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A New Drug for the Treatment of Diabetic Microcirculation: New Formulation of PGE1 α-Cyclodextrin and L. Propionil-Carnitine in Liposomes Covered with Polylysine

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Abstract

The Prostaglandin E_1 and L. Propionil-carnitine are used today for the vascular disease treatment of various origin. The Prostaglandin E_1 injected intravenously has a half-life is very short because metabolized predominantly by pulmonary filter and therefore does not arrive in sufficient amount in the capillaries. You have to remember that hyperglycemia impairs endothelial cells especially at the peripheral level, kidney, retina, skin microcirculation and muscle. The association PGE_1 and L. Propionil-carnitine, transported by liposomes high adhesiveness not being metabolized in the lungs, is able to perform its function pharmacological on endothelial cells damaged by various diseases, such as diabetes or in TAO, especially in the capillaries where blood flow is slowed down. We intend to study the pharmacological response of our new liposomal formulation of PGE_1 more L. Propionil-carnitine on peripheral microcirculation of diabetic patients presenting pathologies. Purchase new Formulation PGE_1 e L. Propionil-carnitine produced by a GMP firm.

Keywords: *PGE_1; Diabetes; liposomal; TAO/Burger's Disease; Microcirculation*

Introduction

Diabetes is a chronic disease characterized by the presence of high levels of blood glucose (hyperglycemia) and due to an altered amount or insulin function. Insulin is the hormone produced by the pancreas, which allows the glucose entry into cells and its subsequent use as an energy source. When this mechanism is altered, the glucose accumulates in the bloodstream. There are various types: Type 1 diabetes accounts for about 10% of people with diabetes and usually occurs in childhood or adolescence. For this reason, the Type 1 diabetes is classified among the diseases so-called "autoimmune", that is due to an immune reaction directed against the body itself. Type 2 diabetes is the most common form of diabetes and represents about 90% of cases of this disease. The cause of Type 2 diabetes is unknown but characteristic is the presence in the blood of antibodies directed against antigens present at the level of insulin-producing cells, said ICA, GAD, IA-2, IA-2SS. The cause is still unknown, although it is certain that the pancreas is able to produce insulin, but the cells of the organism cannot then in use. Typically, the disease occurs after 30-40 years and many risk factors have been recognized associate to its onset. These include: the family history of diabetes, lack of exercise, being overweight and belonging to certain ethnic groups.

Type 2 diabetes usually goes undetected for many years because the hyperglycemia develops gradually and is initially not severe enough to give the classic symptoms of diabetes. Usually the diagnosis is done randomly or in conjunction with a situation of physical stress, such as infection or surgery. The risk of developing the disease increases with age, with the presence of obesity and lack of physical activity: this observation allows to predict prevention strategies "primary" that interventions can prevent the onset of the disease and that have their cornerstone in the application of a proper lifestyle, including nutritional and exercise. Gestational diabetes: Gestational diabetes is defined every situation in which a high level of measuring circulating glucose for the first time during pregnancy. This condition occurs in about 4% of pregnancies. The definition is independent of the type of treatment used, it's just the dietary or the need for

insulin and implies a higher frequency of checks for pregnant and the fetus. The symptoms of the onset of the disease depend on the type of diabetes. In the case of Type 1 diabetes usually are witnessing an acute onset, often in connection with a febrile episode, with thirst polydipsia), increased amounts of urine (polyuria), is feeling fatigue (asthenia), weight loss, dry skin, increased frequency of infections.

