular concentration of the active form of HIF-1α and, as a result, reduces the expression of VEGF. Thus, under physiological conditions, angiogenesis is strictly controlled.[15,16] As perinatal hypoxaemia has been identified as an important aetiological factor in IH,[17] increased production of HIF-1 and consecutively VEGF (as has been shown in proliferating IH[12]) seems a 'logical' consequence.[9,18,19]

There is evidence from recent publications that the expression of VEGF is not only controlled by the oxygen partial pressure in the tissue (via HIF-1α), but also by adrenergic

stimulation (Fig. 2): as demonstrated in different *in vitro* and *in vivo* models catecholamines such as adrenaline and noradrenaline can induce the expression of VEGF.[20–24] Src is a mediator downstream to PKA[20] which belongs to a family of cytoplasmic tyrosine kinases involved in the control of diverse molecular processes. It activates the extracellular signal-related kinases (ERK)/mitogen-activated protein kinases (MAPK) cascade.[20] ERK and MAPK are serine/threonine kinases phosphorylating nuclear transcription factors, thus regulating the expression of multiple genes involved in the control of cell proliferation. VEGF itself exerts its proangiogenic effects, at least partially, by activating the ERK/MAPK cascade.[21,25] Thus, the proliferation of endothelial cells is stimulated via β_2-adrenoceptors by two different mechanisms: (i) stimulation of β_2-adrenoceptors directly leads to an activation of ERK/MAPK (probably via src as mediator[20]) and (ii) it also induces increased release of VEGF, which itself can activate the ERK/MAPK cascade.[21,25]

Conversely, beta blockers like propranolol lead to a reduced expression of VEGF and thus to an inhibition of angiogenesis.[20,22,24,26] Furthermore, it has been demonstrated in animal experiments that carvedilol, also a nonselective beta-antagonist like propranolol, reduces the expression of HIF-1α.[27,28] Assuming that dysregulation of endothelial cell proliferation represents an important pathogenetic factor for IH, the anti-VEGF activity of beta blockers would explain their remarkable effect on proliferating haemangiomas. Interestingly, a similar effect has recently been demonstrated for corticosteroids,[29] which have been the mainstay of IH therapy until now.

Impact on the expression of matrix metalloproteinases

MMPs are a family of soluble and membrane-anchored proteinases involved in degradation and transformation of extracellular matrix proteins. They play a key role in different physiological and pathophysiological processes, e.g. cell proliferation, migration and adhesion, embryogenesis, wound healing and also in angiogenic processes relevant for tumour growth and metastasis.[30] Under physiological conditions the activity of MMPs is strictly regulated on different levels: transcription, activation of inactive precursors (cymogens), interaction with components of the extracellular matrix and inhibition by endogenous inhibitors such as TIMPs.[31]

Elevated concentrations of MMP-2 and MMP-9 were found in tissue samples and in the blood of infants in the proliferative phase of haemangiomas.[9,32] MMP-9 is important for the migration of endothelial cells and tubulogenesis.[33] It has been shown that inhibition of MMP-9 impedes angiogenesis of human microvascular endothelial cells.[34]

There is ample evidence that the expression of MMP-9 and MMP-2 is regulated via β-adrenoceptors[23,24,33,35] (Fig. 2). β-adrenoceptor agonists such as adrenaline or noradrenaline increase the expression of MMP-2 and MMP-9; this effect can be inhibited by propranolol.[23,24,33,35] Reduced expression of MMP-9 by propranolol leads to an inhibition of tubulogenesis of endothelial cells[33] and thus also provides a conclusive mechanism for the antiangiogenic effect of propranolol.

