

# The procyanidin-induced pseudo laminar shear stress response: a new concept for the reversal of endothelial dysfunction

**Roger CORDER, Richard C. WARBURTON, Noorafza Q. KHAN, Ruth E. BROWN, Elizabeth G. WOOD and Delphine M. LEES**

William Harvey Research Institute, Barts and the London, Queen Mary's School of Medicine and Dentistry, Charterhouse Square, London EC1M 6BQ, U.K.

## A B S T R A C T

Reduced endothelium-dependent vasodilator responses with increased synthesis of ET-1 (endothelin-1) are characteristics of endothelial dysfunction in heart failure and are predictive of mortality. Identification of treatments that correct these abnormalities may have particular benefit for patients who become refractory to current regimens. Hawthorn preparations have a long history in the treatment of heart failure. Therefore we tested their inhibitory effects on ET-1 synthesis by cultured endothelial cells. These actions were compared with that of GSE (grape seed extract), as the vasoactive components of both these herbal remedies are mainly oligomeric flavan-3-ols called procyanidins. This showed extracts of hawthorn and grape seed were equipotent as inhibitors of ET-1 synthesis. GSE also produced a potent endothelium-dependent vasodilator response on preparations of isolated aorta. Suppression of ET-1 synthesis at the same time as induction of endothelium-dependent vasodilation is a similar response to that triggered by laminar shear stress. Based on these results and previous findings, we hypothesize that through their pharmacological properties procyanidins stimulate a pseudo laminar shear stress response in endothelial cells, which helps restore endothelial function and underlies the benefit from treatment with hawthorn extract in heart failure.

## INTRODUCTION

HF (heart failure) is an intractable condition that frequently becomes refractory to current treatment regimens [1]. The poor prognosis of patients with HF is of major concern, because of the increasing number of aged patients suffering from this condition and the burden this will be for healthcare providers over the coming decades [1–5]. Insights that identify new approaches to treatment could provide substantial benefit through improved quality of life and reduced frequency of hospitalizations.

In recent years endothelial dysfunction has become accepted as a component of most cardiovascular pathologies, and it is now recognized as a key factor contribut-

ing to the poor haemodynamic status of patients with HF [6,7]. Abnormal endothelial function is often portrayed as a simple reduction in NO (nitric oxide)-mediated endothelium-dependent vasodilator responses, when this is just one feature of a more complex pattern of changes. Indeed, as vasodilator function decreases, there is increased vasoconstriction due to excess ET-1 (endothelin-1) synthesis [8,9]. These alterations in vascular tone are coincident with a spectrum of pro-inflammatory and prothrombotic vascular changes [8,9]. Although agents such as angiotensin-converting-enzyme inhibitors ameliorate endothelial function to a degree [6,7], they are no more effective at preventing HF in patients with hypertension than other antihypertensive

**Key words:** endothelium, hawthorn, grape seed, endothelin-1, heart failure, nitric oxide.

**Abbreviations:** CE, catechin equivalents; ET-1, endothelin-1; GSE, grape seed extract; HF, heart failure; LSS, laminar shear stress; MI, myocardial infarction; NO, nitric oxide; TK, tyrosine kinase.

**Correspondence:** Professor Roger Corder (email r.corder@qmul.ac.uk).

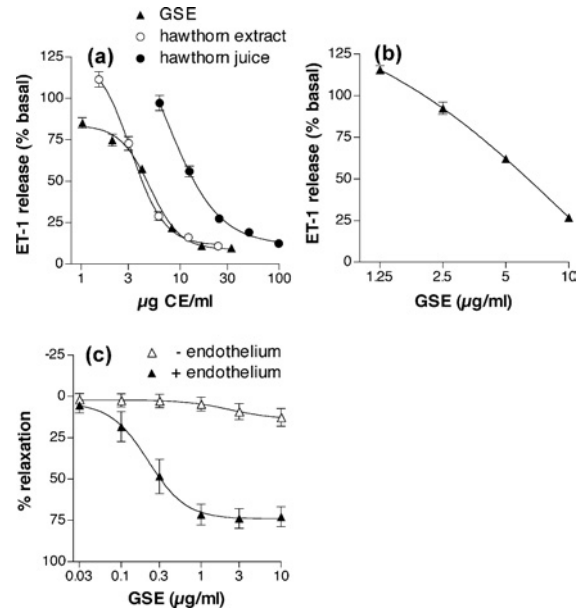
agents [10]. Therefore new treatments that reverse endothelial dysfunction are needed [1].