In Type 2 diabetes, however, the symptoms are more nuanced and usually do not allow a quick diagnosis, so often your blood sugar is high but without clinical signs of Type 1 diabetes. Diabetes can cause acute or chronic complications. Acute complications are more common in Type 1 diabetes and are related to the almost total lack of insulin. In these cases, the patient may experience ketoacidosis coma, due to accumulation of products of altered metabolism, ketones which cause loss of consciousness, dehydration and serious blood disorders. In Type 2 diabetes acute complications are rare, but are very frequent chronic complications that affect different organs and tissues, including the eyes, kidneys, heart, blood vessels and peripheral nerves. The complications of diabetes is Type 1 and 2 are:

1. Diabetic retinopathy: is damage to the small blood vessels that supply the retina with loss of the sight. In addition, people with diabetes are more likely to develop eye diseases such as glaucoma and cataracts.
2. Diabetic nephropathy: it is a gradual reduction of the filter function of the kidney that, if left untreated, can lead to renal insufficiency up to the need for dialysis and/or kidney transplant.
3. Cardiovascular disease: the risk of cardiovascular disease is 2 to 4 times higher in people with diabetes than in the general population resulting in industrialized countries, more than 50% of deaths from diabetes. This leads us to consider the cardiovascular risk in diabetic patients equal to that given to a patient who had a cardiovascular event
4. Diabetic neuropathy: is one of the most frequent complications and according to the World Health Organization is manifested at different levels in 50% of diabetics. It can cause loss of feeling, pain of varying intensity and limb injuries, requiring amputation in severe cases. Can lead to malfunctions of the heart, eyes, stomach and is one of the main causes of male impotence.
5. Diabetic foot: the modifications of the structure of blood vessels and nerves can cause ulceration and problems in the lower limbs, especially the foot, due to loads that. This may necessitate the amputation of limbs and statistically is the leading cause of lower limb amputation for non-traumatic
6. Complications in pregnancy: pregnant women, diabetes can result in adverse consequences on the fetus, congenital malformations with a high birth weight, up to a high risk of perinatal mortality. The high frequency of vascular complications requiring close monitoring of target organs (eyes, kidneys and lower limbs). For this reason, it is necessary for people with diabetes to undergo regular check-ups, even in the absence of symptoms.

Microcirculation refers to the blood circulation in the capillaries and agree that the diameter of these vessels go from 40 to 5 micra. At the beginning of the capillaries from the arterial side there are muscular structures, called pericytes, which regulate the flow while in most peripheral level the capillaries and from the venous side are constituted only by endothelial cells. Microangiopathy is a disease that affects the microcirculation and this being different from organ to organ there are several types of microangiopathy. The diabetes affects the endothelial cells throughout the body causing different diseases.

Microangiopathy is a disease in diabetic patients and it is installed over time and therefore can be considered a chronic disease. At the level of the microcirculation hyperglycemia causes:

1. Increased apoptosis of endothelial cells;
2. The decreased ability of reproduction of endothelial cells;
3. The increase of endothelin which reduces the diameter of the capillaries;
4. Slow blood flow in the capillaries;

The consequence of the above phenomena is primarily tissue oxygenation decrease. The theoretical basis for the use of prostaglandins in Critical Limb Ischemia (CLI) are well known [1]. The Prostaglandin E_1 and Prostacyclin expand and improve the local flow. The prostaglandin E_1 has an angiogenic and anti-inflammatory effect and improves endothelial function. The PGE_1 stimulates endothelial cells VEGF receptors and facilitates the reproduction to maintain sufficient capillarization in the tissue. The problem existing is that Prostaglandin E_1 injected intravenously has a half-life very short (less 1 min.) because metabolised predominantly by pulmonary filter and

therefore does not arrive in sufficient amount in the capillaries, and the amount injected in increased there are side effects (tachycardia, hypotension, etc.). In fact, the therapeutic administration of PGE_1 causes a vasodilatation but also a marked hypotension. Both PGE_1 that Iloprost, Prostacyclin analogue, have been evaluated in several clinical trials in patients with CLI. The results of all these studies have failed to demonstrate the true clinical utility of the treatment of arterial disease with prostanoids. Treatment with PGE_1 has been approved by the Min of Health, as already mentioned, has never had much success. For that reason have been proposed Prostacyclin analogs that have a half-life greater (Iloprost). But also this search and was performed on patients with systolic pressures at the level of the tibial lower than 50 mmHg and not in patients with microangiopathy and gave results somewhat uncertain [2].