Induction of apoptosis in capillary endothelial cells of haemangiomas

Apoptosis is strictly regulated via a complex interplay of caspases, procaspases and proteins of the B-cell lymphoma 2 (bcl-2) family. During the proliferative phase the rate of apoptosis is low in IH. However, during the involution phase it increases by a factor of 5 while the expression of the apoptosis-inhibiting protein bcl-2 decreases in parallel.[36] Only about one-third of the apoptotic cells are of endothelial origin.[37] Blockade of β-adrenoceptors by propranolol can induce apoptosis of different cell types (Fig. 2) *in vitro*, e.g. endothelial cells[38] or pancreas carcinoma cells,[39] respectively. The β_2-adrenoceptor was identified as probable receptor, as the β_1-selective beta blocker metoprolol showed significantly lower apoptosis-inducing effects, and the β_2-selective beta blocker butoxamine exhibited a considerably stronger induction of apoptosis compared with propranolol.[39] The authors hypothesize that beta-adrenergic antagonists are capable of disengaging the inhibition of apoptosis caused by beta-adrenergic agonists (mediated by src, MAPK and caspase cascade), resulting in an increased apoptosis rate.[39] Induction of apoptosis thus represents another possible mechanism of action of propranolol in the treatment of IH.

Conclusion

The striking effect of propranolol on growing IH can be attributed to three molecular mechanisms: vasoconstriction, inhibition of angiogenesis, and induction of apoptosis. They correspond to early (brightening of haemangioma surface), intermediate (growth arrest), and long-term (regression) clinical observations. Apoptosis is not complete, as partial regrowth of haemangiomas after discontinuation of propranolol has been observed. Propranolol has been used in young infants for a variety of indications (such as hypertension, congestive heart failure, supraventricular tachycardia, long QT-syndrome, thyrotoxicosis), at doses up to 8 mg kg^{-1} daily.[40] During 40 years of extensive clinical experience no serious cardiovascular event was recorded for children on chronic beta-blocker therapy.[41] Complications of propranolol treatment of IH reported so far (hypotension, sinusbradycardia, hypoglycaemia)[42] were not life-threatening, but certainly warrant careful monitoring of all infants with IH before and during propranolol therapy. These effects appear, however, minor compared with the serious side-effects of more 'conventional' antiangiogenic agents such as interferon-α, which is associated with spastic diplegia in up to 25%,[43] or the serious side-effects of systemic corticosteroid therapy. Based on case reports and uncontrolled studies the effective dosage for propranolol for IH is 2 mg kg^{-1} daily in three divided doses, and treatment should be continued until the end of the proliferation phase.[2,44–46] However, to assess optimal dose and duration of treatment further studies are required.

What's already known about this topic?

- Propranolol is a new therapeutic option with impressive efficacy for life- or function-threatening infantile haemangiomas (IH).

What does this study add?

- This review summarizes the current knowledge on the possible pharmacological targets of propranolol in IH and presents a conclusive model of the different molecular mechanisms of action involved.

