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Artículo original

Efecto del trasplante autólogo de células madre hematopoyéticas en la velocidad de conducción nerviosa en pacientes con diabetes mellitus tipo 2

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RESUMEN

Objetivo: evaluar el efecto del trasplante autólogo de células madre hematopoyéticas en la neuropatía diabética de pacientes con diabetes mellitus tipo 2.
Material y método: se reclutaron pacientes ambulatorios con diabetes tipo 2 y neuropatía diabética definida según los criterios del consenso de San Antonio, sin otras comorbilidades activas. A los pacientes se les administró Neupogen Roche vía subcutánea por cuatro días, y posteriormente se asignaron al azar a un grupo que recibió tratamiento (se les administraron células madre hematopoyéticas autólogas por vía intramuscular en los gastrocnemios) y un grupo placebo (se les administro plasma autólogo por vía intramuscular en la misma zona). Los pacientes se evaluaron al inicio y a los tres meses con velocidad de conducción nerviosa de las extremidades inferiores. El éxito del trasplante se definió como mejoría en la velocidad de conducción nerviosa.
Resultados: se incluyeron 20 pacientes, 15 en el grupo tratado y 5 en el grupo placebo. Ambos grupos tuvieron mejoría en la velocidad de conducción nerviosa del peroneo izquierdo, independientemente del número de células CD34+ infundidas. Las diferencias entre ambos grupos no fueron estadísticamente significativas.
Conclusiones: aunque encontramos una mejoría en la velocidad de conducción nerviosa con el trasplante autólogo de células madre hematoprogenitoras, creemos que deben realizarse más estudios para determinar el efecto del trasplante en la neuropatía diabética.
Palabras clave: diabetes mellitus tipo 2, neuropatía diabética, velocidad de conducción nerviosa, trasplante de células autólogas hematopoyéticas

ABSTRACT

Background: To evaluate the effect of autologous hematopoietic CD34+ cell transplantation (HCT) on diabetic neuropathy in patients with type 2 diabetes mellitus.
Materials and Methods: We recruited ambulatory patients with type 2 diabetes and diabetic neuropathy as defined by the consensus of San Antonio without other active comorbidities. After s.c. filgrastim administration for 4 days, patients were randomized to a treatment group (intervened with autologous intramuscular HCT on the gastrocnemius region) or to a placebo group (intervened with autologous intramuscular plasma on the same region). Patients were evaluated by blinded personnel at the beginning and 3 months later by nerve conduction velocities (NCV) of lower extremities. Successful outcomes were defined as an improvement in NCV.
Results: Twenty patients were included, 15 in the treatment group and 5 in the placebo group. Both groups had successful outcomes in the left peroneal NCV, independent of the number of CD34+ cells transplanted. The differences between groups were not statistically significant.
Conclusions: Although we found improvement in NCV with autologous HCT, we propose further studies to determine the effect of transplantation on diabetic neuropathy.
Key words: Type 2 diabetes mellitus, diabetic neuropathy, nerve conduction velocity, autologous hematopoietic stem cell transplantation.

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Type 2 diabetes is one of the main causes of mortality in our country, constituting a great economical burden for the health system[1,2] and an important factor for the development of physical and social disabilities. One of these is lower limb amputation as a consequence of peripheral vascular disease, sensorimotor peripheral diabetic neuropathy or both. In fact, type 2 diabetes is the principal cause of lower limb amputation in our country.[3]

Several pathophysiological theories have been proposed for the development of diabetic chronic complications,[4] including deficiencies of trophic or vasodilator factors, accumulation of toxic metabolic byproducts, anomalous activation of intracellular pathways, or even loss in the ability of stem cells to differentiate and/or incorporate into physiological repairing processes.[5] In this sense, the use of stem cells (of totipotential, hematopoietic or endothelial lineages) has been reported in animal and human models of limb ischemia[6,7,8] as an alternative method for inducing neoangiogenesis, with outstanding outcomes. Nevertheless, there are no reports in the use of stem cells for the treatment of human sensorimotor peripheral diabetic neuropathy. Therefore, we performed this study to evaluate the effect of autologous hematopoietic CD34+ cell transplantation (HCT) on nerve conduction velocity (NCV) in patients with type 2 diabetes with diabetic neuropathy.

