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Resumen

La investigación sobre el endotelio vascular en los últimos 40 años ha provisto ideas para entender la enfermedad vascular. Este nuevo conocimiento ha encontrado su camino en la medicina clínica. En esta revisión nos ocupamos de ciertas áreas de la investigación en las que se ha obtenido avances significativos en la prevención y el tratamiento cardiovascular, así como algunas interrogantes que aún permanecen sin respuesta.

Palabras clave: Endotelio vascular, enfermedad vascular, investigación cardiovascular.

Abstract

Over the last 40 years, research on the vascular endothelium has provided important clues for the understanding of vascular disease. This new knowledge is finding its way into clinical medicine. In this review we deal with some areas where significant advances in the prevention and treatment of cardiovascular research has been achieved and with some of the remaining questions.

Keywords: Vascular endothelium, vascular disease, cardiovascular research.

An Fac med. 2014;75(4):333-8 / doi: <http://dx.doi.org/doi:10.15381/anales.v75i4.10851>

The vascular endothelium, the innermost layer of blood and lymphatic vessels, covers the whole surface of the vascular system and provides the interface between circulating blood or lymph and the vessel wall. It is one cell thick and, following its discovery in the 19th century, it was long believed to be an inert layer that merely facilitated the circulation of fluids around the body. It was Florey who, while describing his early pioneering work on the ultrastructure of the vascular endothelial cell, predicted that important discoveries could be made when pursuing the study of these cells despite the fact that the endothelium had until then been considered to be just a kind of cellophane wrapping⁽¹⁾. He was right.

In the last 40 years, research on the vascular endothelium has been very productive and its results have greatly contributed to our understanding of the normal functioning of the vasculature as well as providing important clues for unraveling the mystery of cardiovascular disease, its origin, development, complications and its prevention

or treatment. The endothelium is now considered to be an organ with significant physiological roles rather than an inert surface. Figure 1 shows the number of publications on this subject over the last four decades as well as some key discoveries that have directly contributed to the developing interest in the vascular endothelium. These include the discovery of the vasodilator prostacyclin, that of endothelium-derived relaxing factor and its identification as nitric oxide (NO). The author has described elsewhere his own contribution to this research⁽¹⁰⁾.

The purpose of this brief review is to revisit some areas in which research is at present generating significant new information or where translation into clinical medicine is taking place. These include the significance of the balance between prostacyclin and thromboxane A₂ for vascular homeostasis, the potential use of aspirin and related compounds in the prevention of cancer, endothelial dysfunction and the use of prostacyclin as a drug for the management of pulmonary hypertension.

The unexpected finding of the vasodilator prostacyclin while we were looking for the vasoconstrictor thromboxane A₂ in the vascular wall⁽¹⁰⁾ revealed that two compounds with opposing biological functions, derived from the same precursor (arachidonic acid), are synthesized by cyclooxygenase enzymes in the platelets and the vascular wall. This led us to the hypothesis that a balance exists between the generation of these two compounds (thromboxane A₂ from the platelets and prostacyclin from the vessel wall) and that this is not only important for the understanding of the homeostasis of platelet-vessel wall interactions, but also for the understanding of disease. A closely-related question concerns the net effect achieved in the vasculature following treatment with aspirin and aspirin-like drugs, which have the ability to inhibit the synthesis of both prostacyclin and thromboxane A₂. This has proven to be an enduring question, the answer to which is only becoming clear in the last few years.

