

Propranolol Decreases the Viability and Triggers Apoptosis in Hemangioblastoma Cells from Von Hippel-Lindau Patients

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Abstract

In our previous paper, propranolol is used in hemangioblastomas primary cultures from patients with von Hippel-Lindau disease. This is a rare inherited oncologic condition characterized by the growth of tumors affecting mainly the central nervous system, but also kidneys, pancreas, adrenal glands, retinas and endolymphatic sacs (inner ear). Up to date, the only treatment for these patients is surgery. The search for drugs able, at least, to stop the development of hemangioblastomas, has led our group to propose propranolol, a non-specific beta blocker, as a drug to test. According to the results of this work, propranolol would act by decreasing hypoxia signaling pathway, which is constitutively active in VHL patients, normalizing the hypoxia target genes involved in angiogenesis, survival and stemness in the hemangioblastoma cells. The results *in vitro* are promising and, in the absence of serious side effects, propranolol could be assayed as a therapy in the clinical practice for VHL patients.

Keywords: Von Hippel-Lindau disease (VHL); PVHL; Hypoxia inducible factor; Hemangioblastoma; CNS tumors; Propranolol

Introduction

Von Hippel-Lindau (VHL) disease is a rare type of oncological disease with an incidence of 1/36,000 individuals in the general population [1,2]. The most frequent tumors are hemangioblastomas (HB) of the central nervous system (CNS) and retina, as well as renal cell carcinoma [3,4]. In addition, pheochromocytomas, pancreatic neuroendocrine tumors, pancreatic serous cystadenomas, endolymphatic sac tumors and papillary cystadenomas are associated with the disease. Moreover, rare allocations of hemangioblastoma growth have been described, such as liver, gastrointestinal tract and retroperitoneal peripheral sites [5-7].

VHL disease is inherited in autosomal dominant pattern. Patients are heterozygous for mutations in VHL, a tumor suppressor gene located on the short arm of chromosome 3 (3p25-p26). Tumors develop when a loss of heterozygosity occurs in addition to the mutated copy of VHL at birth [8]. pVHL binds to HIF-1 α and HIF-2 α targeting them for degradation in the proteasome [9]. In the absence of functional pVHL HIF accumulates and translocates to the nucleus triggering the hypoxia responsive genes program involved in cell proliferation, angiogenesis, extracellular matrix degradation, vascular tone, stemness and glycolytic metabolism. All HIF target genes are normally silenced in normoxia. However, cells from VHL tumors have a constitutively active HIF program due to the absence of functional pVHL.

So far, the therapeutic options for VHL patients are derived from surgery [10]. CNS tumors do not respond to systemic therapy used for metastatic cancers [11]. Therefore, the lack of therapies urges requirement for effective drugs with reduced side effects for VHL patients.

Propranolol, a non-specific β -blocker used for the treatment of arrhythmias, hypertension, migraines, and other cardiac and neurological diseases, recently has proved to be the best option for the treatment of infantile hemangioma (IH) [12,13]. In relation to this, our group demonstrated that endothelial cells treated with propranolol showed decreased expression of pro-angiogenic proteins which are HIF-1 α targets [14]. The main aim was to test whether propranolol is effective in controlling the growth of HB *in vitro*. The results led us to consider the hypothesis that propranolol could be an efficient treatment for hemangioblastomas through inhibition of HIF in highly vascularized tumors, in which HIF is constitutively expressed.

Methodology

In the paper by Albiñana et al (2015) recently published, hemangioblastoma-derived cells were treated with propranolol, evaluating cell viability and apoptosis, as well as the impact of propranolol on HIF-1 α and HIF-2 α protein levels, normally very high in these cells, as observed in Western blots. Quantitative PCR was conducted to measure the mRNA expression of HIF target genes [1]. In addition, vascular endothelial growth factor (VEGF), the most important proangiogenic factor, was measured by ELISA in the supernatants of *in vitro* cultured Hemangioblastoma cells. Concerning the inclusion criteria, all the hemangioblastoma derived cultures were obtained from surgeries performed by the same neurosurgeons at the same expertised neurosurgery centre. The patients were clinically and genetically characterized as von Hippel-Lindau, and were being followed at the VHL clinical unit. Previously to the *in vitro* essays, the tumors used to obtain the cultures, were studied by the pathologists and classified as hemangioblastomas.