The favourable findings of a recent meta-analysis of clinical trials using extracts of hawthorn (*Crataegus oxyacantha*) to treat HF raised a number of questions for further investigation [11,12]. Notably, the need to understand the mechanism of action, the optimal dose to provide the maximum benefit and what to monitor in future trials to demonstrate a response to treatment is required. Hawthorn extract is a rich source of flavan-3-ol oligomers called procyanidins [13]. These phytochemicals are also the main component of GSE (grape seed extract) [14]. In the present study, we show that hawthorn extract and GSE are equipotent as inhibitors of ET-1 synthesis by cultured endothelial cells. Based on these findings and other published data, we present evidence to support the hypothesis that these actions of procyanidins represent a new concept for modifying endothelial dysfunction, which may lead to a new approach to treating HF.

## METHODS

Hawthorn juice prepared as 100% pressed juice from fresh plants was from Salus (Warrington, U.K.) and manufactured by W. Schoenenberger GmbH (Magstadt, Germany). Hawthorn berry extract, as a tincture in 30% ethanol (equivalent to 250 mg of fruit/ml), was from Neal's Yard Remedies (London, U.K.). To eliminate any soluble components that might interfere in cell experiments, the procyanidin constituents of these hawthorn preparations were extracted using disposable C<sub>18</sub> Sep-Pak cartridges (Waters, Milford, MA, U.S.A.) [15]. After reconstitution, Sep-Pak extracts were diluted in cell culture medium. GSE containing approx. 80% oligomeric procyanidins by weight was obtained from Polyphenolics (Madera, CA, U.S.A.). Total polyphenol content of each test substance was measured with Folin and Ciocalteu's reagent using catechin as a reference, and expressed as CE (catechin equivalents;  $\mu\text{g/ml}$ ) [15]. The effects on ET-1 synthesis of reconstituted samples of hawthorn juice, hawthorn extract and GSE were tested on cultures of bovine aortic endothelial cells as described previously [15,16]. GSE was also evaluated on confluent cultures of normal human aortic endothelial cells (Promo-Cell, Heidelberg, Germany) using the same methodology. In each experiment, ET-1 release for each treated well was expressed as a percentage of basal release from cells incubated with cell culture medium alone.

The endothelium-dependent vasodilator activity of GSE was determined using rings of rat thoracic aorta [17]. Vascular rings were prepared carefully, in order to avoid damaging the endothelium, and suspended in organ baths. After a period of equilibration the rings of aorta were pre-contracted with phenylephrine (1  $\mu\text{mol/l}$ ) and the



**Figure 1** Concentration-dependent inhibition of ET-1 synthesis in (a) bovine aortic endothelial cells treated for 6 h with GSE, hawthorn berry extract or hawthorn juice and (b) human aortic endothelial cells treated with GSE, and (c) endothelium-dependent vasodilator effect of GSE on rat thoracic aorta

In (a),  $n = 6-9$  for each test condition. In (b),  $n = 4$  for each concentration. In (c),  $n = 6$  for each response determined without or with endothelium.

presence of a functional endothelium was verified with acetylcholine (10  $\mu\text{mol/l}$ ) prior to testing the GSE. In some experiments, the endothelium was removed by rubbing the aorta with forceps. Complete removal of the endothelium was confirmed by the absence of response to acetylcholine (10  $\mu\text{mol/l}$ ).

All data were analysed using GraphPad Prism (GraphPad Software, San Diego, CA, U.S.A.). Results are means  $\pm$  S.E.M. Significant differences were determined by ANOVA with Fisher's protected least-significant difference (PLSD) as a post-hoc test using Statview (SAS Institute, Cary, NC, U.S.A.). IC<sub>50</sub> and EC<sub>50</sub> values are the concentrations of test substances inhibiting ET-1 synthesis by 50% or causing a 50% relaxation of rings of rat aorta respectively.

## RESULTS

Hawthorn juice and hawthorn extract produced concentration-dependent decreases in ET-1 synthesis by bovine aortic endothelial cells (IC<sub>50</sub> values, 26.6 and 4.1  $\mu\text{g}$  of CE/ml respectively; Figure 1a). GSE (IC<sub>50</sub> value, 4.6  $\mu\text{g}$  of CE/ml) was as effective as hawthorn extract (Figure 1a). Significant inhibition ( $P < 0.01$ ) of ET-1 release from bovine aortic endothelial cells was obtained with GSE and hawthorn extract at concentrations  $\geq 3 \mu\text{g}$

