Effect of celiprolol on prevention of cardiovascular events in vascular Ehlers-Danlos syndrome: a prospective randomised, open, blinded-endpoints trial

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Summary

Background Vascular Ehlers-Danlos syndrome is a rare severe disease that causes arterial dissections and ruptures that can lead to early death. No preventive treatment has yet been validated. Our aim was to assess the ability of celiprolol, a β_1-adrenoceptor antagonist with a β_2-adrenoceptor agonist action, to prevent arterial dissections and ruptures in vascular Ehlers-Danlos syndrome.

Methods Our study was a multicentre, randomised, open trial with blinded assessment of clinical events in eight centres in France and one in Belgium. Patients with clinical vascular Ehlers-Danlos syndrome were randomly assigned to 5 years of treatment with celiprolol or to no treatment. Randomisation was done from a centralised, previously established list of sealed envelopes with stratification by patients' age (≤32 years or >32 years). 33 patients were positive for mutation of collagen 3A1 (*COL3A1*). Celiprolol was uptitrated every 6 months by steps of 100 mg to a maximum of 400 mg twice daily. The primary endpoints were arterial events (rupture or dissection, fatal or not). This study is registered with ClinicalTrials.gov, number NCT00190411.

Findings 53 patients were randomly assigned to celiprolol (25 patients) or control groups (28). Mean duration of follow-up was 47 (SD 5) months, with the trial stopped early for treatment benefit. The primary endpoints were reached by five (20%) in the celiprolol group and by 14 (50%) controls (hazard ratio [HR] 0·36; 95% CI 0·15–0·88; p=0·040). Adverse events were severe fatigue in one patient after starting 100 mg celiprolol and mild fatigue in two patients related to dose uptitration.

Interpretation We suggest that celiprolol might be the treatment of choice for physicians aiming to prevent major complications in patients with vascular Ehlers-Danlos syndrome. Whether patients with similar clinical presentations and no mutation are also protected remains to be established.

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Introduction

Ehlers-Danlos syndrome consists of a heterogeneous group of inherited connective tissue disorders. The vascular type is the most severe because of its complications: vascular dissection or rupture and hollow organ (uterus, intestine) rupture, which are both caused by fragility of connective tissue.[1] Median survival is 40–50 years, with the first complication usually seen by age 20 years; 90% of patients have a major event before age 40 years.[1] Until now, no treatment has been proven to prevent clinical events.

The disease results from heterozygous mutations in the *COL3A1* gene, causing structural defects in the pro1(III) chain of collagen type III, which are characterised by decreased thermal stability, reduced secretion, and abnormal proteolytic processing.[2] The vascular type is transmitted as an autosomal dominant trait. Clinically, it is characterised by four major and nine minor diagnostic criteria.[3] The combination of any two major diagnostic criteria has a high specificity, but further biochemical testing and mutational analysis of the *COL3A1* gene is recommended to formally confirm the diagnosis.

We previously identified that patients with vascular Ehlers-Danlos syndrome have decreased intima-media thickness that is associated with increased mechanical stress applied to excessively fragile tissues.[4] This arterial phenotype can explain the risk of arterial dissection or rupture and led us to propose treatment by celiprolol, a cardioselective β blocker with β_2 agonist vasodilatory properties,[5,6] Celiprolol was reported to reduce heart rate, mean, and pulsatile pressures in essential hypertension,[7] and could therefore decrease the continuous and pulsatile mechanical stress on collagen fibres within the arterial wall. We postulated that celiprolol could decrease vascular complications such as dissection or rupture in such patients. We aimed to assess the preventive effect of celiprolol for major cardiovascular events in patients with vascular Ehlers-Danlos syndrome.

Methods

Participants

Our study, the Beta-Blockers in Ehlers-Danlos Syndrome Treatment (BBEST) study, used a multicentre, prospective,

randomised, open trial with blinded evaluation of clinical events (PROBE) design.

Participants were enrolled at eight centres in France and one in Belgium (Ghent). Enrolment and exclusion criteria are summarised in the panel. Inclusion criteria were adapted from Villefranche diagnostic criteria,[3] consisting of three major and ten minor criteria. Patients aged from 15 years and 2 months to 65 years presenting with either one major criterion and two minor criteria or four minor criteria were enrolled. If the patients presented with only minor criteria, their inclusion was confirmed only after the decision of a member of the steering committee and with the mandatory opinion of a geneticist.

Patients with vascular Ehlers-Danlos syndrome were ineligible for the BBEST study if they were pregnant, were a woman with childbearing potential on inadequate contraception, had previous treatment with a β blocker for vascular complications, or had contraindications related to celiprolol. Patients in the two last categories benefited from the same follow-up as the BBEST study (cohort group). This study was approved by the ethics committee of Saint-Germain en Laye, France. Written informed consents were obtained from all patients.

Randomisation

Randomisation and arterial investigations were done in the department of pharmacology, Georges Pompidou European Hospital, France, or in the department of genetics, Ghent University Hospital, Belgium. If all criteria were fulfilled, randomisation was done from a centralised, previously established list of sealed envelopes with stratification by patients' age (≤32 years or >32 years). Envelopes were prepared by the sponsor (Assistance Publique, Hôpitaux de Paris), independent of the investigators, and were opened in numerical order.