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References

1 Drolet BA, Swanson EA, Frieden IJ *et al.* Infantile hemangiomas: an emerging health issue linked to an increased rate of low birth weight infants. *J Pediatr* 2008; **153**:712–15.
2 Léauté-Labrèze C, de la Roque ED, Hubiche T *et al.* Propranolol for severe hemangiomas of infancy. *N Engl J Med* 2008; **358**:2649–51.
3 Westfall TC, Westfall DP. Adrenergic agonists and antagonists. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Brunton LL, Lazo JS, Parker KL, eds), 11th edn. New York: McGraw-Hill, 2006; 271–95.
4 Westfall TC, Westfall DP. Neurotransmission: the autonomic and somatic motor nervous systems. In: *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Brunton LL, Lazo JS, Parker KL, eds), 11th edn. New York: McGraw-Hill, 2006; 167.
5 Guimaraes S, Moura D. Vascular adrenoceptors: an update. *Pharmacol Rev* 2001; **53**:319–56.
6 Berard M, Sordello S, Ortega N *et al.* Vascular endothelial growth factor confers a growth advantage *in vitro* and *in vivo* to stromal cells cultured from neonatal hemangiomas. *Am J Pathol* 1997; **150**:1315–26.
7 Chang J, Most D, Bresnick S *et al.* Proliferative haemangiomas: analysis of cytokine gene expression and angiogenesis. *Plast Reconstr Surg* 1999; **103**:1–9.
8 Giatromanolaki A, Arvantidou V, Hatzmichael A *et al.* The HIF-2α/VEGF pathway activation in cutaneous capillary haemangioms. *Pathology* 2005; **37**:149–51.
9 Kleinman ME, Greives MR, Shurgin SS *et al.* Hypoxia-induced mediators of stem/progenitor cell trafficking are increased in children with haemangioma. *Arterioscler Thromb Vasc Biol* 2007; **27**:2664–70.
10 Qu Z, Liebler JM, Powers MR *et al.* Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. *Am J Pathol* 1995; **147**:564–73.
11 Takahashi K, Mulliken JB, Kozakewich HPW *et al.* Cellular markers that distinguish the phases of hemangioma during infancy and childhood. *J Clin Invest* 1994; **93**:2357–64.
12 Przewratil P, Sitkiewicz A, Wyka K *et al.* Serum levels of vascular endothelial growth factor and basic fibroblast growth factor in children with hemangiomas and vascular malformations – preliminary report. *Pediatr Dermatol* 2009; **26**:399–404.
13 Przewratil P, Sitkiewicz A, Andrzeijwska E. Local serum levels of vascular endothelial growth factor in infantile hemangioma: intriguing mechanisms of endothelial growth. *Cytokine* 2010; **49**:141–7.
14 Folkman J. Angiogenesis inhibitors generated by tumors. *Mol Med* 1995; **1**:120–2.
15 Alberts B, Johnson A, Lewis J *et al.* Blutgefäße und Endothelzellen. In: *Molekularbiologie der Zelle* (Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P, eds; translation by Jaenicke L), 4th edn. Weinheim: Wiley-VCH Verlag, 2004; 1492–7.
16 Hickey MM, Simon MC. Regulation of angiogenesis by hypoxia and hypoxia-inducible factors. *Curr Top Dev Biol* 2006; **76**:217–57.
17 Colonna V, Resta L, Napoli A *et al.* Placental hypoxia and neonatal haemangioma: clinical and histological observations. *Br J Dermatol* 2010; **162**:208–9.
18 Boye E, Jinnin M, Olsen BR. Infantile hemangioma: challenges, new insights, and therapeutic promise. *J Craniofac Surg* 2009; **20** (Suppl. 1):678–84.
19 Léauté-Labrèze C, Taïb A. Efficacité des bêtabloquants dans les hémangiomes capillaires infantiles: signification physiopathologique et conséquences thérapeutiques. *Ann Dermatol Venereol* 2008; **135**:860–2.
20 Fredriksson JM, Lindquist JM, Bronnikov E *et al.* Norepinephrine induces vascular endothelial growth factor gene expression in brown adipocytes through a beta-adrenoceptor/cAMP/protein kinase A pathway involving src but independently of Erk1/2. *J Biol Chem* 2000; **18**:13802–11.
21 Iaccarino G, Ciccarelli M, Sorriento D *et al.* Ischemic neoangiogenesis enhanced by beta2-adrenergic receptor overexpression – a novel role for the endothelial adrenergic system. *Circ Res* 2005; **97**:1182–9.
22 Lutgendorf SK, Cole S, Costanzo E *et al.* Stress-related mediators stimulate vascular endothelial growth factor secretion by two ovarian cancer cell lines. *Clin Cancer Res* 2003; **9**:4514–21.
23 Thaker PH, Han LY, Kamat AA *et al.* Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat Med* 2006; **12**:939–44.
24 Yang EV, Sood AK, Chen M *et al.* Norepinephrine up-regulates the expression of vascular endothelial growth factor, matrix metalloproteinase (MMP)-2, and MMP-9 in nasopharyngeal carcinoma tumor cells. *Cancer Res* 2006; **66**:10357–64.
25 Parenti A, Morbidelli L, Cui XL *et al.* Nitric oxide is an upstream signal of vascular endothelial growth factor-induced extracellular signal-regulated kinase 1/2 activation in postcapillary endothelium. *J Biol Chem* 1998; **273**:4220–6.
26 Al-Wadei HA, Al-Wadei MH, Schuller HM. Prevention of pancreatic cancer by the beta-blocker propranolol. *Anticancer Drugs* 2009; **20**:477–82.
27 Shyu KG, Liou JY, Wang BW *et al.* Carvedilol prevents cardiac hypertrophy and overexpression of hypoxia-inducible factor-1α and vascular endothelial growth factor in pressure-overloaded rat heart. *J Biomed Sci* 2005; **12**:409–20.
28 Shyu KG, Lu MJ, Chang H *et al.* Carvedilol modulates the expression of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in a rat model of volume-overload heart failure. *J Card Fail* 2005; **11**:152–9.
29 Greenberger S, Boscolo E, Adini I *et al.* Corticosteroid suppression of VEGF-A in infantile hemangioma-derived stem cells. *N Engl J Med* 2010; **362**:1005–13.
30 Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 2003; **92**:827–39.

