Endothelium as a pharmacological target

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Over the last few years, the increasing knowledge of the endothelium has highlighted its integral role in a number of pathologies. Endothelial cells are pivotally involved in the recruitment and adhesion of leukocytes and platelets, and they express adhesion molecules and growth factors. This review highlights the recent advances made in the understanding of the endothelium and discusses the endothelium as a potential target in a variety of diseases, including cardiovascular diseases, cancer and inflammatory diseases.

Keywords Adhesion molecule, cancer, cardiovascular disease, endothelial cell, integrin, leukocyte, nitric oxide, platelet, VEGF

Introduction

Knowledge about endothelium has improved considerably in the last few years, highlighting its role as a pharmacological target in pathologies as different as thrombosis and cancer. The endothelium as a target has entered into the field of cancer therapy by different approaches. Firstly, the relevance of neo-vasculogenesis in tumor dissemination established vascular endothelial growth factor (VEGF) as a target for interfering with metastasis recruitment. Secondly, based on the possibility of inducing a break in immune tolerance against autologous angiogeneic endothelial cells, innovative strategies for cancer therapy have been proposed: immune therapy of tumors using xenogeneic endothelial cells as a vaccine will be discussed. Recently, the characterization of endothelial organ specificity has pointed to the possibility of exploiting the heterogenous character of vascular endothelium to prevent metastasis dissemination. Lastly, cytoskeleton proteins as vectors of mechanotransduction have enabled the inclusion of endothelial actin partners as possible targets for pharmacological approaches in cerebrovascular ischemic disease, atherosclerosis and cancer. Indeed, the endothelium-based adhesion process has raised the possibility to select drugs affecting leukocyte and metastasis recruitment, such as nitric oxide (NO) donors, treating cardiovascular and metabolic diseases, such as diabetes, as well as cancer progression.

Endothelium anti-adhesion approaches

Endothelial cells lining the vascular wall express adhesion molecules at their surface, which help to recruit circulating leukocytes for tissue immune surveillance. In the course of infectious disease, due to microbial invasion, or during inflammatory reaction, specific cytokines activate leukocytes, which then secrete massive amounts of reactive oxygen species at the level of the endothelial surface. This event is at the origin of enhanced endothelial adhesiveness and, with the progression of inflammation, responsible for dysfunctional endothelium. Adhesion to the endothelium is therefore a crucial phenomenon in the immune defense of the organism. Because of its potential deleterious consequences, it is modulated *in vivo* by several factors, such as NO or reactive oxygen species detoxifying enzymes, such as glutathione peroxidase (GPx) [1]. Recent findings suggest that inhibition of molecular signaling cascades may constitute a potential target for the interruption of cancer progression. Within the endothelial cell, the actin cytoskeleton is linked on one side to metastasis recruiting adhesion molecules, and on the other side to the extracellular matrix, via integrins, thus constituting the core of an adhesion transducing unit. The mechanical modification of endothelial actin filaments and microfilaments are crucial elements in integrin [2] and adhesion molecule signaling [3]. Finally, recent observations link cell cycle, mechanical factors and tumoral microenvironment to the promotion of neoplastic transformation and dissemination [2,4,5].

Endothelial cells and cancer progression

It appears that tumor cells utilize the endothelial adhesion molecule repertoire to invade tissues. Over the past decade, advances have been made in our understanding of tumor growth and invasiveness. However, the mechanisms by which primary tumor cells become specifically invasive for one or more tissues of different organs is still unclear and under investigation. Several studies have focused on the capacity of a primary tumor to modify its microenvironment, which involves the interplay of integrins with the extracellular matrix and its subsequent solubilization by tumor cells [6,7]. Equally important, is the capacity of tumor factors to modify the host's cell cycle clock, as established for oncoproteins of viral origin such as HIV-protein, Tat or HPV16-E7 [8-10]. As already mentioned, the discovery of VEGF as a critical element for tumor growth [11,12] has drawn attention to the implication of the vascular endothelium itself in cancer. Interestingly, arguments have suggested that cytotoxic agents and, more specifically, chemotherapeutics might display anti-angiogeneic activity in the course of tumor metastasis dissemination [13].

