Preliminary Findings about Effectiveness of Aminapthone Therapy in Diabetic Microangiopathy

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Abstract:
Objective: microangiopathic complications of Diabetes (Retinopathy, Nephropathy and Neuropathy) are an invalidant and social clinical problems in Diabetics. Aim of this study has been to verify if a treatment with an “endothelial-protector drug” like aminapthone, added to standard therapy, could be useful in controlling and delaying diabetic complications evaluated by nail fold periungueal videocapillaroscopy, retinal fluoroangiography and OCT (Ocular Coherence Tomography)

Materials and Methods: Database from 30 patients arrived to Department of Cardiology of Acireale hospital (Catania) affected by IDDM (Insulin-Dependent Diabetes Mellitus-20 patients) and NIDDM (Non Insulin Dependent Diabetes Mellitus-10 patients) have been collected in this retrospective observational clinical trial lasting from January 2012 to April 2015. All the patients were Diabetics from, at least, 10 years and, at enrollment time, presented microangiopathic alterations in nail fold periungueal videocapillaroscopy, OCT and retinal fluoroangiography. All the thirty patients have been treated with aminapthone (75 mg cps b.i.d) for the first three months followed by almost other three months with 75 mg cps/day. Nail fold periungueal videocapillaroscopy, OCT and retinal fluoroangiography have been collected after 1, 3 and 6 months from enrollment time.

Results: All the patients shown an improvement in videocapillaroscopic examination together with ophthalmoscopic examination immediately after 1 month of treatment with aminapthone and this improvement was more evident after 3 and 6 months of treatment

Conclusion: Aminapthone’s therapy for 6 months has shown to control microangiopathic micro vessels modifications induced by Diabetes in nail fold periungueal videocapillaroscopy, in retinal fluoroangiography and in OCT. It could represent a useful new supportive treatment, together with standard treatment and diet, to postpone Diabetes clinical complications.

Keywords: Aminapthone; Diabetic Microangiopathy; Endothelin 1; E-Selectin; Nail fold Periungueal Videocapillaroscopy; Retinal Fluoroangiography

Abbreviations:
OCT: Optical Coherence Densitometry

Introduction:
Diabetic microangiopathy is characterized as a disorder of small vessels [1]. Its prevalence has rising tendency even in children population and is positively associated with duration of diabetes mellitus [1]. According to Diabetes Control and Complication Trial (DCCT Research Group 1993) important risk factors for microvascular complications are cigarette smoking and genetic susceptibility to hypertension at early stages of diabetes and poorer glycemic control, higher blood pressure and unfavorable lipid profile at later stage [1]. The small vessels (microcirculation comprises arterioles, capillaries, venules and lymphatics, all <100 mm in diameter) are crucial for maintaining tissue metabolism and structural and functional changes in the microcirculation are present in diabetes mellitus irrespective of the organ studied (retinal, kidney, CNS and skin) [2]. The pathophysiology of diabetic microangiopathy is
complex because it involves not only metabolic but also genetic factors [2]. For example, it has been shown that subjects with diabetes heredity have impaired microvascular responses to both endothelium and not endothelium-dependent stimuli in the skin microcirculation in spite of normal body dimension, normal glucose tolerance and normal insulin sensitivity [3]. Early on in the course of the disease, microvascular perfusion is increased in many organs under resting conditions [4,5] and a cutaneous microvascular over perfusion occurs in the limbs, but most of the blood flow under normal thermal conditions passes through arteriovenous shunts, bypassing the nutritive capillary bed and leading the “so-called capillary ischemia” [4,6,7].

Endothelial dysfunction, characterized by an imbalance between endothelium-derived vasodilator and vasoconstrictor substances, plays an important role in the pathogenesis of vascular complications in diabetes, including microangiopathy [2]. Almost two different steps seem to be involved in the microcirculation imbalance: “leukocyte recruitment cascade” and “Endothelin-1 over expression” [2,8-10].

The recruitment of leukocytes from circulating blood into tissues is crucial for the inflammatory response: during this process a number of well-studied adhesion molecules on the endothelium sequentially interact with their ligands expressed on the cell surface of leukocytes [8]. The interaction between adhesion molecules and ligands occurs in a cascade-like fashion, driving leukocytes from the circulation to the extravascular space, that is, through the steps of leukocyte rolling, firm adhesion and transmigration (Figure 1).