Study design

Patients were randomly assigned to a 5-year intervention, receiving either celiprolol or no treatment. Celiprolol was uptitrated by 100 mg steps every 6 months to a maximum of 400 mg. Recommended treatment was as twice daily. In case of excessive response or intolerance (fatigue, heart rate lower than 55 beats per minute, or systolic blood pressure lower than 100 mm Hg with symptoms) uptitration was postponed or the drug was downtitrated. Patients were asked not to stop treatment without medical advice. They could taper their dose by steps of 100 mg in case of excessive fatigue. Patients randomly assigned to no treatment received the same attention as those assigned to celiprolol. β blockers were not used in this group. If there was an indication of slow heart rate or decreased blood pressure, then diltiazem or verapamil were given.

Haemodynamic measurements were done in a dedicated, air conditioned room. Blood pressure was measured with a Colin oscillometric device (Press-Mate 8800, Omron, Rosny, France). Central blood pressure was measured with a Sphygmocor device (Atcor medical, Sydney, Australia). The right common carotid artery was measured with a high-precision echotracking device (Wall Track System, Esaote PIE Medical, Maastricht, Netherlands), as previously described.[4]

51 patients received genetic testing before or after their inclusion, but testing was not compulsory. After consent was obtained, search for mutations in *COL3A1* was done either from complementary DNA (cDNA) obtained from total RNA extracted from cultured skin fibroblasts (18 patients) or from genomic DNA (gDNA) extracted from peripheral blood cells obtained from EDTA (edetic acid) samples (16 patients). *COL3A1* analysis was done from cDNA with a confirmation from genomic DNA for 17 patients; genetic testing was not done in two patients.

Protocols for *COL3A1* mutation explorations were adapted from those previously described.[8,9] After PCR amplification with ten pairs of primers for the cDNA

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***Panel:* Enrolment criteria**

Inclusion criteria
- Age 15–65 years
- One or more major criteria and two minor criteria OR four minor criteria

Disease features

Major criteria
- Personal or first-degree relative history (parent-child, brother-sister) of arterial rupture or dissection (excluding aneurysm)
- Personal or first-degree relative history (parent-child, brother-sister) of uterine or intestinal rupture
- Previous known mutation of *COL3A1*

Minor criteria
- Facial dysmorphy, thin and translucent skin
- Acrogeria
- Club foot
- Hypermobility of small joints
- Tendon rupture
- Lower limb varicosity
- Arteriovenous fistula
- Pneumothorax
- Gingival recession
- Absence of the inferior lingual frenula

Exclusion criteria for the PROBE design
- Patient already presented an arterial rupture or dissection and treated by β blocker
- Celiprolol is contraindicated
- Pregnancy
- Women with childbearing potential on inadequate contraception

Exclusion criteria for the PROBE design and follow-up cohort
- Refusal to participate in the study
- Inability to move

analysis and 33 pairs of primers when genomic DNA was used, direct sequencing analysis was done with sequencing agents and kits and a 3730 DNA analyser from Applied Biosystems (Carlsbad, CA, USA). DNA sequences assembly and analysis was done with Sequencher software version 4.8. Mutation was confirmed by a second PCR and was reconfirmed on a second independent peripheral blood sample.

The duration of follow-up was 5 years or until the first qualifying event. Patients were asked to visit Georges Pompidou European Hospital or Ghent University Hospital (seven patients) every 6 months during the first 2 years, then every 12 months during the last 3 years. At each visit, a full clinical examination, drug monitoring, event records during standardised questionnaires, and arterial measurements (including cervical ultrasound scans) were obtained. Every 12 months patients had a systematic workup including CT scan and MRI. A standardised telephone interview was done at interim dates.

All clinical events were submitted to the event committee, composed of four experts in different specialties, who were not involved in the patients' care. Event committee members, masked to the adjudicated treatment, assessed clinical complications and classified events as primary or secondary endpoints, or deemed them as not meeting endpoint definitions. Short medical reports summarising the clinical events were masked for any possible identification (eg, date, name, hospital). Any further medical documents were prepared in the same way. The committee met after every four submitted events. In case of discrepancies, a consensus was reached by asking for supplementary documents and by discussion. The primary endpoint was a composite of cardiac or arterial events (rupture or dissection, fatal or not) during follow-up. Secondary endpoints were intestinal or uterine rupture, and all severe clinical events related to vascular Ehlers-Danlos, as adjudicated by the event committee.