SUBJECTS

We recruited ambulatory patients with type 2 diabetes and diabetic neuropathy based on the definition of the consensus of San Antonio. Exclusion criteria were patients older than 75 years; hypercoagulable states; left ventricular ejection fraction <30%; cardiovascular disease; neoplastic disease; active infection; diabetic ketoacidosis or hyperosmolar hyperglycemic state; or gangrene of any extremity requiring immediate surgery. Patients were drawn out of the study if they failed to follow-up.

MATERIALS AND METHODS

Eligible patients had an initial determination of a lipid profile and $GHbA_{1c}$ determination. They were invited to have electrophysiologic testing of the lower extremities (Nicolet Biomedical, Madison, USA) which evaluated peroneal nerve conduction from the fibular head to the ankle, and posterior tibial nerve conduction from the popliteal fossa to the ankle, with a single, non-recurrent stimulation, lasting 0.2 miliseconds and with a mean intensity of 256 V for peroneal nerve and 273 V for tibial nerve.

Patients were randomly assigned to a treatment group (group 1) or a control group (group 2). All patients received s.c. filgrastim (granulocyte-colony stimulating factor, Neupogen®, Roche, Bogota, Colombia) 5 mg/kg a day for a period of 4 days. Group 1 patients had a subsequent harvesting of 100 ml of bone marrow from the sacral region with a Jamshidi needle under sedation. The product was immediately processed in a refrigerated centrifuge SIGMA 3K15 (SIGMA Laborzentrifugen GmBH, Osterode am Harz, Germany) at 3500 rev/min for 15 min at 8°C with pentastarch 6% to obtain a mononuclear cell layer that was later diluted in 50 ml of 5% albumin. In order to know how many CD 34+ cells were obtained, 0.5 ml of the product was diluted in CD45FITC (fluorescein isothiocyanate)/ CD34PE (phycoerythrin)/7ADD (7-amino actinomycin D) and analyzed in a BD FACScalibur™ cytofluorometer (Becton Dickinson, California, USA). We established a cut-off level of $2x10^6$ CD34+ cells as adequate for transplantation. CD34+ cells were delivered in a sterile 50 ml Falcon tube to the operating room. Group 1 randomly received CD34+ cells by 30-50 intramuscular injections (0.5-1 mL each) of the solution in the gastrocnemius region of either limb, applied with a 25-gauge needle with a depth of 1 cm. Group 2 randomly received 20 ml of autologous plasma without CD34+ cells by the same method in the same region of either limb.

Patients in both groups were followed weekly for a period of 12 weeks for adverse effects (pain, infection) as well as for the adjustment or inclusion of medications (oral hypoglycemic agents, insulin, statins) to achieve metabolic control at the end of the study, defined as $GHbA_{1c}$ level <7%, total cholesterol less than 5.22 mmol/L, LDL cholesterol less than 2.62 mmol/L, and HDL cholesterol higher than 1 mmol/L for men and 1.16 mmol/L for women. Antibiotics and analgesics were started if considered necessary. At the end of the study, there were final determinations of NCV of the lower extremities, lipid profile and $GHbA_{1c}$ determination where applicable. The main outcome of the study was the "development" of new nerve fibers as evaluated by NCV, defining a successful outcome as an improvement in NCV.

All subjects gave informed consent and we obtained approval of our institutional Ethics Committee. Comparison between paired variables were treated with the Wilcoxon signed rank test. Numerical changes (deltas) of the paired variables were correlated with each other using Spearman´s rho. We used SPSS for Windows 10.0 as the statistical program. A *p* value less than 0.05 was considered significant. Results are expressed in standard error of the mean (SEM) unless otherwise stated.

RESULTS

Twenty patients were included, 15 patients in group 1 and 5 patients in group 2 (see table 1). One patient of the group 1 was lost to follow up. There were more men than women in both groups. Though patients in group 1 were older and patients in group 2 had longer standing diabetes, the difference was not statistically significant.