**Figure 1:** Leukocyte Recruitment to the Vessel Wall

The selectin family of adhesion molecules mediates the capture and rolling steps of leukocytes along the endothelial cells [8]. The selectin consists of three members of C-type lectins (P, E and L-selectin) [8]. After the selectins have initiated leukocyte rolling along the surface of endothelium, a different sets of adhesion molecules comes into play to reduce the leukocyte rolling velocity and allow to leukocyte to firmly adhere to the endothelial surface [8]. This firm adhesion step is largely mediated by molecules of immunoglobulin superfamily such as intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) expressed by endothelial cells and by those expressed constitutively by leukocytes or by many other types of cells. Upon achievement of stable adherence to the endothelial surface, the leukocytes extravasate between endothelial cells along the intercellular junctions. PECAM-1 (Platelet Endothelial Cell Adhesion Molecule) and VAP (Vascular Adhesion Protein) mediates leukocytes transmigration [8]. Various lines of evidence indicate that the shedding of selectins is enhanced on the endothelium during the progression of diabetes and that the soluble form of selectin proteins has the potential to be a clinically useful biomarker of the severity of Diabetic Retinopathy [8]: E-Selectin, in particular, may also serve as a proangiogenic factor [8].

Once that leukocytes have transmigrated from endothelial junctions an hyper production of ET-1 (Endothelin 1) have been released by the endothelium. ET-1 is one of the most potent vasoconstrictor described and has been suggested to be involved in the development of cardiovascular disease [2]. It possess pro-inflammatory and profibrotic effects [2]. Enhanced of endogenous ET-1 has been demonstrated in hypertension, coronary artery disease and heart failure [10-14]. In diabetic microangiopathy one important feature of endothelial dysfunction is an increased in production and biological activity of the vasoactive and proinflammatory peptide ET-1 [2]. Elevated levels of ET-1 are found in patients with type 2 diabetes [15-17]. Furthermore ET-1 may contribute to the development of endothelial dysfunction, and consequently insulin resistance,
by increasing the production of Reactive Oxigen species, mainly superoxide anion, in the vasculature [18-20].

A large body of evidence from animal models and human studies indicates that ET-1 is casually involved in the pathogenesis of diabetic microangiopathy also if the potential of ET-1 blockers in the treatment of diabetic microangiopathy has not been investigated in human studies [2].

Targeting the ET-1 system might be of importance in the treatment of complications related to diabetic microangiopathy [2]. Taking into account the role of endothelial adhesion molecules (specifically E-Selectin) and ET-1 in the pathogenesis of diabetic microangiopathy and that mostly of the diabetic complications such as retinopathy, nephropathy and neuropathy have their basis in disturbed microvascular function we added to standard therapy an “endothelial protector drug”, such as aminapthone (2-hydroxy-3-methyl-1,4-naphthohydroquinone-2-p-aminobenzoate).

Aminapthone is a synthetic molecules derived from four amino benzoic acids which is currently employed for “capillary disorders” and for “chronic venous insufficiency” [21,22].

This “endothelial protector drug” has demonstrated the ability to downregulates ET-1 production in ECV 304 cells by interfering with transcription of pre-pro ET-1 (PPET-1) gene expression [23]. At the same time, cytofluorimetry has shown that aminapthone significantly reduce the expression of E-Selectin (endothelial-leukocyte adhesion molecules 1: ELAM 1) both in resting and in IL-1 beta activated ECV 304 cells in a dose dependent manner [24]. In vivo, in patients affected by systemic sclerosis, 12 weeks of aminapthone treatment has demonstrated the ability to down regulate sELAM-1 (soluble E-Selectin Adhesion Molecules) and sVCAM (soluble Vascular Cell Adhesion Molecules) [25]. Otherwise aminapthone treatment for 48 weeks in patients affected by Rajnaud Phenomenon secondary to Systemic Sclerosis has recently proved to significantly improved Rajnaud Phenomenon when added to standard therapy [26]. In a rat model of monocrotaline-induced pulmonary hypertension the administration of aminapthone (30mg/Kg/day or 150 mg/Kg/day) significantly lowered rat mortality and plasma ET-1 concentration [27].

Recently in vitro and in vivo studies shown that aminapthone significantly prevent excessive Endothelin 1 release in sclerotherapy [28]. Some in vitro direct anti-inflammatory effects on endothelial cells has been demonstrated by aminapthone application [29]. Furthermore, aminaphtone therapy has recently shown to be useful for proteinuria reduction in patient with diabetes type I [30].