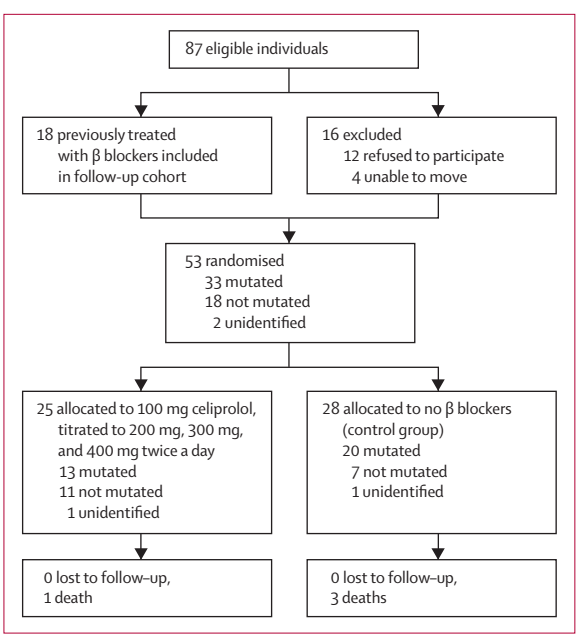

Figure 1: **Trial profile**

Statistical analysis

We postulated that celiprolol could reduce primary endpoints by 60%. Sample size calculation showed that 40 primary events were needed to achieve a power of 80%, α risk of 5%, with a two-sided significance level of 0·05. On the basis of the retrospective analysis of the centres' files, together with interpretation of the clinical and genetic features of the disease,[1] the rate of qualifying events in the controls was estimated to be 20% per year. 50 patients per group were needed to achieve this aim. An independent biostatistician (JSH) planned and did interim analyses after the first eight primary events and subsequently after each four primary events, with the triangular test.[10]

Continuous and discrete variables were compared with Wilcoxon rank tests. Change in haemodynamic variables was assessed through the slope of their change with time (per year) until the end of follow-up. Survival curves were compared with the Kaplan-Meier method and with a two-tailed log-rank test. Treatment interaction with mutation, age, or systolic blood pressure on event-free survival was estimated from Cox proportional hazard model in the whole population. In the mutated population, we split the population according to median systolic blood pressure or age to assess with the Kaplan-Meier method their effect on event rates. Thresholds for prognosis of baseline indices were established with receiver operating curve analysis. The analysis was by intention to treat, on the basis of the whole study population—ie, the 25 patients allocated to 100 mg celiprolol and the 28 patients allocated to no β blockers. Data are expressed as mean (SD). All tests were two-sided and p values lower than 0·05 were deemed significant. All analyses were done with PEST 4·4, NCSS 2007, and SAS 9·1 software. This study is registered with ClinicalTrials.gov, number NCT00190411.

Role of the funding source

Assistance Publique-Hôpitaux de Paris was the sponsor of the study and managed the methods. Aventis Pharmaceutical provided celiprolol unconditionally and had no other involvement in the study. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

87 patients were eligible and enrolled between Oct 2, 2003, and March 28, 2006. Figure 1 summarises the flow of participants through the trial. Patients who had had β blockers for arterial dissection or rupture or another cardiovascular indication were included in the follow-up cohort. In accordance with PROBE study design, patients

were randomly allocated to either celiprolol or no treatment. Table 1 lists patient characteristics. Two young patients (age 15 and 22 years) in a family whose mother had died of aortic dissection (age 38) had only one major and one minor criterion or two minor criteria according to Villefranche diagnostic criteria.[3] Mean age at entry was 35 (SD 12) years. Most patients were women at a ratio of two to one female to male, and important phenotype characteristics were equally balanced between celiprolol and control groups. 29 (55%) patients had previous clinical events (arterial rupture or dissection, uterine or intestinal rupture), and 11 (21%) had a family history of clinical events (eight patients had both, two on the celiprolol and six controls).

33 patients had proven mutations of *COL3A1*, 13 of 25 on celiprolol and 20 of 28 controls (webappendix pp 1–3). Of patients with mutations, demographic and arterial characteristics did not differ from those of the whole study population (webappendix pp 4–5).

Mean follow-up duration was 47 months (SD 15) with a median of 50 months (IQR 36–59). The minimum follow-up was 4 months (in this case the first primary event was death by iliac rupture), and the maximum was 64 months. The shortest follow-up in patients without events was 36 months.

Table 2 details all primary and secondary endpoints. 50 clinical events were submitted to the event committee with 27 verified for primary endpoint, 11 verified for only secondary endpoint, and 12 events did not qualify. In patients with more than one validated event, only the first qualifying primary or secondary event was used for analysis.

The study was ended prematurely after a consensus decision of the safety monitoring board, the methodologist of our institution, and the principal investigator because significant differences were recorded between the two groups in the whole population after 64 months. One important point in the decision was that it was also judged futile to continue the trial because of the small number of patients still free of events in the least favourable group—ie, the group with the largest number of events.

In five (20%) of 25 patients on celiprolol a primary endpoint was recorded, compared with 14 (50%) of 28 controls (hazard ratio [HR] 0·36; 95% CI 0·15–0·88; p=0·040—ie, 64% reduction in risk; figure 2). Combined primary and secondary endpoints affected six (24%) of 25 patients on celiprolol and 17 (61%) of 28 controls (HR 0·31; 0·14–0·71; p=0·010) in favour of celiprolol—ie, 69% reduction in risk (figure 2). The Cox model explaining event-free survival (primary plus secondary endpoints) identified two associated variables: treatment (HR 0·33; 0·13–0·85; p=0·020) and presence of mutation (0·48; 0·19–1·22; p=0·122). Neither age nor systolic blood pressure affected event-free survival (data not shown).

Of the participants who died, causes formally identified in two controls; one (case 1) had an iliac artery rupture and then dissection of the ascending aorta after emergency implantation of an abdominal aortic endoprothesis for bilateral leg ischemia and the other (case 2), a man aged 45 years, died within 1 hour after acute lumbar pain. In the celiprolol group, a 19-year-old man died suddenly after acute chest pain radiating to the right arm. The day before his death, he had practised shot-put at school. Large artery dissection or rupture was the most common primary event (ten of 19 patients) at various sites (cervical arteries in three, iliac arteries in three, arteriovenous fistulae of the carotid artery and cavernous sinus in two, and intestinal arteries in two; table 2). Before inclusion, case 18 (table 2) had an episode of haemoptysis, which led to the diagnosis. Haemoptysis has been reported as a complication of vascular Ehlers-Danlos syndrome.[11] Case 20 (secondary events, table 2) had carotid dissection detected on routine MRI, which

See Online for webappendix

	Celiprolol (n=25)	Control (n=28)
Demographic and haemodynamic		
Age (years)	36 (13)	35 (11)
Sex (male/female)	8/17	10/18
Weight (kg)	55 (9)	61 (14)
Height (cm)	165 (9)	169 (10)
Body-mass index (kg/m²)	20 (2)	21 (4)
Body surface area (m²)	1·60 (0·17)	1·69 (0·22)
Heart rate (beats per min)	70 (13)	71 (11)
Brachial systolic blood pressure (mm Hg)	113 (10)	117 (13)
Brachial diastolic blood pressure (mm Hg)	67 (10)	69 (10)
Diagnostic criteria		
Phenotype		
Facial dysmorphy	17	19
Thin and translucent skin	20	20
Bruising	18	25
Acrogeria	15	21
Club foot	5	5
Hypermobility of small joints	17	23
Tendon rupture	2	6
Lower extremity varicose	6	11
Gingival resection	7	4
Arteriovenous fistula	1	2
Pneumothorax	3	2
Absence of the inferior lingual frenula	4	3
Genotype		
COL3A1 mutation	13 (unidentified in one patent)	20 (unidentified in one patent)
Previous clinical events		
Only personal history of arterial rupture or dissection, uterine or intestinal rupture	14	15
Only first degree relative history of arterial rupture or dissection, uterine or intestinal rupture	6	5
Both personal and first degree relative history of arterial rupture or dissection, uterine or intestinal rupture	2	6

Data are mean (SD), or number.

Table 1: **Demographic, haemodynamic characteristics, and diagnostic criteria of the study population**

	Sex, age (years)	*COL3A1* mutation	Group	End point	Clinical symptoms	Environmental trigger	Admission to hospital
Primary endpoint							
1	Male, 31	Yes	Control	Death or iliac artery rupture	Major	No	Yes
2	Male, 28	Yes	Control	Hypogastric artery rupture	Major	Yes	Yes
3	Female, 51	Yes	Control	Spontaneous cerebral haematoma	Major	No	Yes
4	Female, 38	Yes	Control	Spontaneous haematoma of psoas muscle with blood suffusion	Major	No	Yes
5	Male, 25	Yes	Control	Carotid dissection	Major	No	Yes
6	Male, 28	Yes	Control	Death or aortic dissection	Major	No	Yes
7	Female, 24	Yes	Control	Carotid dissection	Minor	No	Yes
8	Female, 34	Yes	Control	Carotid-cavernous sinus fistula	Major	No	Yes
9	Female, 53	No	Control	Vertebral artery dissection	Minor	No	Yes
10	Female, 31	Yes	Control	Carotid-cavernous sinus fistula	Major	No	Yes
11	Female, 59	No	Control	Superior mesenteric artery dissection	Minor	No	Yes
12	Female, 67	Unknown	Control	Carotid dissection	Minor	No	Yes
13	Female, 42	Yes	Control	Primitive iliac artery dissection	Minor	No	Yes
14	Male, 45	Yes	Control	Sudden death after acute lumbar pain	Major	No	No
15	Female, 40	Unknown	Celiprolol	Intestinal perforation	Major	No	Yes
16	Male, 19	Yes	Celiprolol	Sudden death after acute chest pain	Major	Yes	Yes
17	Female, 41	No	Celiprolol	Iliac artery dissection	Minor	No	Yes
18	Male, 19	Yes	Celiprolol	Haemoptysis	Major	Yes	Yes
19	Female, 36	No	Celiprolol	Anterior tibial artery dissection	Minor	No	Yes
Secondary endpoint							
20	Female, 39	Yes	Control	Asymptomatic carotid dissection	Minor	No	No
21	Female, 27	Yes	Control	Colic perforation	Major	No	Yes
22	Male, 24	Yes	Control	Colic perforation	Major	No	Yes
23	Male, 17	Yes	Celiprolol	Spontaneous tear of pectoral muscle	Major	No	No

***Table 2*: Primary and secondary endpoints, by patient**

was absent 6 months before and was classified as a secondary endpoint because it was only accompanied by mild cervical pain (not leading to admission). For case 15 (unknown mutation status; table 2), intestinal perforation was caused by ischaemia after mesenteric artery dissection and secondary rupture.

Table 3 presents clinical event rates by enrolment and Villefranche diagnostic criteria. Most clinical events were in patients with at least one major and two minor enrolment criteria or two major Villefranche diagnostic criteria.

Figure 3 shows Kaplan-Meier estimates of event-free survival for the 33 patients with *COL3A1* mutations. The primary endpoint was noted in two of the 13 patients on celiprolol compared with 11 of the 20 controls (HR 0·24; 0·08–0·71; p=0·041). Combined primary and secondary endpoints were recorded in three of 13 patients on celiprolol and 14 of 20 controls (HR 0·25; 0·10–0·64; p=0·017 in favour of celiprolol; figure 3). We did additional analyses by splitting the mutated population into two groups by median age (32 years) or baseline systolic blood pressure (114 mm Hg) and undertook Kaplan-Meier analysis in each group (webappendix p 6). For combined primary and secondary endpoints in patients younger than 32 years HR was 0·34 (0·10–1·11, p=0·092) and for patients 32 years or older HR was 0·00 (p=0·040; six events in the controls *vs* none in the celiprolol group aged ≥32 years). For such combined endpoints in patients with baseline systolic blood pressure lower than 114 mm Hg HR was 0·18 (0·04–0·79, p=0·036) and for patients with baseline systolic blood pressure greater than this value, HR was 0·34 (0·10–1·20, p=0·151).

None of the baseline characteristics predicted outcomes in the untreated group except for the presence of *COL3A1* mutation (HR 4·06; 1·47–11·21; p=0·042). This mutation did not predict outcomes in treated patients (HR 1·32; 0·23–7·64; p=0·764). In the treated group, low baseline diastolic pressure (<62 mm Hg) and high pulse pressure (>50 mm Hg) also predicted a poor response to celiprolol. All six patients who had clinical events under treatment had one of these conditions, compared with only five of 19 who were free of events. In patients with the mutation, all three events were in those with low diastolic and high pulse pressures.

Slopes of change with time for variables in the whole population (table 4) show that brachial systolic pressure increased substantially, as did pulse pressure after celiprolol whereas, systolic pressure and pulse pressure fell in controls. Elastic modulus also increased in the celiprolol group compared with controls. Carotid

distensibility decreased in the celiprolol group but increased in controls. Most carotid variables diverged between the two groups (table 4). Changes in haemodynamic variables for the mutated population were similar and even larger than those of the whole study population (webappendix pp 7–8). Blockers of the renin angiotensin system did not change the effect of celiprolol, although no event was indentified in patients treated with both celiprolol and such drugs (webappendix p 9).

No patients were lost to follow-up. One woman discontinued the study after 15 months' follow-up because of unexpected pregnancy. She also had severe fatigue after starting 100 mg celiprolol and decided to stop the drug soon after. Two other patients given celiprolol had mild fatigue that was related to dose uptitration—to 300 mg. No clinical hypotension or bradycardia was reported. One case of leg oedema was reported in a 58-year-old woman on celiprolol together with amlodipine and pravastatin. Her oedema was attributed to amlodipine and not to celiprolol since it lessened when amlodipine was stopped. Treatment compliance, assessed through pill count, was good. Self-reported compliance to treatment was low in two young patients (age 19 and 18 years); however, this poor compliance was not confirmed by pill count.

Discussion

Our trial shows the effective prevention of major complications in patients with vascular Ehlers-Danlos syndrome. Treatment of patients with celiprolol compared with no treatment reduced arterial events, such as rupture or dissection, by three times. Results were nearly identical in patients with and without the *COL3A1* mutation. Thus, celiprolol was effective even after adjustment for genotype. Treatment with celiprolol was well tolerated, and the target dose of 400 mg twice a day was reached in all but two patients and only one had to stop taking celiprolol because of fatigue.

Clinical studies have previously addressed phenotypic characteristics and genetic features of vascular Ehlers-Danlos syndrome but no randomised trial of treatments has been done so far. Patients are often treated empirically with drugs such as β blockers[12] or renin-angiotensin-aldosterone blockers[13] that have protective effects in patients with Marfan's syndrome. However, the pathophysiology of Marfan's syndrome is different from that of vascular Ehlers-Danlos syndrome. Marfan's syndrome is due to a deficiency of fibrillin-1 and abnormal elastin synthesis, altering elastic properties of the aortic wall: decreased distensibility, increased stiffness index, and increased pulse wave velocity in the ascending and abdominal aorta.[14] Vascular Ehlers-Danlos syndrome is characterised by a deficiency of synthesis, secretion, and structure of procollagen type III affecting the entire arterial tree, together with the skin and the intestine. Electronmicroscopy of homozygous *COL3A1* mutant mice[15] showed substantial qualitative and quantitative changes of collagen III and cell-matrix attachments, together with abnormalities in collagen I fibrillogenesis. Abnormalities of the carotid wall in patients consists of decreased intima-media thickness

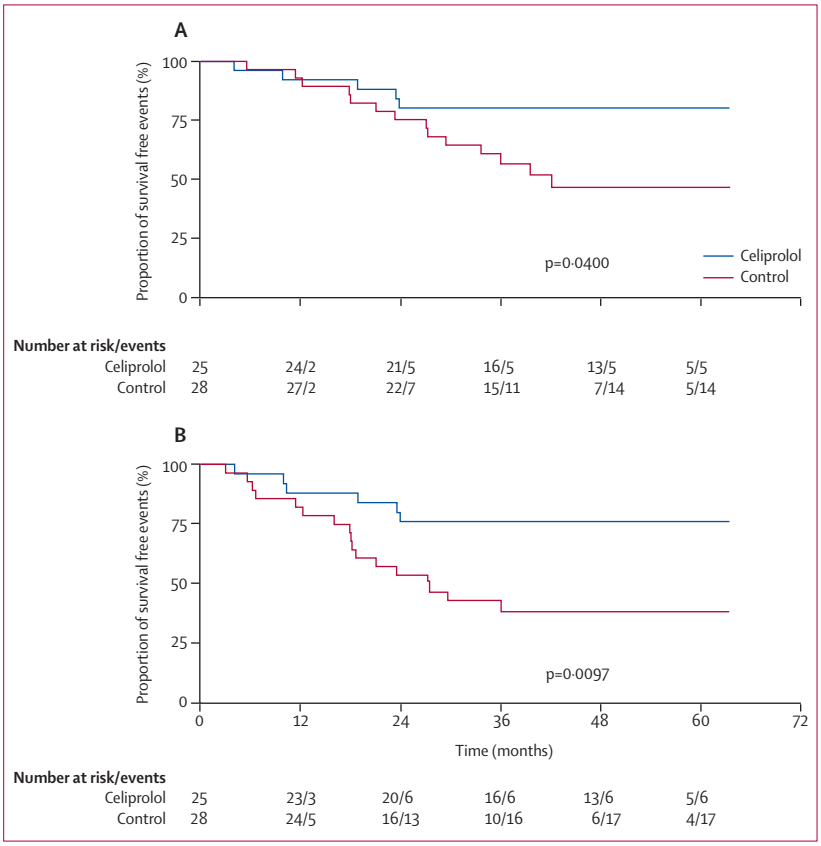

Figure 2: **Kaplan-Meier curves of event-free survival in 53 patients with vascular Ehlers-Danlos**
Primary endpoint (A). Primary and secondary endpoints (B).

	Celiprolol (n=25)		Control (n=28)	
	Yes	No	Yes	No
Enrolment criteria				
Three major criteria	0	0	2	0
Two major criteria	2	9	11	5
One major criterion and at least two minor criteria	3	8	4	4
One major criterion and one minor criterion	0	1	0	1
At least four minor criteria	1	1	0	1
Total	6	19	17	11
Villefranche diagnostic criteria				
Four major criteria	2	6	7	6
Three major criteria	3	6	7	1
Two major criteria	0	5	2	1
One major criterion and at least two minor criteria	1	1	1	2
Only two minor criteria	0	1	0	1
Total	6	19	17	11

Table 3: **Clinical events according to enrolment criteria and Villefranche diagnostic criteria**

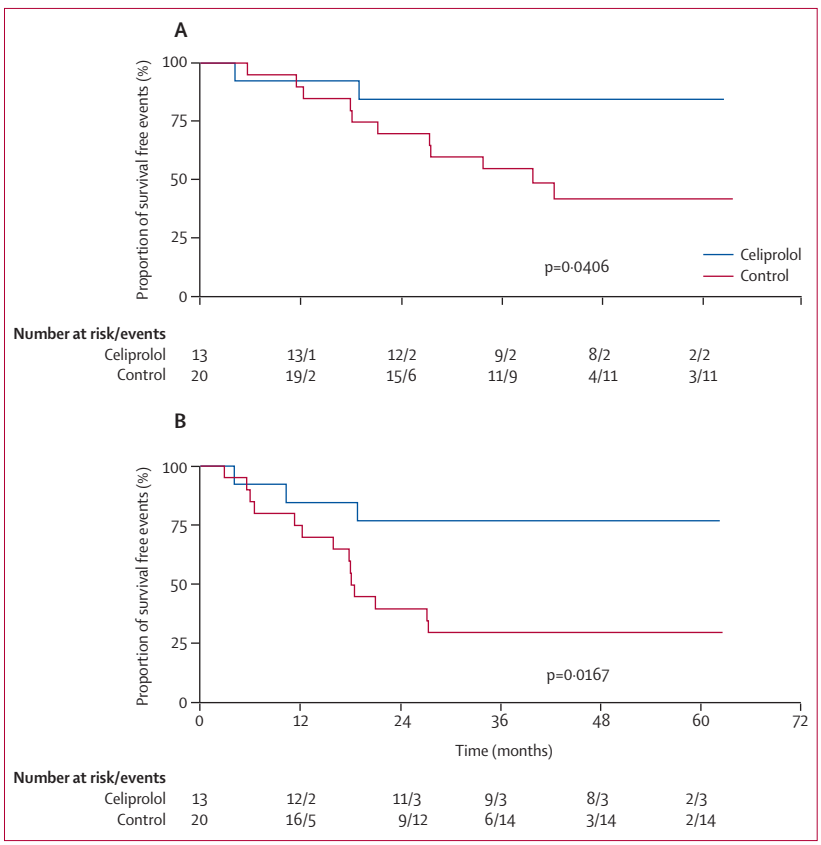

Figure 3: **Kaplan-Meier curves of event-free survival in 33 patients with positive *COL3A1* mutation**
Primary endpoint (A). Primary and secondary endpoints (B).

associated with increased mechanical stress of excessively fragile tissues.[4] These features are not present in patients with Marfan's syndrome in which stiffening and dilatation of the aorta predominate.[14,16]

Our results have relevance in the context of recent advances on transforming growth factor β (TGFβ) signalling pathway in diseases such as Marfan's and Loeys-Dietz syndrome.[17–20] In Marfan's syndrome, an increased bioavailability of TGFβ in response to the defect in its chelation by abnormal fibrillin has been proposed as a key factor in the pathogenesis of arterial lesions. Treatment with losartan, a direct angiotensin AT_1-receptor antagonist and indirect inhibitor of TGFβ, or with perindopril, an ACE inhibitor, prevents arterial complications in rodents[17] and in human beings.[19,21] In Loeys-Dietz syndrome, the mechanism by which mutations in the TGFβ receptors cause the multisystem effects is complex and poorly understood.[18] The signalling pathway downstream of the TGFβ receptor was paradoxically enhanced. Thus, activation of TGFβ is generally accepted as a key factor in the pathogenesis of arterial lesions in Marfan's and Loeys-Dietz syndromes.[18,19]

However, the causal relation between activation of TGFβ and arterial lesions might be more complex. Indeed, TGFβ is also an important growth factor in wound healing. TGFβ1 and 2 are necessary for collagen synthesis and TGFβ3 for organisation of scar tissue.[22] Local delivery of TGFβ1 has also being associated with stabilisation of experimental aortic aneurysms in rats.[23] Gohel and colleagues[24] showed that healing of skin ulcers was related to increased concentrations of TGFβ1. Recent evidence[25] confirmed that TGFβ inhibition is not always accompanied by vascular protection because mice exposed to TGFβ antibodies and angiotensin II infusion develop fatal aortic aneurysms.

Whether TGFβ has a key role in the pathogenesis of arterial lesions in vascular Ehlers-Danlos syndrome is not clear. A report[26] of raised TGFβ concentration in patients with Ehlers-Danlos syndrome does not show it to be pathogenic, since raised TGFβ concentration might indicate only a physiological response of the TGFβ pathway to impaired collagen synthesis, and be the consequence of repeated skin or arterial healing. Taken together, these studies highlight the complexity and the context-dependent roles of TGFβ in vascular disease.[27]

The changes in the mechanical properties of the carotid artery after celiprolol (table 4, webappendix pp 7–8) might provide some insights into the mechanisms of prevention of arterial dissection and rupture. Indeed, we noted that common carotid artery stiffness increased in response to celiprolol (ie, distensibility was reduced and Young's elastic modulus, which indicates the stiffness of the arterial wall material, increased). There are strong associations between β-adrenergic receptors and TGFβ pathways. The stimulation of β_2-adrenergic receptors is associated with activation of TGFβ,[28] and overexpression of TGFβ is also associated with overstimulation of the β_2-adrenergic pathway.[29] Chronic β_2 stimulation might enhance collagen synthesis through increased expression of TGFβ. Indeed, β_2 stimulation by clenbuterol in rats boosted mRNA expression of TGFβ1, 2, and 3, and platelet-derived growth factor subunit B.[28] Unopposed α-adrenergic stimulation by displacement of endogenous agonist from β-receptors and baroreflex stimulation can also contribute to TGFβ stimulation.[30] TGFβ could enhance the production of type I and III collagen and lead to fibrosis.[31–33] Thus, in response to celiprolol, an upregulation of collagen synthesis might have strengthened the arterial wall, reducing its susceptibility to rupture.

When we designed the study, we postulated that celiprolol, a cardioselective β blocker with β_2 agonist vasodilatory properties, would reduce central blood pressure[7] and thus mechanical load on collagen fibres within the arterial wall, ultimately prevent arterial dissection and rupture. However, celiprolol did not decrease brachial systolic and diastolic blood pressures and heart rate. Moreover, systolic and pulse pressures substantially increased after treatment, which is consistent with findings in healthy normotensive people.[34] Celiprolol's lack of blood pressure lowering in normotensive people was explained by its β_2-adrenoceptor agonist properties.[35] The balance between β_1 and β_2

	Baseline	Slope of change per year	p
Brachial indices			
Systolic blood pressure (mm Hg)			0·10
Celiprolol	113 (10)	0·7 (6·1)	
Control	117 (13)	−2·1 (5·1)	
Diastolic blood pressure (mm Hg)			0·25
Celiprolol	67 (10)	−2·3 (4)	
Control	69 (10)	−1·4 (6·1)	
Mean blood pressure (mm Hg)			0·92
Celiprolol	83 (9)	−1·1 (3·6)	
Control	85 (10)	−1·6 (4·8)	
Pulse pressure (mm Hg)			0·04
Celiprolol	46 (10)	2·9 (7·1)	
Control	48 (11)	−0·7 (6·8)	
Heart rate (beats per min)			0·10
Celiprolol	70 (13)	2·8 (9·6)	
Control	71 (11)	−1·8 (10·6)	
Carotid indices			
Carotid pulse pressure (mm Hg)			0·22
Celiprolol	34 (8)	3·4 (6·7)	
Control	38 (14)	−0·6 (9·7)	
Internal diameter (mm)			0·29
Celiprolol	5.1 (0.5)	0·1 (0·3)	
Control	5.5 (0.7)	0 (0·2)	
Intima-media thickness (μm)			0·11
Celiprolol	510 (90)	−17 (32)	
Control	494 (114)	1·4 (41)	
Distensibility ($kPa^{-1} \cdot 10^{-3}$)			0·057
Celiprolol	52 (24)	−7·2 (12)	
Control	49 (28)	1·4 (13·5)	
Young's elastic modulus (kPa)			0·03
Celiprolol	231 (113)	58 (73)	
Control	328 (304)	6 (97)	
Circumferential wall stress (kPa)			0·11
Celiprolol	56 (12)	2·3 (7)	
Control	67 (21)	−2·6 (12·8)	

Values are mean (SD).

Table 4: **Slope of change with time in supine brachial blood pressure, heart rate, and common carotid artery variables of randomised patients**

ligand properties depends on the level of sympathetic activation. In hypertensive people, celiprolol is a β_1 antagonist with mild vasodilation through β_2 agonist properties.[36] In healthy volunteers, the β_2 agonist properties predominate.[34] Most of our patients were normotensive at inclusion. Thus, the protective effect of celiprolol was unlikely to be through blood pressure lowering. However, we cannot exclude that it could have prevented excessive rises in blood pressure and heart rate during exercise, as do all β blockers.[35] In summary, celiprolol might have provided more stable haemodynamic conditions and led to a less fragile arterial wall. Renin inhibition by celiprolol could have an effect, through lower activation of TGFβ. Indeed, celiprolol is a β_1 antagonist and could, in theory, inhibit renin secretion, an action antagonised by its β_2 partial agonist properties.

We also found a harmful role for low diastolic pressure and high brachial pulse pressure at inclusion in treated patients. We did not anticipate this finding since treatment was well tolerated. Future studies should pay close attention to low diastolic or high pulse pressure before the start of treatment.

We could not reach our target number of patients. Nevertheless, the enrolled population was very large for such a rare disease. In controls, the rate of major events was close to that expected. The effect of celiprolol was larger than expected (–64% to –69%), partly compensating for reduced statistical power. The PROBE design was a trade-off resulting from insufficient funding to obtain a placebo. However, that endpoints were major indisputable clinical events, together with strict procedures of evaluation, eliminates most bias.

The inclusion criteria were set by a group of specialists after the Villefranche classification.[3] In 2000, when the study was conceived, molecular testing for patients with clinical vascular Ehlers-Danlos syndrome was not routine in all centres and if inclusion had to be made on positive mutation, the study would not have been done at all. Only 33 of 53 participants had proven mutations, whereas all participants fulfilled the clinical definition of the disease, which was a limitation of the study. Screening for other mutations causing phenotypic copies of vascular Ehlers-Danlos syndrome was done only in patients with signs suggestive of an alternative diagnosis, but genotyping is not available everywhere. Our inclusion criteria corresponded to recruitment of specialist centres and thus the results of the study gain external validity and applicability. Whether patients with clinical presentation and no mutation are also protected has not been established. The results of the BBEST study apply only to patients fulfilling the inclusion criteria. Analysis according to genotype was not prespecified; thus, results in the mutated population must be assessed cautiously, although results in the mutated population were identical. Finally, we could not test the evoked mechanisms of action of celiprolol on TGFβ and collagen synthesis.

Contributors

SL, PB, HP, ADP, and JNF contributed to study concept and design. KTO and JDB collected the data. KTO and EB contributed to the haemodynamic measurements. XJ, ALF, ADP, and JDB made the genetic testing. JP, JDB, PC, DPG, and GG were involved in patient enrolment. SL and JE contributed to endpoint adjudication. JSH did the statistical analysis. SL, PB, and KTO analysed the data, interpreted the data, and wrote the report. JE, JDB, JNF, JSH, XJ, and HP extensively reviewed the paper. All authors revised the report for important intellectual content and have seen and approved the final version.

Conflicts of interest

We declare that we have no conflicts of interest.

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