Main Results

The results obtained accordingly suggest that propranolol acts by decreasing HIF protein levels, and in this way, the HIF dependent genes are down regulated: among them, VEGF, EPO or SOX-2 (a stemness gene). Consequently, in the absence of essential factors for survival, the tumor cells, stop dividing and die by apoptosis (we detected an increase in caspases activity, more specifically in Caspase 3/7 activity). A scheme showing the hypothetical mechanism of action of propranolol investigated in the present paper is presented in (Figure 1).

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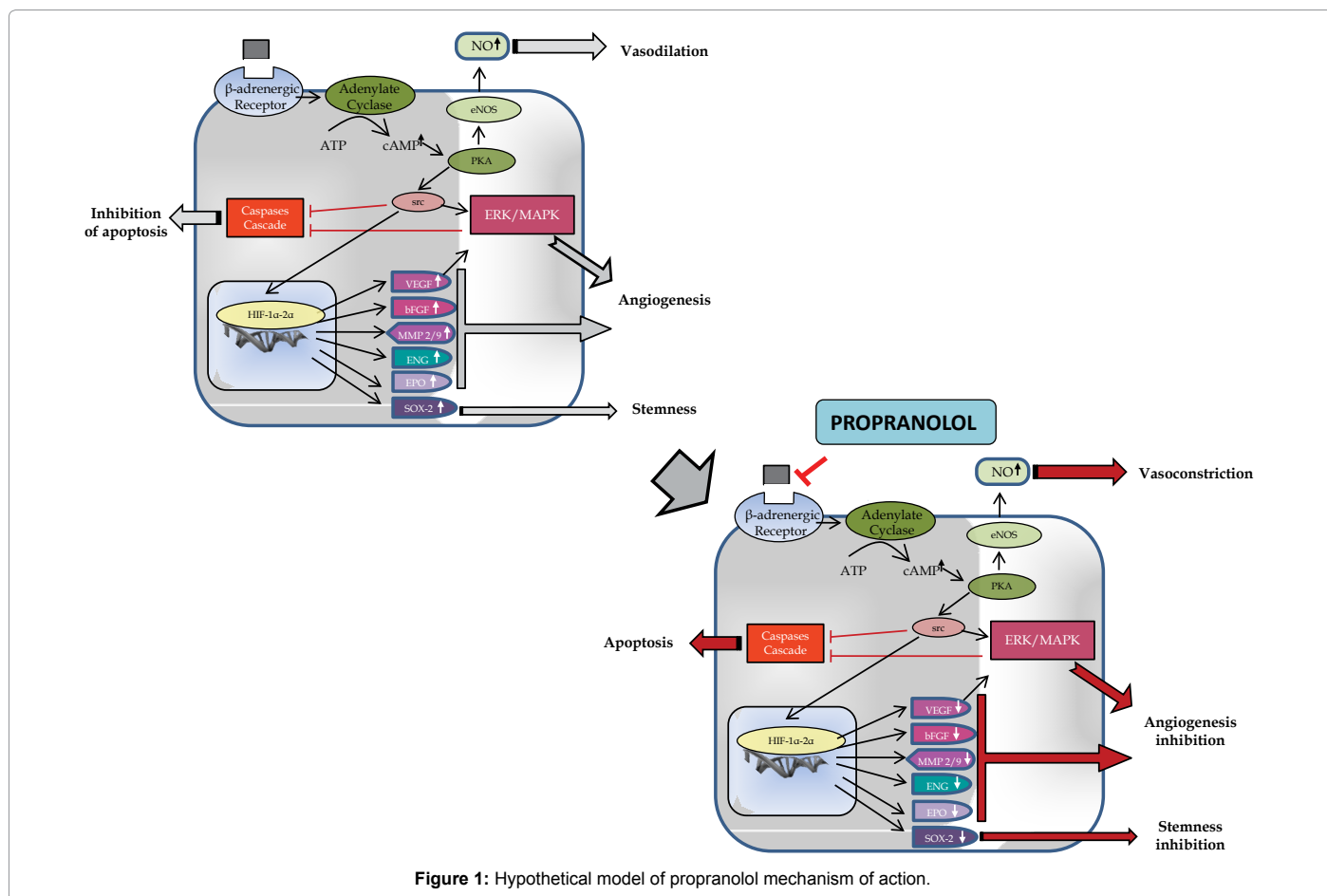


Figure 1: Hypothetical model of propranolol mechanism of action.