Considering that micro vascular complications (rthritis, Nephropathy, Neuropathy) are very frequent in Diabetics and that one of the most common causes of blindness in people over 50 age is Diabetic Retinopathy, and considering too our previous experience, we usually added to standard Diabetes therapy aminapthone treatment. The data collection allows us to better understand if this “adjuvant” therapy could delay the onset of microvascular complications.

**Material and Methods:**
This is a retrospective, case controlled study performed in Diabetic patients afferring to Department of Cardiology of Acireale Hospital in Catania from January 2012 to April 2015. All patients are under glycemic and lipidemic control before and during the trial and also the hypertensive profile was under control before and during the study.

Between all the case reports collected for microangiopathy in our Department we have selected 30 homogeneous cases affected by IDDM lasting from more than 5 years (20 patients - 6 women and 14 men – mean age 51.05 years) and by NIDDM (10 patients – 6 women and 4 men – mean age 72.2 years) with documented microangiopathic alterations (nail fold periangueal videocapillaroscopy, retinal fluorangiography and OCT evaluation). Nail fold periangueal videocapillaroscopy has shown to be a non-invasive diagnostic and prognostic method to control micro vascular abnormalities related to diabetic microangiopathy [31,32] and ophthalmoscopic examination as fluorangiography and Optical Coherence Tomography have demonstrated to be useful in control diabetic retinal microvascular abnormalities progression [33-39].

We usually used these procedures to follow-up microangiopathic diabetic complications and, according to our standard procedures; we repeated these observations after 1, 3 and 6 months. So that the observations have been done on existing reports by a single rater blinded at different time-points (1 month vs 3 vs 6).

According to our previous experience [30] we usually added aminapthone to standard therapy in all diabetic patients with the following schedule: 2 cps/day for 90 days followed by 1 cps/day for other 90 days. All the thirty patients collected have been treated according to the aforementioned treatment protocol for 180 days and collected almost 3 different evaluations during the trial.
(basal, after 30, 90 and 180 days). All the patients case records were assessed at the beginning of the trial and after 1, 3, and 6 months for nail fold videocapillaroscopy examination using a videocapillaroscope: the optical microscope was connected to a digital camera and computer. Inclusion criteria was the presence of pathological changes (morphological and functional) of the nail fold videocapillaroscopy like hemorrhages, edema and morphological abnormalities of the microvascular architecture. Since there are not a validate international score for bleeding and edema in videocapillaroscopy for diabetic microangiopathy we use the following score: For bleeding we use the score “number of the fingers with bleeding” and for edema the score “absence of the micro vessels visibility due to edema”.

Clinically edema of the hands has been evaluated for every time: micro vessel visibility at videocapillaroscopy clearly moved according to reduction of edema of the hands. The same patients have been reevaluated after 30, 90 and 180 days of treatment with aminaphthone for videocapillaroscopic evaluation: an improvement of the hemorrhages, perivasal edema and abnormalities of the microvascular architecture has been classified like “treatment success”.

All the diabetic patients enrolled in the trial have also ophthalmoscopic evaluation (fluorescein angiography and Optical Coherence Tomography) at time 0 (basal) and after 180 days (end of the trial). OCT is a non invasive method that is easily tolerated by patient which is used in diagnosing and assessing various macular pathologies [37-39]. Also in this case inclusion criteria were retinal microaneurisms, intra-retinal hemorrhages and retinal hard exudates. Every diabetic patient was reevaluated, from ophthalmoscopic point of view, at the end of the trial (180 days). Adverse effects to the test drug have also been collected.

Results:
Nail fold periungueal videocapillaroscopy shown an early and significative improvement immediately at first control visit (30 days) in all the treated patient in terms of hemorrhages (number of fingers with bleeding 6.56 ± 1.93 at T0; vs 3.96 ± 0.8 at T30: p<0.0001 T-Student Test) and oedema (absence of micro vessel visibility due to oedema 21 pts at T0 vs 12 pts at T30: p<0.05 T-Student Test) (Figure 2-Figure 9).

Figure 2: Time 0 - Nail fold periungueal videocapillaroscopy (Pictures are Representative of All Patients)

Figure 3: Time 30 - Nail fold Periungueal Videocapillaroscopy (Pictures are Representative of All Patients)
The same and more evident results have been obtained after 90 days (number of fingers with bleeding T90 = 2.12 ± 0.9: p<0.0001 vs T0 and absence of micro vessel visibility due to the oedema 10, 5 patients: p<0.05 vs T0 – T-Student Test).