31 Nagase H, Woessner JF. Matrix metalloproteinases (minireview). *J Biol Chem* 1999; **274**:21491–4.
32 Zhong S, Yang G, Xia C *et al.* Expression of matrix metalloproteinase and its tissue inhibitor in haemangioma. *J Huazhong Univ Sci Technolog Med Sci* 2009; **29**:614–19.
33 Annabi B, Lachambre MP, Plouffe K *et al.* Propranolol adrenergic blockade inhibits human brain endothelial cells tubulogenesis and matrix metalloproteinase-9 secretion. *Pharmacol Res* 2009; **60**:438–45.
34 Jadhav U, Chigurupati S, Lakka SS *et al.* Inhibition of matrix metalloproteinase-9 reduces *in vitro* invasion and angiogenesis in human microvascular endothelial cells. *Int J Oncol* 2004; **25**:1407–14.
35 Sood AK, Bhatty R, Kamat AA *et al.* Stress hormone-mediated invasion of ovarian cancer cells. *Clin Cancer Res* 2006; **12**:369–75.
36 Mancini AJ, Smoller B. Proliferation and apoptosis within juvenile capillary hemangiomas. *Am J Dermatopathol* 1996; **18**:505–14.
37 Razon MJ, Kräling BM, Mulliken JB *et al.* Increased apoptosis coincides with onset of involution in infantile hemangioma. *Microcirculation* 1998; **5**:189–95.
38 Sommers Smith SK, Smith DM. Beta blockade induces apoptosis in cultured capillary endothelial cells. *In Vitro Cell Dev Biol Anim* 2002; **38**:298–304.
39 Zhang D, Ma Q, Shen S *et al.* Inhibition of pancreatic cancer cell proliferation by propranolol occurs through apoptosis induction. *Pancreas* 2009; **38**:94–100.
40 Garin EH, Araya CE. Treatment of systemic hypertension in children and adolescents. *Curr Opin Pediatr* 2009; **21**:600–4.
41 Love JN, Sikka N. Are 1-2 tablets dangerous? Beta-blocker exposure in toddlers. *J Emerg Med* 2004; **26**:309–14.
42 Lawley LP, Siegfried E, Todd JL. Propranolol treatment for hemangioma of infancy: risks and recommendations. *Pediatr Dermatol* 2009; **26**:610–14.
43 Michaud AP, Bauman NM, Burke DK *et al.* Spastic diplegia and other motor disturbances in infants receiving interferon-alpha. *Laryngoscope* 2004; **114**:1231–6.
44 Buckmiller LM, Munson PD, Dyamenahalli U *et al.* Propranolol for infantile hemangiomas: early experience at a tertiary vascular anomalies center. *Laryngoscope* 2010; **120**:676–81.
45 Manunza F, Syed S, Laguda B *et al.* Propranolol for complicated infantile haemangiomas: a case series of 30 infants. *Br J Dermatol* 2010; **162**:466–8.
46 Sans V, Dumas de la Roque E, Berge J *et al.* Propranolol for severe infantile hemangiomas: follow-up report. *Pediatrics* 2009; **124**:423–31.

