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Hematopoietic Progenitor Cells From Patients with Chronic Mountain Sickness Lack the JAK2V617F Mutation, Show Hypersensitivity to Erythropoietin and Are Inhibited by Statins.

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Abstract

Background
Chronic Mountain Sickness (CMS) is a clinical entity that occurs in native or long-life residents above 2500 meters of altitude. The disease is characterized by massive erythrocytosis, hyperviscosity syndrome (headache, dyspnea and cyanosis) severe hypoxemia and cardiopulmonary symptoms. The etiology is unknown and no association has been found with Erythropoietin (EPO), Epo receptor (EpoR), Hypoxia Inducible Factor 1a (HIF-1a), von Hippel Lindau (VHL), as well as PHD1, PHD2, PHD3 or PTEN genes. Therapy relies on phlebotomy and oxygen support. Acetazolamide, Medroxyprogesterone and Enalapril have also been tested, but their use has not been largely implemented. Since HMG-CoA inhibitors such as farnesyltransferase inhibitors (Larghero, Blood 2005) may inhibit the in vitro autonomous erythropoiesis of polycythemia vera patients, we studied in CMS the therapeutic potential of statins that have similar pharmacologic activity.

Patients and Methods
Normal controls (NC, n= 10) and patients were native Bolivians from the city of La Paz, Bolivia (3600–4000 mt altitude). The diagnosis of CMS (n=15) was made according to the consensus statement on this disease (Leon-Velarde, 2005). The diagnosis of Polycythemia Vera (PV, n= 5) or secondary erythrocytosis (SE, n= 10) was done according to WHO criteria or established clinical guidelines. Serum Erythropoietin (sEpo) was assessed by chemiluminescent assay. Burst forming units-erythroid (BFU-E) assay was performed by plating 10^5 BM mononuclear cells in methylcellulose with or without recombinant human rhEpo (2IU/ml) and Simvastatin (20 mM). Evaluation of apoptosis by Annexin V/7-AAD and $JAK2^{V617F}$ mutational analyses were performed as described (Guerini et al, Leukemia 2008).

Results
CMS patients (median age 48 years, range 29–58) had median values of hemoglobin and hematocrit (Hb 20.3 gr/dl, range: 19.1–22 and Hct, 62%) significantly higher than observed in NC (Hb 16.2 gr/dl, range 14.8–16.5 and Htc 52%), respectively ($p< 0.001$) and significantly lower than SE patients (Hb: 22.8 gr/dl, range 20.2–25 and Htc: 71%), ($p<0.001$). Hb and Hct were not different in CMS and PV patients ($p= 0.875$). In CMS sEpo values (median 22 mIU/mL, range: 16.1–45.1) were significantly higher

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compared to NC (median 10.7 mIU/mL, range 7–18.8) ($p<0.001$) and lower compared to SE patients (median 82.9 mIU/mL, range 44.8–135) ($p<0.001$); as expected, PV patients showed very low sEpo levels (median 3 mIU/ml, range 2.5–5.2). The $JAK2^{V617F}$ mutation analysis proved negative in all NC, CMS and SE patients and positive in PV. In the absence of exogenous rhEpo, a median of 0, 10, 0, 45 BFU-E colonies were obtained from NC, CMS, SE and PV patients. When rhEpo was added, 21, 40, 47 and 130 BFU-E were counted, respectively; this difference was significant when comparing NC and PV to CMS ($p<0.001$; $p< 0.001$ respectively), but not in the case of SE vs. CMS ($p= 0.227$). Interestingly, in PV and CMS patients, BFU-E colonies remain remarkably viable between day 14–21 while viability declined rapidly in NC and SE colonies after day 14. The prolonged viability and higher sensitivity to rhEpo of BFU-E obtained from CMS and PV erythroid progenitors was also confirmed by plating BM mononuclear cells with suboptimal doses of rhEpo (0.03 to 1 IU/ml). Moreover, when simvastatin (20 μM) was added in vitro to rhEpo driven BFU-E colonies, it induced a median inhibition of 29% in NC as compared to 37, 56 and 44 in CMS, SE and PV ($p <0.013$: $p<0.001$; $p<0.001$, respectively). Finally, 11 CMS patients who had a concomitant hypercholesterolemia (median cholesterol level 238 mg/dl, range 206–310) had the opportunity to be treated with statins (atorvastatin, 20–40 mg/day). Before starting treatment with atorvastatin, all patients, who had median Hb and Htc values of 19.9 gr/dl and 63 % respectively, performed phlebotomy. After a median follow up of 18 months with atorvastatin, the median Hb and Htc values were 17.1 gr/dl and 54.6 %, respectively. The need of phlebotomy was apparently reduced, from 4–6 sessions/year to 1.

Conclusions

Our results underline that a) hematopoietic progenitor cells from CMS patients may promote an autonomous erythroid colony growth and show hypersensitivity to hrEpo b) statins may induce in vitro a significant inhibition of this accelerated erythropoiesis so that they could play a therapeutic role in the treatment of this and other chronic myeloproliferative disorders.

Disclosures: No relevant conflicts of interest to declare.