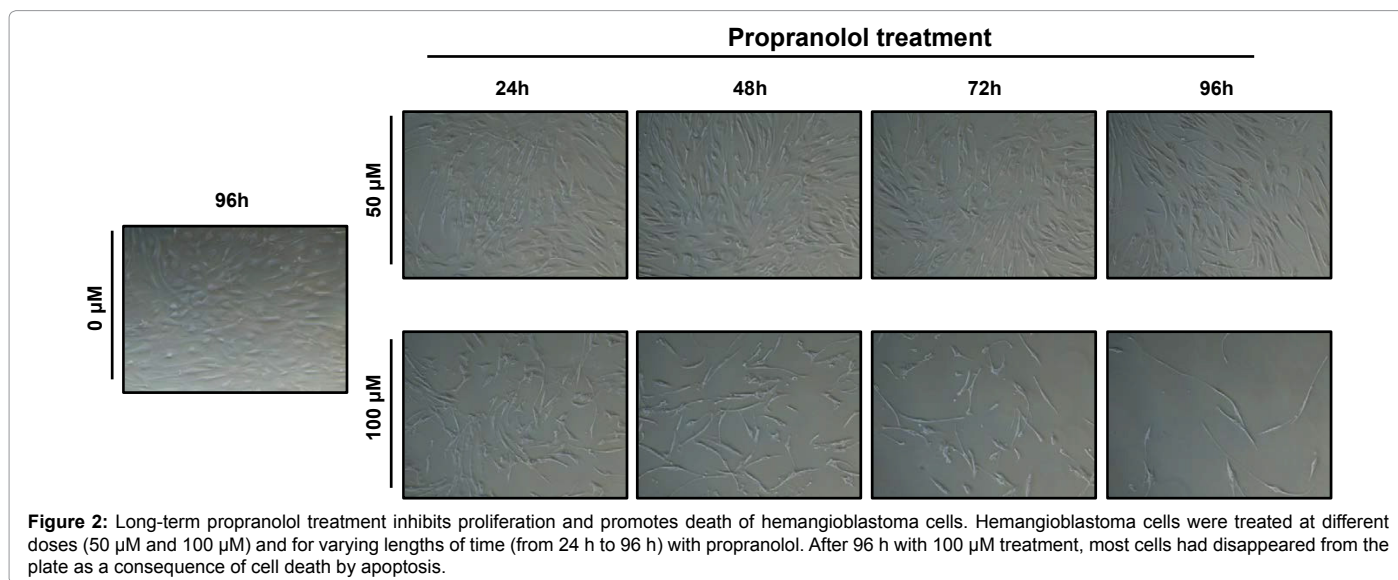


Figure 2: Long-term propranolol treatment inhibits proliferation and promotes death of hemangioblastoma cells. Hemangioblastoma cells were treated at different doses (50 μ M and 100 μ M) and for varying lengths of time (from 24 h to 96 h) with propranolol. After 96 h with 100 μ M treatment, most cells had disappeared from the plate as a consequence of cell death by apoptosis.

Given the results obtained with propranolol, leading to a viability reduction consequence of an increase in death by apoptosis in a short time (24-48 h), a long term propranolol treatment was followed. To this purpose, we started cultures with 50,000 cells per P-6 chamber well, recording images in a time course follow-up. Propranolol treated cells, stopped proliferation, and then cell death induction was apparent from the empty spaces present in the culture plates. After 5 days (96

h) of continuous propranolol treatment, there were fewer than 5,000 cells remaining in the 100 μ M propranolol-treated cultures, compared with 300,000 healthy cells in the untreated cultures. The remaining propranolol-treated cells, on the other hand, exhibited an atypical and apoptotic appearance (Figure 2).

Altogether, our conclusion is that propranolol decreases the viability

of VHL-derived hemangioblastoma cells by stopping proliferation and inducing cell death by apoptosis. This result would be compatible with the regression observed in IH, and it would suggest that propranolol could delay the proliferation of hemangioblastomas in VHL patients.

Our results suggest that propranolol reduces the growth of HIF-dependent tumors, and may be a promising therapeutic drug to delay surgery in VHL patients, especially since propranolol is a drug with a safety profile known for a long time, without serious side effects.

To the best of our knowledge, this is the first study treating hemangioblastoma derived cells with propranolol in vitro. Previous therapies in VHL, have used known antiangiogenic drugs, obtaining limited response concomitant with many adverse side effects. To test the effectiveness of this drug in clinical practice are necessary clinical trials with VHL patients.

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