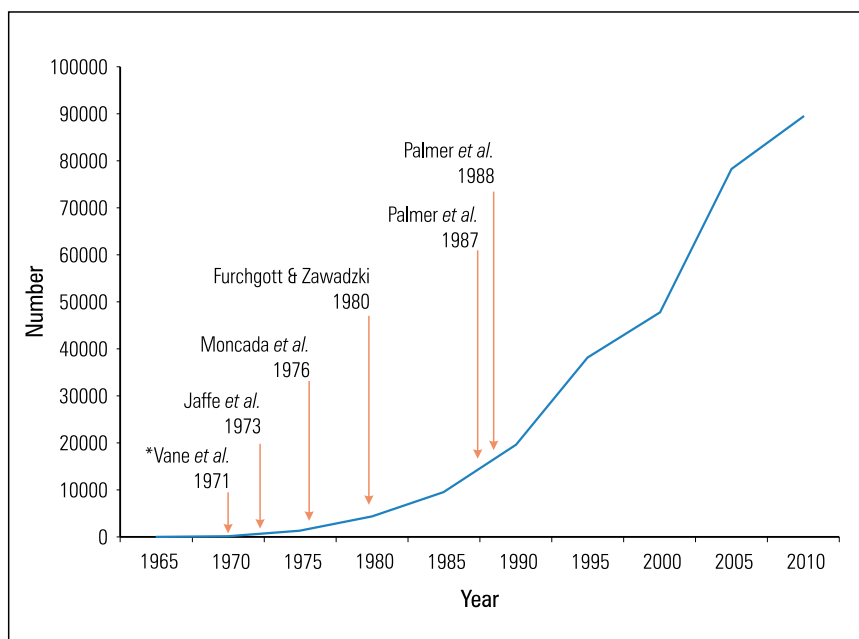


Figura 1. Some key publications in the field of vascular endothelium research and number of publications in the field over time ⁽²⁻⁹⁾.

The unique action of aspirin was unraveled in the late 1970s when it was demonstrated that the platelet cyclooxygenase, unlike that of the vessel wall, is exquisitely sensitive to aspirin and that the acetylation by aspirin of a serine residue at the active site of the enzyme is irreversible and lasts for the duration of the life of the platelets ⁽¹¹⁻¹³⁾ which are unable to synthesize new proteins. This, together with the demonstration that a small dose of aspirin is more effective than a large dose in increasing cutaneous bleeding time in humans ⁽¹⁴⁾, led to the understanding of the now well-recognized protective effect of low doses of aspirin against vascular disease. This protective effect has been demonstrated in a large number of clinical trials in different cardiovascular conditions ^(15,16). The work on aspirin lent support to the hypothesis of the significance of the balance between prostacyclin and thromboxane A_2 , since what a low dose of aspirin achieves by selectively inhibiting generation of thromboxane A_2 is to shift the balance in favor of prostacyclin.

Further support for the hypothesis came from an unexpected source. In the 1990s it was discovered that cyclooxygenase exists in two forms, one constitutive (called COX1) which generates prostaglandins for physiological functions, and a second, inducible form (called COX2) which is expressed during pathological conditions and generates prostaglandins involved in inflammation ⁽¹⁷⁾. Each enzyme is encoded by a different gene and their molecular structure is sufficiently different to warrant the pursuit of selective inhibitors of the COX2 enzyme. It was believed that these types of compounds would possess anti-inflammatory activity without the side effects (particularly gastric side effects) that bedevil the classical non-steroidal anti-inflammatory drugs (NSAIDs). In the event, such compounds were synthesized and the objective of achieving similar anti-inflammatory activity to the traditional aspirin-like drugs with reduced gastric side effects was achieved ^(18,19). However, during the development of these compounds a potential problem was

identified ⁽²⁰⁻²²⁾ which was later confirmed in patients, namely that COX2 inhibitors increase the risk of cardiovascular events ^(18,19). Studies indicated that this serious side effect was due to inhibition of the generation of prostacyclin in the vasculature, leading to an increase in blood pressure and thus to a pro-thrombotic state. Over the last few years animal experiments ^(23,24) and clinical studies have produced overwhelming evidence in support of this suggestion, confirming that this is not a side effect related to any specific molecule but is associated with the pharmacological action of the whole class of compounds and is dependent on the strength and duration of inhibition of the synthesis of prostacyclin ⁽¹⁹⁾. Fittingly, a concomitant inhibition of COX1 with low-dose aspirin protects against this side effect through inhibition of the generation of thromboxane A_2 ^(23,25).

Two problems remain to be fully clarified, the first of which is whether the generation of prostacyclin in the vasculature is due to an inducible COX2 resulting from a subliminal inflammatory condition of the vasculature, or is due to a constitutive enzyme. There is a body of evidence in favor of the latter ⁽¹⁹⁾. However, recent evidence indicates that it may be a mixture of the two enzymes ^(26,27), a fact that would be in agreement with the early observation that the concentration of 6-oxo PGF1 α , the stable end product of the metabolism of prostacyclin, is elevated in patients with atherosclerosis ⁽²⁸⁾.