Metabolic control. There were no statistical differences in $GHbA_{1c}$, total cholesterol, HDL cholesterol and LDL cholesterol between groups both at the beginning or end of the study (see table 2). By our definition, with appropriate medications, 4 out of 11 patients of group 1 and 2 out of 5 patients of group 2 achieved $GHbA_{1c} < 7\%$; 7 out of 11 patients of group 1 and all patients of group 2 achieved total cholesterol less than 5.22 mmol/L; 5 out of 11 patients of group 1 and 3 out of 5 patients of group 2 achieved LDL cholesterol less than 2.62 mmol/L; and 2 out of 6 men and 4 out of 5 women of group 1 achieved the targeted HDL cholesterol, as well as 1 out of 3 men and 2 out of 4 women of group 2.

Paired changes in the metabolic parameters were only significant for $GHbA_{1c}$ levels in group 1 (p=0.03).

Changes in nerve conduction velocities and stem cell transplantation. The mean CD34+ cell count was $11.27\pm6.49 \times 10^6$/kg. Changes in NCV in both groups are described in table 3. There were no statistical differences between both groups neither at the beginning nor at the end of the study, nor paired changes were significant. Changes in NCV were independent of the intervened leg. By our criteria, an improvement in NCV was seen in both groups for the left peroneal NCV, and in group 2 for the right tibial NCV.

The number of CD 34+ cells negatively correlated with the changes in NCV for all 4 determinations, but only the one with the right peroneal NCV was significant (r=-0.85, p=0.01). There were no other significant correlations between variables.

During the study, the sole side effect reported by patients in group 1 was transient lower limb edema, particularly in the site of injections.

DISCUSSION

This was a randomized, controlled trial that described the effect of the transplantation of CD34+ cells (and other non-characterized mononuclear bone marrow cells) on NCV in type 2 diabetes patients. We demonstrated that with HCT there were patients who showed improvement in NCV in both groups, which leads us to speculate about the necessary role of harvesting and reinjecting autologous CD34+ cells into the patients for obtaining such a result. Maybe the sole use of filgrastim could mobilize stem cells into the circulation, that, by stimuli not described so far, could exert beneficial effects in injured tissues. Maybe the sole stimulation with filgrastim and/or the inflammatory stimuli provoked by the repetitive intramuscular injections could induce a beneficial microenvironment in group 2. Data supporting this hypothesis is the fact that there was no correlation between the intervened leg and the changes observed in NCV.

Even when both groups achieved similar levels of $GHbA_{1c}$ at the end of the study (the group 2 showing a greater change in metabolic parameters than group 1), the improvement in metabolic control did not yield a better result in NCV. It would be interesting to note if there is an effect on metabolic parameters after CD34+ transplantation without external intervention.

This is the first report of sensorimotor peripheral diabetic neuropathy in humans treated with HCT. NCV was chosen as a surrogate marker of sensorimotor peripheral diabetic neuropathy since it is a supportive objective, non-invasive, reliable and recommended test.[4] It is important to mention that, so far, the only resources that can "improve" NCV are aldose reductase inhibitors and pancreatic transplantation.[4] In this study, some patients did show improvement in the NCV, but we found an apparent deleterious effect on the change in NCV by increasing number of CD34+ cells. The mechanism of change is unknown so far. A previous report by Hasegawa et al[9] showed improvement in NCV in streptozotocin-induced diabetic rats after HCT at 4 weeks of observation. These effects were blunted with the administration of anti-vascular en-

dothelial growth factor neutralizing antibodies, and were independent of neoangiogenesis.

A disadvantage of the study was the small number of patients involved. This small number, added to the wide distribution of the values in the groups, cannot permit the generalization of results. Also, depending on which side you look at it, the treatment of some patients was targeted towards achieving the best metabolic control that could be obtained. This could potentially blur the effect of the transplantation on metabolic parameters, but we felt that given the vascular nature of the patients involved, it would be unethical to leave the patients without any further adjustment.

Other disadvantage was the short duration of the study. We chose a three-month model as an empirical period of time, since, as described above, in animal models changes in NCV were demonstrated in a shorter period of time. Maybe in humans the regeneration or new formation of nerve fibers takes a longer period of time. Also, we do not know if we are applying the right kind of cell. Speaking of diabetic neuropathy, maybe the utilization of neural stem cells (CD133+ CD34- CD45-)[10] could provide better results. Also, it would be interesting to determine the presence of specific neuronal markers (NeuN, nestin)[11] in injured tissues after HCT.

Our results, we conclude, further stimulate prospective studies looking at the effect of HCT on diabetic neuropathy.

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