In 1991, our group experienced the treatment of microangiopathy of different origin, IIb and stage III sec. Fontaine (diabetic, from connective tissue, and in Buerger's disease) with a continuous Iloprost infusion, via elastomeric pump, for a period of 28 days. The results of these studies we did observe that it was possible to obtain a marked improvement of symptoms due to microangiopathy. Infusion therapy with prostanoids was used both before and after surgical revascularization [3]. The goal of therapy was to reduce the phenomena closely related to microangiopathy.

In fact therapy with Iloprost or with PGE_1 causes vasodilation, reduces platelet aggregation and restores the functions of endothelial tissue, but is not able to increase the flow in the presence of stenosis or obstruction of macrocirculation. The PGE_1 has, as has already been said, a short half-life, but that compared to Iloprost, has the advantage of better reactivate the system functionality endothelial, acting on the VEGF receptor, and at the same time causes a marked vasodilation and if injected directly into the artery in the districts with decreased blood flow causes a marked peripheral vasodilation. But this practice cannot be used for a routine treatment. If injected intravenously its short half-life, lower per minute limits its therapeutic action. By some researchers has been observed that the therapeutic effect improves when PGE_1 is associated together with the l-propionyl-carnitine in the treatment of microangiopathies of various origin and in Buerger's disease to stage II or III. To determine a pharmacological action on the microcirculation, damaged by diabetic pathology, it has been proposed to carry the PGE_1 from liposomes that cross the pulmonary filter and have the characteristic of adhering to endothelial cells, especially in sites where there is a microangiopathy and blood flow slows down, and for a low blood pressure and because the endothelial cells are damaged by diabetes [4].

The new formulation was performed at the Department of Chemistry and pharmaceutical technology direct from Prof. Fadda. (See attachment # 1 chemical and physical exam). The liposomes of phosphatidylcholine have the ability to overcome the filter lung to adhere to the endothelial cells of the capillaries species in districts where the blood flow is slowed down and release the drug directly at the level of the peripheral microcirculation.

In the experiments performed in diabetic rats with streptozocin we observed that the administration of liposomes coated with polylysine containing PGE_1 more L. Propionil-carnitine determined compared to untreated rats:

1. Decrease endothelial apoptosis in the kidneys in the lungs in the muscle
2. Increase of VEGF
3. Lower water consumption, and better renal function

These observations lead us to propose the use of PGE_1 α-cyclodextrin and L. Propionil carnitine transported by liposomes of phosphatidylcholine coated with polylysine in clinical use for the treatment of diabetic foot and microangiopathy in general.

Objectives

The objective of the study is to demonstrate that administration of PGE_1 together with L. Propionil-carnitine transported by liposomes is able to determine the healing or at least a net improvement of microangiopathies induced by diabetes. In particular, we wish to observe whether it is possible to obtain a result pharmacological stable over time.

Material and Methods

Because diabetic microangiopathy are chronic diseases is essential to observe the results of treatment during treatment and after at least 15 to 30 days from the end to record the results obtained. In fact, the PGE_1 acts by stimulating the endothelial cells and therefore has to be considered that the positive response is observed during administration but that is only if the skin oxygenation and blood flow in the capillaries increases and remains stable it can be stated that it has obtained a positive outcome pharmacological [5].

For this reason, patients will undergo a thorough general and specialized peripheral circulation with Echocolor Doppler, Digital Plethysmography, Capillaroscopy, evaluation of skin oxygenation in four points of the toes and hands, and retinal circulation with fluorescein angiography and it is likely, as well as already established by our personal clinical experience, which should be a period of 15-20 days after initiation of therapy, to observe the positive clinical results. The experiences of all of the various researchers who have infused PGE_1 indicate that you need at least a month to get a positive clinical outcome.

Even pharmaceutical companies that sell PGE_1 α Ciclodestrina recommend treatments administered intravenously for 30 days at doses of 60 μg/day. It is proposed a treatment with total dosages that contain doses of PGE_1 7 (seven) times lower and dosages of L. Propionil-carnitine 50 times lower [6].

Selected treatments, drug information, preclinical and clinical trials: activity, toxicity, pharmacokinetics.

We intend to study the pharmacological action of PGE_1 is associated with the L. Propionil-carnitine transported by liposomes in microangiopathies present in patients suffering from diabetes Type 1 and 2.

Preclinical experiences

1. Performed on cell cultures have shown that it was possible to get yourself a normalization of the morphology of the cells damaged by high doses of glucose in the medium (see "experiment *in vivo*" and Annex # 1Fase1 report cell cultures).
2. Research done on diabetic rats, infusion of PGE_1 more L. Propionil-carnitine transported by liposomes has shown positive effects on endothelial cell apoptosis and tissue histology (see Annex No. 2 Fase1 report diabetic rats).
3. Research on toxicity in rats has shown that there are no toxic effects (see Annex No. 3 Fase1 Report diabetic rats).

Clinical experiences made of patients who did not respond to any medical treatment, carried out by Prof. Brotzu at the Nursing Home S. Antonio, Via Chironi 3 Cagliari in 2012. They used the same doses and the same methods of administration that are followed in the testing officer [7].

Patient's selection

Specialist examination, evaluation and acceptance to participate in the trial:

1. Routine blood-chemistry including, CBC, urine examination, Blood uric acid, creatinine, total cholesterol, HDL, LDL, Triglycerides, PT, PTT, D-dimer, fibrinogen, serum electrophoresis, C-reactive protein.
2. Distance traveled so far running on a treadmill;
3. Echocolor Doppler femoral artery, Popliteal and Tibial front and rear. Plethysmography, pulse Oximetry and capillary skin of the limbs;
4. Study with 3D laser and photographic documentation of any ulcer;
5. Fluoroangiography, computerized measurement of retinal circulation;
6. Study of Diabetic Neuropathy. Sensitivity to vibrations, Monofilament Semmes-Weinstein;

Five infusions of PGE_1 + L. Propionil carnitine- carried by liposomes on phosphatidylcholine.

Infusion: 0.72 mg/kg of PGE_1 + 10 mg/kg of L. Propionil-carnitine transported by 20 mg. of liposomes of phosphatidylcholine, with elastomeric pump in 48 hours.

N°PRAT	Name	sex age	Pathology	Ulcer	Other Path	Stage	n°Inf.	Results
281	S.G	m 68	d2			II	2	++
282	C.F.	m 63	Art, O.	15 cmq	obesity	IIIb	2	++
279	S.A.	m 86	d2	20 cmq	finger necrosis	IVa	3	+++
229	A.A.	m 70	d2			II	1	++
366	M.G.	m 86	Art. O.			III	2	++
1308	S.V.	m 84	d2	3 cmq		IIIa	2	+++
367	C.G.	m 46	d1	25 cmq	finger necrosis	Iva	2	+
368	P.G.	m 77	d2	3cmq		IVa	2	++
618	F.A.	m 75	d2			II	1	++
719	C.P.	m 69	d2	5cmq	finger necrosis	Ivb	2	+
3374	F.A.	m 37	TAO			III	1	+++
823	S.B.	m 74	d2		neuropathy	II	1	+
717	M.D.	m 92	d2	ulcere	neuropathy	IVb	2	++
718	M.B.	f 87	d2			IIIb	2	+++
3771	C.F.	m 74	d2		neuropathy	III	1	++
824	F.L.	f 20	d1	7 cmq		IIIb	2	+++
1244	S.G.	m 64	d2		neuropathy	II	1	++
3624	M.P.P.	m 56	d2			II	1	+++
1249	M.M.R.	f 74	Art. Ob.			III	2	++
1640	B.A.	m 66	Art. Obl			III	2	+
1637	R.E.	m 64	Art.o.			III	2	+++
1915	A.A.	m 81	d2			II	1	++
2052	S.G.	f 64	Art.O.			II	1	++
1912	T.N.	m 85	d 2			III	2	++
1913	M.S.	f 69	d2		neuropathy	III	2	++
2051	C.V.	m 91	d2			IVa	1	++
2049	P.A.	m 83	d2			III	1	++
3599	G.G.	m 68	d2			II	1	++
3470	C.L.	m 91	d2	15 cmq	neuropathy	IVb	3	+++
3930	G.E.	m 78	d2	8 cmq	neuropathy	IVb	2	++
3053	L.S.	f 35	d1		neuropathy	II	1	+++