If that is correct, then COX2 overexpression would be part of an inflammatory condition and thus a defensive mechanism. The second problem relates to the question of whether classical NSAIDs also carry the risk of cardiovascular side effects. This remains a highly controversial issue which may be resolved in further clinical trials. However, it is reasonable to assume that the cardiovascular risk of these drugs will be associated with the degree and duration of inhibition of COX2 and that the ratio between COX1 and COX2 will be de-

terminant in their relative tendency to cause this side effect^(29,30). Indeed, the use of diclofenac (which has a ratio of COX1:COX2 inhibition similar to that of the COX2 inhibitor celebrex) is associated with increased cardiovascular risk, while the use of naproxen (which is a more selective COX1 inhibitor) is not⁽³¹⁾. The data on ibuprofen, which is also a more selective COX1 inhibitor, remains controversial⁽³²⁾. In summary, the concept of the balance between prostacyclin and thromboxane A₂ in the homeostasis of the vascular system has been validated and its relevance in health and disease is now well understood and is guiding further development of therapies. Recently, however, a genetic variant of the gene responsible for the encoding of COX2 (PTGS2), associated with lower COX2 activity, has been identified in humans. The relationship between this condition and cardiovascular risk has so far proven to be controversial⁽³³⁻³⁶⁾. One of the reasons for this may be that this genetic variant, although associated with a decrease in excretion of 6-oxo PGF1 α , seems also to be associated with decreased concentrations of thromboxane A₂; this complicates interpretation of the results using the prostacyclin/thromboxane A₂ balance hypothesis.

One of the most exciting discoveries in the use of NSAIDS has been the finding that these compounds prevent the development of different forms of cancer. This effect, which was identified some years ago in large prospective clinical trials^(37,38), was later attributed to the inhibition of prostaglandin synthesis, specifically that of prostaglandin E₂ (PGE₂), generated by a COX2 enzyme induced by inflammation associated with pre-malignant lesions⁽³⁹⁾. This prostaglandin was believed to be responsible, at least in part, for the neoplastic transformation through its activation of pro-survival pathways⁽⁴⁰⁻⁴³⁾. This led to the testing of COX2 inhibitors in the chemoprevention of colorectal cancers, in which a protective action was demonstrated. These trials were, however, marred by concerns

related to the potential cardiovascular side-effects of these drugs, which hampered their full evaluation⁽⁴⁴⁾. Studies which demonstrated that the enzyme converting PGH₂ to PGE₂, the so-called microsomal prostaglandin E synthase-1 (mPGE-1), is overexpressed in inflammation and couples with COX2 to enhance PGE₂ generation. This has led more recently to the suggestion that selective inhibitors of this enzyme may be an important therapeutic target that will result in selective inhibition of the pathological PGE₂, allowing PGH₂ to be converted into the physiologically active prostaglandin, prostacyclin^(45,46). Overexpression of mPGE-1 has been shown in different forms of cancers and its presence is significantly correlated with a worse prognosis, at least in colorectal cancers^(47,48). Although animal studies in which deletion of this enzyme has been carried out show controversial results in relation to cancer^(49,50), the development and early *in vitro* testing of selective inhibitors of this enzyme is proceeding^(51,52), and clinical trials are likely to clarify before long the viability of this hypothesis.

The origin of the inflammatory reaction in premalignant lesions has been linked to platelet activation. Evidence for this originally came from the long-term follow-up clinical trials mentioned above in which the efficacy of aspirin as an antithrombotic agent was investigated⁽⁵³⁻⁵⁵⁾. It was noticed that ingestion of aspirin, even at the low doses used to protect against arterial thrombosis, reduced the incidence of mortality due to cancer, particularly of those of the gastrointestinal tract. These results pointed to the platelets as a culprit⁽⁵⁵⁾ — a suspicion that has been strengthened by several observations including the fact that aggregating platelets can produce inflammation and induction of COX2^(56,57), and that the doses of aspirin that are protective do not reach plasma concentrations sufficiently high to inhibit COX2 and are therefore likely to be inhibiting the platelet COX1^(52,58). If these results are correct, they point towards a key

role of platelet activation not only in atherosclerosis and thrombosis, where their role is now fully accepted, but also in the process of neoplastic transformation. Both effects take place via a two-step process which involves the activation of a COX1 and other pathways in the platelets, followed by the induction of COX2 in a number of cells participating in the development of the atherosclerotic plaque or the tumor.

As far as prevention or antineoplastic therapy is concerned, low-dose aspirin therefore emerges as a particularly attractive option for antithrombotic and antitumor therapy, clearly superior to the more selective COX2 inhibitors which possess cardiovascular side effects and also superior to the classical NSAIDS, none of which shares with aspirin its unique selectivity of inhibition of the platelet COX1 enzyme.