of CE/ml or hawthorn juice  $\geq 12 \mu\text{g}$  of CE/ml. GSE also inhibited ET-1 synthesis by human aortic endothelial cells with comparable potency ( $\text{IC}_{50}$  value,  $6.4 \mu\text{g/ml}$ ; significant reductions occurred at 5 and  $10 \mu\text{g/ml}$  compared with control;  $P < 0.01$ ; Figure 1b), and produced an endothelium-dependent relaxation of isolated rat thoracic aorta ( $\text{EC}_{50}$  value,  $0.31 \mu\text{g/ml}$ ; Figure 1c). Concentrations of GSE  $\geq 0.3 \mu\text{g/ml}$  caused a significant vasodilation ( $P < 0.01$ ) when the endothelium was intact, but these were without effect when the endothelium was removed. The approximately 10–20-fold greater potency on isolated aorta compared with incubations with cultured endothelial cells probably reflects the relative instability of procyanidins in bicarbonate-buffered culture medium, as responses of aortae were determined 1–2 min after addition to the organ bath compared with a 6 h incubation with cultured endothelial cells prior to collection of media samples for ET-1 measurements.

## DISCUSSION

Increased ET-1 production and decreased endothelium-dependent vasodilation are key features of endothelial dysfunction in HF [6,7,18]. ET-1 increases systemic and pulmonary vascular resistance and contributes to myocardial ischaemia in HF [7,18]. Plasma levels of ET-1 and big ET-1 can be predictive of mortality in HF [19–21]. Moreover, ET-1 excess is exacerbated by the loss of NO-dependent vasodilator function, and endothelial function declines as the clinical severity of HF worsens [6,7]. Initial clinical trials of endothelin receptor antagonists in patients with HF showed promise, but subsequent studies have been disappointing [1,22]. This is probably due to the inability of endothelin antagonists to reverse other components of endothelial dysfunction.

The main cause of HF is previous myocardial infarction (MI) [23]. Regular exercise is the only lifestyle factor that reduces the incidence of HF after MI [23], and it may also reduce mortality in patients with established HF [24]. In part, this is due to improved endothelial function, as exercise training improves NO-dependent vasodilation, which, in turn, reduces the severity of symptoms of HF [6,7]. These observations emphasize the importance of improving endothelial function as a strategy to treat or prevent HF. Although arginine supplements are reported to boost endothelium-dependent vasodilator responses [9], this is not a consistent observation [25]. A specific treatment that rapidly reverses deficits in NO-dependent vasodilation and suppresses ET-1 synthesis has yet to be described.

Hawthorn extract is known to cause vasodilator responses and improve coronary blood flow [13]. In the present study, we show that procyanidin-rich natural products, such as extracts of grape seed or hawthorn, inhibit ET-1 synthesis and stimulate endothelium-depen-

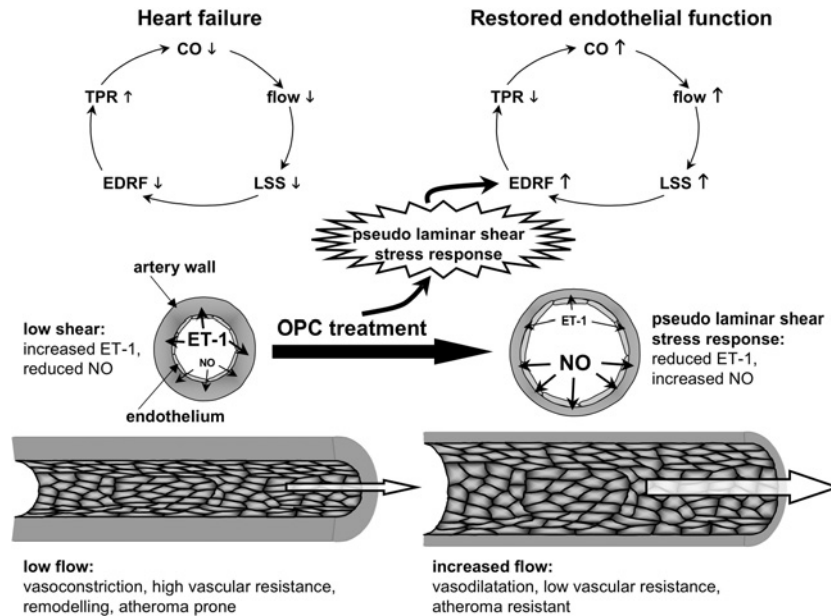
dent vasodilation. This may well explain the clinical benefits from hawthorn extracts in HF [11].

Unequivocal evidence that oligomeric procyanidins are absorbed in sufficient quantities to modify endothelial function has yet to be described [26]. This lack of substantive information is, in part, due to difficulties associated with extracting procyanidins from plasma, and because oligomeric procyanidins are a heterogeneous mixture such that total procyanidin concentration in plasma samples cannot be readily quantified. As a result there is little data describing the absorption, metabolism or elimination of procyanidins. Nevertheless, consumption of procyanidin-rich chocolate is reported to improve endothelial function, whereas chocolate depleted of these bioactive flavonoids is without effect [27].

The mechanism underlying the effects of procyanidins on the endothelium has yet to be defined. In the context of normal endothelial function, LSS (laminar shear stress) is the most important regulatory influence as it induces vasodilation through NO and prostacyclin synthesis and inhibits vasoconstriction by suppressing ET-1 production [28,29]. LSS induces re-organization of the endothelial cytoskeleton, release of mediators and altered expression of a spectrum of genes [30]. Gene array studies have elegantly demonstrated that physiological levels of LSS suppress the mRNA levels of genes associated with vascular dysfunction and increase the expression of protective genes [31,32]. These findings add further support to the view that LSS confers vascular protection by converting the endothelium into an atheroma-resistant phenotype. Integration of the endothelial response to LSS is dependent on TK (tyrosine kinase) signalling in focal adhesion complexes [30].

LSS is the frictional force generated by blood flowing over the endothelium and is therefore proportional to blood flow. Progressive loss of endothelial function with age parallels the deterioration in cardiac performance [4,5]. Reduced LSS is likely to be a key factor underlying this change, precipitated by abnormalities in cardiac function and sedentary lifestyles. Hence decreased cardiac output with reduced blood flow will lead to diminished endothelium-dependent vasodilation, which contributes to increased systemic vascular resistance. This creates a vicious circle, which, if uninterrupted, not only predisposes to HF, but will also increase its severity (Figure 2). In patients with HF, the beneficial effects of regular exercise may well be due to the increased LSS occurring during exercise.

The actions of procyanidins on endothelial function bear many similarities to the response evoked by LSS. Thus aortic rings suspended in organ baths release NO in the absence of LSS and synthesis of ET-1 by cultured endothelial cells is inhibited, two well-described responses to LSS [28,29]. Moreover, treatment of endothelial cells with a procyanidin-rich red wine extract produces morphological changes with re-organization of



**Figure 2** Schematic outline of the impact of low cardiac output on endothelial function in heart failure and the vascular response to treatment with procyanidins

CO, cardiac output; LSS, laminar shear stress; EDRF, endothelium-dependent relaxing factor (NO); TPR, total peripheral resistance; OPC, oligomeric procyanidins.

the cytoskeleton. This results in a pattern of phosphotyrosine staining and co-localization with F-actin [15,16], which is indistinguishable from that induced by LSS [33]. Based on these observations, we hypothesize that oligomeric procyanidins act as phytochemical inducers of the LSS response, and it is this property that helps reverse the vascular dysfunction in HF (Figure 2).

How procyanidins trigger this pseudo LSS response is unclear, but there are several lines of evidence that point to a specific mechanism. Procyanidins are composed mainly of epicatechin units. Epicatechin monomers and oligomers (procyanidins) are potent antioxidants. However, the endothelium-dependent vasodilation induced by procyanidins has a clear structure–activity relationship that is optimal with oligomeric procyanidins (particularly epicatechin trimers, tetramers and pentamers), but not mimicked by epicatechin monomers or dimers and, therefore, unrelated to antioxidant properties [14]. Inhibition of ET-1 synthesis exhibits a similar sensitivity [15,16].

A study of the tyrphostin family of TK inhibitors revealed the divergent selectivity of TK inhibitors for specific pathways involved in the regulation of morphological changes in endothelial cells [34]. The variable changes in morphology with different tyrphostins indicate the interplay between activating and inhibiting TKs [34]. Since structurally similar plant flavonols to procyanidins act as TK inhibitors [35], it is likely that these phytochemicals induce the pseudo LSS response in endothelial cells by interfering with TK signalling. Alternatively, inhibition of a specific tyrosine phosphatase could underlie the pseudo LSS response, as this would

lead to activation of TK-dependent pathways that are normally regulated by the inhibitory influence of such phosphatases. Further research on procyanidins may lead to synthetic analogues with similar properties and improved bioavailability.

In summary, the beneficial effects of hawthorn extract in HF may involve induction of a pseudo LSS response, which triggers vasodilation and reduces cardiac afterload. This would break the vicious circle of reduced cardiac output leading to ever greater endothelial dysfunction. Future studies should investigate fully the ability of procyanidins to modify vascular function and determine whether these natural products, or synthetic analogues, can provide a much-needed new approach for treatment of the haemodynamic abnormalities and associated endothelial dysfunction that occur in HF.

## ACKNOWLEDGMENTS

This work was supported by grants from the William Harvey Research Foundation, and supplemented by support from Polyphenolics Inc., Madera, CA, U.S.A. R.E.B. was supported by a British Heart Foundation PhD studentship.

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Received 30 June 2004/9 August 2004; accepted 24 August 2004

Published as Immediate Publication 24 August 2004, DOI 10.1042/CS20040189