In cancer, recruitment of tumor cells by tissues requires vascular adhesion molecules to be expressed by organ-specific endothelium [14-22]. Specific tumoral factors, such as the E7 viral oncoprotein, enhance endothelial adhesiveness in an organ-specific pattern in cervical neoplasia [23]. Moreover, new observations have emphasized the capacity of tumor cells to proliferate *in situ* on the endothelial surface, stressing the importance of the adhesive phase rather than diapedesis of neoplastic cells [21]. It seems that attachment to the endothelium not only determines the physical site of metastasis, but also provides the necessary anchorage that facilitates cancer cell proliferation. Thus, drugs interfering with endothelial adhesion molecule expression, which is crucial for leukocyte recruitment modulation in inflammatory disease, might also be of value in reducing the attachment and the diapedesis of circulating metastasis in cancer.

Cytokines present in the tumoral microenvironment contribute to the adhesion of tumor cells, in particular tumor necrosis factor α (TNFα) [24]. Tumor cell adhesion is also increased by exposure to lipopolysaccharide (LPS) [25] and oxidative stress, as neoplastic cells constitutively produce H_2O_2 at concentrations comparable to those released by activated leukocytes [26]. Furthermore, a marked reduction in the levels of glutathione, glutathione peroxidase, catalase, vitamin E and C is observed in uterine cervical carcinoma [27]. This impaired antioxidant status is likely to be the consequence of increased levels of H_2O_2. In liver cancer, recent evidence shows that metastasis recruitment is facilitated by tumor-derived pro-inflammatory mediators. Indeed, H_2O_2 mediates vascular cell adhesion molecule (VCAM)-1 expression in hepatic sinusoidal endothelium in the absence of pro-inflammatory cytokines, linking liver inflammation and mestastasis dissemination. Thus drugs interfering with reactive oxygen species, through the limitation of leukocyte recruitment in inflammatory disease, would also be able to inhibit the attachment and the diapedesis of circulating metastasis in cancer.

Adhesion of leukocytes or tumor cells to endothelial cells depends on the expression of adhesion molecules belonging either to the immunoglobulin super-family, or to the selectin family. In the latter, adhesion relies on oligosaccharide structures on the surface of endothelial cells, expression of which varies in a function- and organ-specific way. Lectins interact with glycoconjugates to initiate the adhesion cascade between circulating leukocytes or tumor cells and the endothelium. The expression of E-selectin in specific tissues can expand metastasis dissemination of tumor cells expressing appropriate ligands *in vivo* [29]. Cell-cell interactions mediated by lectins and leading to diapedesis through the endothelium are proposed as possible targets for invasion inhibitors or immunomodulators [30]. Site-directed drug delivery to activated endothelium using Sialyl Lewis(x)-liposomes targeted to E-selectin as vehicles has been documented [31]. Liposomal encapsulation to administer vectors leading to tumoricidal activity at the site of the endothelial target has also been tested *in vitro* [32]. Furthermore, antisense sequences of α(1,3)fucosyltransferase (FUT) that inhibit selectin-mediated adhesion and liver metastasis in colon carcinoma cells have provided a promising adjuvant approach for treatment of disseminated colon carcinoma [33]. Interestingly, radiation can also be used to guide drugs to specific sites of neoplasia or aberrant blood vessels. Following radiation, oxidative stress and the expression of intercellular adhesion molecule (ICAM)-1, E- and P-selectin as well as β3 integrins are induced in endothelial cells. In all tumor models, both circulating blood cells and endothelial cells respond to oxidative stress. For this reason, delivery of drugs to human neoplasms by the use of radiation-guided peptides has been proposed [34].

Endothelial cells for vaccine therapy

Generation of new blood vessels necessary for neoplastic transformation is reminiscent of normal embryonic development and present in rheumatoid arthritis and retinopathy [35-38]. It is now a decade since the relevance of neo-angiogenesis was established for the persistence of solid tumors and metastasis spreading [35,38-40]. New cell culture techniques have been developed allowing the study of tumor vasculature. Specific markers characterize tumor-derived capillary endothelial cells (TdMEC) and represent useful tools for the investigation of tumor invasion [41,42].

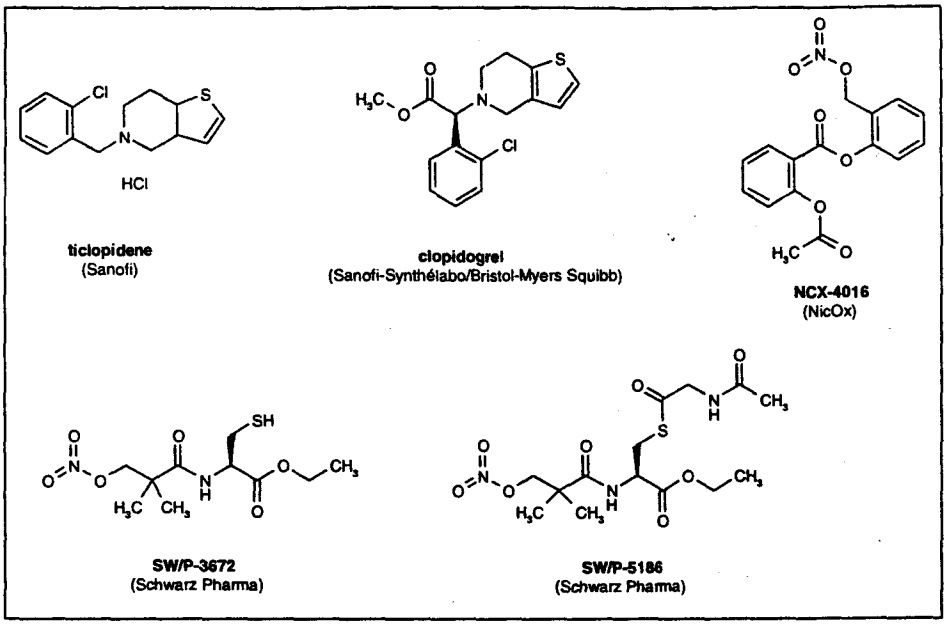
Among the strategies against cancer, the induction of an autoimmune response directed against the tumor endothelium represents one of the most interesting approaches. Within neo-angiogeneic vessels, endothelial cells express proteins at their surfaces that are absent or barely detectable in normal quiescent vascular endothelium, including αvβ3 integrin and receptors for angiogeneic growth factors [36-40]. Blockade of the ligand binding domain of these molecules results in the inhibition of angiogenesis *in vivo* or of endothelial cell proliferation *in vitro*. Utilizing these tools, the feasibility of immunotherapy of tumors based on xenogeneic endothelial cells as a vaccine has been explored [43]. The method consists of breaking immune tolerance against autologous angiogeneic cells in cross-reactions between the xenogeneic homologs and self molecules. In this study, human and bovine endothelial cells were tested as vaccines for their ability to induce antitumor immunity in several tumor models in mice. The results show that fixed xenogeneic endothelial cells induce both protective and therapeutic antitumor immunity. On one hand, the auto-reactive immune response against the microvessels in solid tumors is provoked via the immunization of pre-conditioned mice by xenogeneic endothelial cells. On the other hand, direct antitumor activity and inhibition of angiogenesis is obtained by adoptive transfer of purified immunoglobulins using cross-reactive peptides corresponding to endothelial αv integrin and VEGFR II receptor.

CAD, atherosclerosis and stroke

The endothelium is dysfunctional in atherosclerosis, coronary artery disease (CAD) and stroke. Adequate platelet aggregation, leukocyte recruitment, vasorelaxation and intimal integrity seems to depend on endothelial 'fitness'. In the early 1990s, platelet GPIIb/IIIa receptors were recognized to sustain adhesion of platelets to the subendothelium [44] providing a rationale for their blockade by monoclonal antibodies as an antithrombotic therapy [45]. Acetylsalicylic acid (aspirin), a potent platelet anti-aggregant, is efficacious in the prevention of stroke and myocardial infarction (MI) [46]. Inhibitors of specific platelet agonist-receptor interactions include adenosine diphosphate (ADP) receptor blockers, such as thienopyridine agents, ticlopidine (Sanofi; Figure 1) and clopidogrel (Sanofi-Synthélabo; Figure 1), are efficient in preventing re-occlusion and restenosis following angioplasty and atherectomy [47]. They also reduce cardiovascular death, MI and stroke. In fact, to block platelet aggregation, administration of monoclonal antibodies and RGD analogs that inhibit ligand binding to the platelet glycoprotein IIb/IIIa complex appear to be the most efficient approach [48].

But inhibition of platelet aggregation can be achieved by either inhibition of platelet membrane receptors or by interfering with their signaling with endothelium or activated neutrophils. New agents should therefore preferably interrupt the early stages of platelet activation and initial steps of platelet adhesion to endothelial cells, by inhibiting the platelet-neutrophil interaction occurring at the surface of damaged endothelium. Endothelial cell selectins, such as P-

Figure 1. Structures of compounds with potential in the treatment of cardiovascular disease.



selectin, interact with platelets and leukocytes. Upon adequate stimulation, circulating platelets interact with neutrophils via P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1). Inhibition of this binding may constitute a good target in the treatment of thrombotic reactions, given their inflammatory context [49]. In fact, receptor antagonism provides high specificity, but the inhibition of platelet-induced signal transduction at the level of the endothelial cells also appears to be efficient.

The physiological anti-aggregants are the endothelial factors NO and prostacyclin, which elevate intracellular cGMP or cAMP content, respectively [50]. NO is an important bioactive molecule, which maintains vascular vasodilation, inhibits platelet aggregation and limits leukocyte recruitment by vascular endothelial cells [51]. NO supplementation has been used as a therapeutic approach in ischemic stroke [52]. Alternatively, the endothelial Rho G protein-dependent signaling pathway implicated in adhesion molecule expression in endothelial cells has been targeted [53]. Rho-mediated endothelial actin disruption [54], in an NO synthase (NOS) knockout mouse model, induced a consistent improvement of cerebral blood flow, reaffirming that the endothelial cytoskeleton may be a promising therapeutic target, in this case for the purpose of blood flow normalization in ischemic stroke.

In both CAD and stroke, excessive leukocyte recruitment due to overexpression of adhesion molecules was demonstrated by *in vitro* and *in vivo* studies. *In vitro*, potential anti-atherogeneicity was shown for two novel cysteine-containing NO-donors [55] that inhibited monocyte adhesion and surface expression of endothelial adhesion molecules, such as VCAM-1, ICAM-1 and E-selectin, as well as major histocompatibility (MHC) complex class II antigens expression. The inhibitory effect on adhesion molecule expression following endothelial activation appears to be related to the ability of two molecules, SW/P-3672 (Schwarz Pharma; Figure 1), a fast spontaneous NO releaser and its prodrug, SW/P-5186 (Schwarz Pharma; Figure 1) which liberates NO after bioactivation, to induce NO for prolonged periods of time. More recently, a new NO-releasing aspirin derivative NCX-4016 (NicOx SA; Figure 1), associates anti-inflammatory and anti-thrombotic strategies and displays vasorelaxant effects in normotensive and hypertensive rats [56].

Endothelial progenitors for cell therapy

An increasing number of studies indicate that during development, endothelial and hematopoietic cells derive from a common progenitor, hemangioblasts [57]. The existence of such hemangioblasts in adults was recently suggested as a consequence of the discovery of circulating endothelial cells (angioblasts) in the peripheral blood. Other studies harvested endothelial progenitor cells (EPCs) from undifferentiated mesenchymal cells of aortic rings of 11-week-old human embryos. EPCs generate cardiomyocytes [58], illustrating that the plasticity of endothelial cells during development may be conserved in adulthood - thus opening new perspectives for cell replacement therapies. In order to exploit the morphogenetic capacities of endothelial cells, mature endothelial cells coupled with marrow derived-mesodermal EPCs can be isolated and cultured for expansion [59]. These approaches allow us to envisage valve vessel replacements and directed regional organ-specific angiogenesis for the treatment of ischemic parenchymal and peripheral vascular disease. Furthermore, angioblasts/vascular stem cells could be used to investigate the molecular mechanisms involved in human endothelial cell maturation and acquisition of senescent phenotype. The study of the pathophysiological mechanisms leading to the appearance of the dysfunctional endothelium present in vascular pathologies and vascular regeneration would benefit largely from this approach. Finally, circulating EPCs contribute to neovascularization and are extremely sensitive to angiostatin, and are proposed as putative targets for angiostatin [60].

Conclusion

The new era - targeting endothelium adhesion molecules - consecrates endothelium dysregulation as critical point in thrombosis, atherosclerosis and cancer. NO, the ubiquitous molecule produced by the endothelium not only has changed our way of conceiving biology, but also implies that in the human body, interfering with signaling pathways between organs and tissues may provide important new tools for therapeutic approaches. Growing knowledge on endothelium specificity allows us to envisage the use of xenogeneic cells as vaccines in cancer. Finally, circulating angioblasts represent an original and promising tool for cell therapy.

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