Although the idea of a dysfunctional vascular endothelium was mooted many years ago⁽⁵⁹⁾, only in the last 20 years has it become one of the most studied areas of vascular biology. Indeed, early detection of 'endothelial dysfunction' is proving to be predictive of cardiovascular disease and may indicate ways of preventing its development. Endothelial dysfunction occurs in a number of conditions including hypertension, diabetes (types 1 and 2), coronary artery disease and chronic renal failure⁽⁶⁰⁾. It has been equated with a decrease in generation of NO by the vascular endothelium and it is likely that this may indeed be its major pathophysiological cause. However, more recently, a number of other changes have been identified which indicate that, besides a decrease in availability of NO, endothelial dysfunction also comprises an increase in vasoconstrictor, pro-inflammatory and pro-thrombotic parameters⁽⁶⁰⁾.

The decrease in activity of NO has been attributed largely to a decrease in its availability resulting from the interaction with oxygen-derived species, mainly superoxide anion^(61,62), which may be generated by a number

of enzymes including NADPH oxidase, xanthine oxidase, uncoupling of NO synthase or from the mitochondrial oxidative phosphorylation chain⁽⁶³⁻⁶⁵⁾.

More recently, it has been suggested that increases in the concentration of asymmetric dimethylarginine (ADMA) may be involved in endothelial dysfunction. This compound was discovered some years ago to be an endogenous inhibitor of the NO synthase and shown to be increased in patients with renal insufficiency⁽⁶⁶⁾. Since then, evidence in favor of its role in endothelial dysfunction and in cardiovascular disease has been mounting. Indeed, an increase in plasma concentration of ADMA is associated with hypercholesterolemia⁽⁶⁷⁾, and with increased cardiovascular risk factors in patients with renal failure⁽⁶⁸⁾. Furthermore it is predictive of acute coronary events⁽⁶⁹⁾, overall mortality of patients with chronic renal failure⁽⁷⁰⁾, and mortality in critically ill patients⁽⁷¹⁾. Two independent pieces of evidence have added support to the suggestion that ADMA plays a role in vascular disease. First, it has been shown that in some forms of vascular pathology the intracellular concentration of ADMA is elevated 3 to 9-fold over physiological concentrations; these concentrations, unlike physiological concentrations, are sufficient to inhibit NO synthase, indicating that endogenous inhibitors of NO synthesis are critical factors in vascular dysfunction following injury⁽⁷²⁾. Secondly, a genetic mutation has been identified in the enzyme dimethylarginine dimethylaminohydrolase (DDAH, the enzyme responsible for the metabolism of ADMA) in some individuals with a susceptibility to pre-eclampsia⁽⁷³⁾. In summary, although a great deal of evidence has been generated supporting the concept of endothelial dysfunction, much work is still required to clarify fully the pathophysiological mechanisms involved in this early manifestation of vascular disease. It will be important to establish whether, and to what extent, early intervention has a significant effect on the development of vascular disease.

Although the powerful vasodilator and antiplatelet effect of prostacyclin suggested early on its potential use in clinical conditions associated with thrombosis and vasoconstriction⁽⁷⁴⁾, its main clinical use at present is in the management of primary pulmonary hypertension^(75,76), where it has been shown to improve symptoms, induce remodeling of the pulmonary vasculature and reduce mortality. The difficulties related to its intravenous usage as an unstable compound, requiring continuous administration, led to the development of different formulations of prostacyclin or its analogs for intravenous, subcutaneous and inhaled administration^(75,76). In addition to the use of these compounds, two different approaches have also proven to be useful in the management of primary pulmonary hypertension. These are the use of endothelin receptor antagonists and inhibitors of the enzyme 5-phosphodiesterase to boost the effect of endogenous NO on its receptor, the soluble guanylyl cyclase. These compounds, used alone or in different combinations and schedules, have revolutionized the treatment of this complex and fatal disease to the point that the long-term management with orally-active compounds is now being investigated and prostacyclin and nitric oxide receptor agonists are at present the subject of long-term clinical trials⁽⁷⁶⁻⁸⁰⁾. Furthermore, the proliferative nature of the disease, at least in part associated with the release of platelet-derived growth factor, has led to the development and use of different kinase inhibitors⁽⁷⁹⁾.

In summary, research on the vascular endothelium and in closely-related areas continues to generate a great deal of interest. As this work matures, translational developments into medicine are becoming prominent, and clear clinical benefits are being demonstrated. Almost half a century after Florey, it is still possible to predict that the endothelial cell has many secrets yet to be uncovered and that, when this occurs, further avenues for the prevention and treatment of disease will be identified.

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Conflicts of interest:

None declared by the author.

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