Table 1: *Below are the results on patients treated in 2012.*

It must perform an infusion weekly for five weeks. During the infusion performing both Plethysmography and oxygenation skin of the feet are recorded. At the end of the five infusions are repeated all the clinical examinations previously performed. After 15-30 days a new examination of patients and all tests are repeated and subsequent evaluation of results. The dosage of PGE_1 is equal to one-seventh of that which is recommended by pharmaceutical companies for the treatment of vascular pathologies like. Plan to run the search in 6 months [8].

Randomization criteria for inclusion and exclusion

They include patients with diabetes Type 1 and 2 diabetes sent by the various centers of the city of ....., which present, reducing the distance of travel, neuropathy, ulcers and possibly decrease in serum creatinine and diabetic retinitis.

Duration and population, inclusion and exclusion criteria

Diabetic patients 1 and 2 with angiopathy in type II and stage III, according to Fontaine, who follow a proper control of blood sugar. Patients with TAO we have ceased to smoke. Excluding patients who do not control the glycemic asset [9].

Selection and withdrawal of subjects

Patients who do not wish to follow the treatment plan prepared can withdraw at any time

Results and Conclusions

Primary and secondary endpoints

Increased capacity of travel, reducing neuropathy, reduction or healing of ulcers.

Measures of effectiveness

Increase oxygenation to the skin extremities.

Security

Monitoring of ECG and blood pressure during the infusion of the drug.

Concomitant medications allowed

Therapy for glycemic control. Therapy for the treatment of heart rhythm disorders and any problems in urination.

Statistical aspects

It will assess the changes in the parameters. Increase distance running, transcutaneous oxygen, change in ulcer surface in cm^2, speed of blood flow in the capillaries of the limbs.

Follow up procedures

Patients will be followed during the infusion and then 15-30 days after the end of treatment to assess the validity of therapy.

Definition of treatment failure and associated procedures

No modification of the march distance, and increased oxygenation device.

Procedures for reporting and management of serious adverse events and deaths

We will report any: tachycardia, hypotension and changes in diuresis.

Withdrawal of the study, interruption mode and subsequent follow-up

In the event that you do not follow tachycardia and hypotension, it will suspend the treatments and patients will be followed for 30 days.

Sampling and instrumental investigations details

They will keep records in all the results of Haematological examinations, Echocolor Doppler, skin oxygenation, and photographic documentation.

Subsidies

You can check if in diabetic patients, who have problems of neurological pathology of the central nervous system, there is an improvement after the Circle therapeutic.

Control procedures and quality

The drug used will be manufactured by a pharmaceutical company authorized GMP characteristics. Committees and structures involved in the conduct Hospital Ethics Committee.

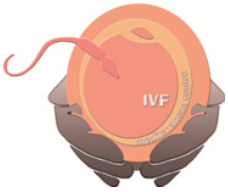
Check the progress of the study

Every five patients treated with the new formulation will proceed to a first check of the results.

Liability of the investigator

The responsibility is generic because it is a drug treatment with drugs already tested and in use and which are known complications. Moreover, the treatment involves the use of an amount of drug substantially lower than that recommended in the instructions. Due to the low dose of PGE_1 injected is impossible that suffering complications of any kind.

IVF Mediterranean Centre



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