**Figure 4:** Time 90 - Nail fold Periungueal Videocapillaroscopy (Pictures are Representative of All Patients)

And at the end of the trial (180 days): (number of fingers with bleeding T180 = 1.13± 0.9: p<0.0001 vs T0 and absence of micro vessel visibility T180 = 6 pts: p<0.05 vs T0 – T-Student Test).

**Figure 5:** Time 180 - Nail fold Periungueal Videocapillaroscopy (Pictures are Representative of All Patients)

Taking into account bleeding score and oedema we clearly shown an improvement in bleeding and in micro vessel visibility due to the oedema at a very time versus T0. Clinically edema of the hands improves during the treatment time.

Also for opthalmoscopic control (fluorangiography and OCT) every patient shown an evident improvement at the end of the trial in terms of retinal micro aneurisms, hard-hexudates, edema and intra-retinal hemorrhages:
No differences have been shown between NIDDM and IDDM patients in terms of results for videocapillaroscopy, OCT and fluorangiography evaluation.

**Discussion:**

Micro vessels instrumental evaluation must be indicated during microangiopathy and, particularly in the Diabetic patient, it must be considered a first stage approach with the aim to prevent microangiopathic complications (Retinopathy; Neuropathy and Nephropathy). This the first retrospective, observational uncontrolled study performed with Aminapthone in Diabetic patients with the aim to understand if aminapthone adjunctive therapy to the standard of care could be useful in reducing the micro vessels damages related to Diabetes.

Instrumental examination (Nail fold periungueal videocapillaroscopy, Retinal Fluor angiography and OCT) performed during 180 days of aminapthone treatment clearly demonstrates the efficacy of the treatment drug administered according to a “consolidate schedule” (75 mg bid for the first 3 months plus 75 mg/day for the second...
three months) in reducing oedema, hemorrhages and micro aneurisms in the nail fold as in the retina. This is not surprising considering the direct aminaphthone efficacy on micro vessels according to its pharmacodinamic profile and its endothelial pharmacological effects [23-29] and according to preview results in Diabetic Retinopathy [40-42].

Probably, the efficacy of aminaphthone against recruitment of leukocytes from circulating blood into tissues, that is crucial for inflammatory response, together with the ability of the drug to counteract the ET-1 over expression in micro vessels endothelial dysfunction, could improve micro vessels damage due to diabetes delay the onset of diabetic microangiopathy complications. Also the recent published case report on a diabetic patient affected by microalbuminuria [30] seems to encourage the use of the drug supportive therapy added to standard therapy to control microangiopathic complications progression in Diabetic patients.

This observational study has a lot of methodological limitations since it was not a prospective, controlled double blind clinical trial. Also the disomogeneous (two different types of patients, IDDM and NIDDM) and the little sample size (30 patients) could represent a study limitation.

Nevertheless the instrumental data collected for every single patient clearly demonstrated an improvement in nail fold and retinal micro vessel at every time (30, 90 and 180 days – p<0.0001 for bleeding and p<0.05 for oedema for any time vs T0 – T-Student’s Test) when compared with the basal observation and this results could represent an interesting preliminary finding to plan other prospective, controlled clinical trial to support this hypothesis.

To the best of our knowledge this is the first “observational study” in which “an endothelial protector drug”, like aminaphthone, shown to be useful in controlling the progression of diabetic microangiopathy complications confirming the hypothesis that “act directly on micro vessels” reversing “micro vessel flogosis cascade” could be an appealing and new pharmacological approach for diabetic patients.

Conclusions:

Aminaphthone, an “endothelial protector drug” has been used for 6 months in diabetic patients as “supportive therapy” added to or care: surprising after 30, 90 and 180 days of treatment all the micro vessels instrumentals evaluation (nail fold videocapillaroscopy, retinal fluoroangiography and OCT) have significantly improved in terms of oedema, hemorrhages and morphological abnormalities of the micro vessels architecture. Since diabetic microangiopathy is the main cause of diabetic complication like Retinopathy, Nephropathy and Neuropathy and since Diabetic Retinopathy represents the main cause of blindness in developed countries the observation of a “visible” improvement in retinal fluoroangiography and in OCT obtained adding aminaphthone therapy to standard of care in IDDM and NIDDM patients could represents a new pharmacological approach for diabetic patients management.

Obviously these are only preliminary results belonging to a retrospective, observational, not randomized uncontrolled study and a well-designed prospective, controlled, double blind clinical trial are needed to support our “preliminary findings”.

References:


