Chapter 9

Future perspectives: Drug development for vascular leak

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« In the field of observation, chance favours only the prepared mind »
-L. Pasteur

INTRODUCTION

Treatment of vascular leak in the clinical setting has been cumbersome thus far. Currently the management of vascular leak involves early recognition and supportive therapy [1,2], which is mainly directed at the prevention of further damage (e.g. swift antibiotic treatment or lung protective ventilation) and maintenance of adequate perfusion by fluid resuscitation [1]. Yet, in many cases severe vascular leak is present at first contact, while in other cases fluid resuscitation does more harm than benefit [3]. The development of effective pharmacological agents that reverse endothelial barrier dysfunction and attenuate vascular leak may therefore be of great benefit here.

Traditionally, the development of pharmacological agents follows design of novel agents based on recognized targets in disease pathophysiology. Here, preclinical research (in vitro or in vivo) results in clinical applications (bench-to-bedside). Alternatively, accidental discovery of drugs or off-target effects of existing drugs may yield direct novel drug applications. Famous drugs flowing forth from this kind of ‘serendipity’ include penicillin [4], warfarin [5] and cisplatin [6]. It was recently estimated that about 25% of available drugs were discovered with aid of serendipity [6], part of which resulted from serendipous clinical observations. Here, clinical observations have boosted experimental research and yielded pathophysiological insights (bedside-to-bench).

In the current chapter we present two clinical observations, which may contain directions for future management of vascular leak. The first case, describing treatment of capillary leak syndrome with imatinib opens perspectives on pharmacological therapy of vascular leak in the near future. The second case is focused on pulmonary lymphangiomatosis, and as such provides novel insight in lung lymphatics. Given the importance of lymphatics for fluid clearance and protection against alveolar flooding [7], this case may serve future research on the pathophysiology of pulmonary edema.
RESULTS

Case 1
Pharmacological agents to reverse endothelial barrier dysfunction and vascular leak are currently absent, limiting clinical management of diseases like sepsis [8], acute respiratory distress syndrome [9] and systemic capillary leak syndrome [10]. The present case suggests that imatinib effectively reverses vascular leak.

A 64-year old woman with unremarkable medical history visited her general practitioner in October 2011 with facial swelling. Specific exposures provoking these symptoms could not be identified, and after several days the swelling disappeared spontaneously. In November she presented at an affiliated hospital with progressive dyspnoea, non-productive cough and fatigue, without fever or orthopnoea. On examination the patient appeared comfortable, without signs of respiratory distress. The blood pressure was 110/54mmHg (baseline measurements: 142/90mmHg in 2010 and 140/80mmHg in 2011), the heart rate 67/minute, temperature 36.9°C, and oxygen saturation 97% at ambient air (FiO₂ 21%). She had a normal jugular venous pressure, but bibasilar rales in the lungs, peri-orbital edema and pitting edema in both legs. She reported a recent increase in bodyweight of 10kg. Blood tests indicated hemoconcentration (hemoglobin 9.6mmol/L or 15.5g/dL) and hypoalbuminemia (albumin 30g/L). Blood count, electrolytes and inflammatory markers were normal, as were renal function and liver enzymes. Chest radiography showed bilateral pulmonary edema, pleural effusion and atelectasis. Cardiac failure was excluded by normal ventricular function on cardiac echography and normal NTproBNP levels (<121ng/L). Other non-infectious causes of general edema, like anaphylaxis, C1-esterase inhibitor deficiency, malignancy (by PET and CT imaging), nephrotic syndrome, protein losing enteropathy, adrenal insufficiency and systemic mastocytosis were excluded. Because of steadily increasing dyspnoea our patient was treated with diuretics and underwent several thoracenteses. Upon further deterioration of clinical condition, she was referred to our academic center for analysis (March 2012).

Systemic capillary leak syndrome was considered, because serum analysis revealed the presence of M-protein (IgGκ 0.21g/dL), a protein associated with capillary leak syndrome [10]. However, the chronic character of the vascular leak and the presence of pleural effusions rendered systemic capillary leak syndrome less likely in our patient. Putative markers of systemic capillary leak syndrome, Vascular Endothelial Growth Factor and Angiopoietin-2 [11], were not detectable (both <0.1ng/mL). To evaluate the severity of the vascular leak in the lungs we measured the pulmonary leak index, which determines the permeability of the lung vasculature based on distribution of radiolabeled protein [12]. The pulmonary leak index in our patient was 23x10⁻³/min, revealing substantially increased permeability of the lung vasculature (normal <14x10⁻³/min), and approaching values found in patients with acute respiratory distress syndrome (>30x10⁻³/min) [12].
Based on these measurements, the increased bodyweight, mild hypotension, hypoalbuminemia, the hemoconcentration, and the absence of a clear eliciting condition, idiopathic vascular leak was diagnosed.

Because imatinib (Gleevec®) was recently shown to protect against vascular leak [13], we started treatment with a low dose imatinib (200mg/day). One month after initiation of imatinib treatment the dyspnoea had largely disappeared, which was objectified by reduced requirement for thoracentesis. Simultaneously, bodyweight normalized and plasma albumin levels increased (Figure 1A), together with disappearance of peripheral edema. Chest radiography demonstrated reduced amounts of pleural fluid (Figure 1B). In August, the pulmonary leak index was $17 \times 10^{-3}$/min, indicating reversal of the increased pulmonary vascular permeability (Figure 1A). The dyspnoea had completely disappeared. No side effects of imatinib were observed. The patient consented to off-label use of imatinib, and provided written informed consent for publication of the case report. The institutional medical ethical committee gave permission for off-label use of imatinib, with reference to compassionate use in the acute setting.

To the best of our knowledge this is the first description of treatment of vascular leak with imatinib in controlled setting. Generalized loss of endothelial barrier integrity forms a key pathophysiological mechanism in (idiopathic) vascular leak. Soluble factors induce contraction of endothelial cells and subsequent gap formation in the endothelial monolayer [8]. The vascular leak that follows disruption of the endothelial barrier carries high mortality in a wide variety of diseases. Laboratory studies recently showed that imatinib protects against endothelial barrier disruption and vascular leak by strengthening endothelial cell-cell contacts and adhesion of endothelial cells to the extracellular matrix [13]. Experimental treatment of our patient with imatinib was followed by normalization of direct (pulmonary leak index, necessity of thoracentesis) and indirect parameters of vascular leak (body weight, albumin levels, hemoglobin levels, dyspnoea).

The observation that imatinib protects against vascular leakage is surprising in the light of previous studies that report peripheral edema as side-effect of imatinib [14]. This paradoxical finding may first of all be explained by duration and intensity of imatinib treatment. Compared to patients treated with imatinib for chronic myeloid leukemia, imatinib treatment in our patient was relatively short, while the imatinib dose given to our patient was low (200mg/day). In addition, the mechanism of edema formation in idiopathic vascular leak likely differs from the mechanism of edema formation as side-effect of imatinib treatment. In idiopathic vascular leak, onset of edema is (sub)acute, and follows dysfunction of the endothelial barrier (either by contraction or apoptosis of endothelial cells) [8]. Although the mechanism of edema formation during imatinib treatment remains incompletely understood, it has been suggested that chronic inhibition (several month to years) of growth factors like Platelet-Derived Growth Factor Receptor impairs pericyte function [15], leading to weakening of the vessel wall. This may explain the more gradual and chronic
onset of edema as observed during long-term imatinib treatment. The use of imatinib in the therapeutic manner employed on our patient may have important implications for conditions that share endothelial barrier dysfunction as common pathophysiological mechanism. The application of imatinib for reversal of endothelial barrier dysfunction may therefore include various conditions like systemic capillary leak, sepsis and acute respiratory distress syndrome, which currently lack specific therapy [8-10].

Causal treatment for vascular leak is currently lacking. Although phase I/II trials are required to assess safety of imatinib in vascular leak-associated conditions, the current case suggests that imatinib may seal the gap.

Figure 1 – Parameters of vascular leak before and after initiation of imatinib treatment. A) Time curves of bodyweight (blue) and albumin levels (red) as indirect parameters of vascular leak, and the pulmonary leak index as direct parameter of vascular leak. The average pulmonary leak index of both lungs is given together with the range of the values measured for the two lungs (error bars). The grey area indicates the period of imatinib treatment (200mg/day). Black triangles represent thoracenteses, of which volumes ranged from 50mL (diagnostic thoracenteses) to 1500mL (therapeutic thoracenteses). B) Chest radiographic examinations for evaluation of pleural fluid. The image on the left reveals the presence of bilateral pleural fluid, while the image on the right shows clear contours of the heart and the diaphragm, suggesting that pleural fluid substantially improved following imatinib.
Case 2

In 2005, a 40-year old woman was referred to our hospital for evaluation of a mediastinal mass and radiological lesions in the spleen, after she had presented with chest pain and increasing fatigue. Surgical exploration of the mediastinum revealed an old, capsulated hematoma, without apparent bleeding focus. Mediastinal biopsies did not yield evidence for malignancy or thoracic endometriosis, while genetic and hematological screening excluded connective tissue disease or coagulopathies as cause of the bleeding. In 2006, she was readmitted for hemoptysis and hematothorax, indicating frequent intrathoracic bleeding. Again, surgical exploration of the thorax did not provide a cause or focus of the bleeding. From 2008 onwards, our patient repeatedly presented with aggravating hemoptysis, accompanied by growing masses in the mediastinum, left lung and spleen as observed on CT. In 2010, histological revision of biopsy specimen yielded the diagnosis diffuse pulmonary lymphangiomatosis.

By that time, our patient suffered from continuous hemoptysis, leading to anemia. Bronchial artery coiling was unsuccessful, whereas the disseminated localization precluded surgical resection. Since VEGF was previously described to mediate lymphatic proliferation [16,17], intravenous treatment with the VEGF-blocking antibody bevacizumab (Avastin®, 1mg/kg every 3 weeks) was initiated. Bevacizumab treatment was followed by cessation of hemoptysis, stabilization of hemoglobin levels (Figure 2A) and impressive reduction of the tumor size (Figure 2B). Post hoc immunohistochemical staining showed increased VEGF-A expression in diseased lymphatic vessels compared to healthy lymph vessels (Figure 2C), despite normal plasma levels of VEGF-A (<0.1 ng/mL). After seven gifts, the bevacizumab treatment was discontinued because of development of hypertension. Until now, ten months after discontinuation, tumor size is stable on CT, and our patient remained without hemoptysis.

Diffuse pulmonary lymphangiomatosis is a rare disorder, characterized by uncontrolled proliferation of lymphatic vessels. Although considered benign, the proliferation can be devastating due to infiltration of surrounding tissues. Reported treatment options are limited and often ineffective [18,19]. In our patient VEGF-blockade by bevacizumab turned out to be effective as treatment of diffuse pulmonary lymphangiomatosis. Thus far, treatment options for lymphangiomatosis have been limited. In 1990, a review on lymphatic disorders stated that “just as the clear fluids of the lymphatics make these vessels invisible to the naked eye, so the medical knowledge and study of the lymphatic system has been nearly invisible” [19]. This lack in medical knowledge has undoubtedly hampered development of lymphangiomatosis treatment in the past. During the last two decades, however, considerable progress has been made in the understanding of lymphatic biology, largely resulting from identification of specific markers of lymphatic endothelium [16]. Among others, understanding of lymph vessel proliferation (lymphangiogenesis) has shed light on the pathophysiology of lymphatic disease. Lymphangiogenesis is driven by VEGF – mainly via
VEGF-C and the lymph-specific receptor VEGFR-3 [16], but also via VEGF-A [17]. While therapeutic interference with VEGF-C/VEGFR-3 is still experimental, ample clinical experience exists with VEGF-A inhibition. Therefore, we treated our patient with the VEGF-A blocking antibody bevacizumab, resulting in fast reduction of tumor size and immediate clinical improvement. A recent case report already suggested involvement of VEGF in lymphangiomatosis, but could not provide mechanistic evidence [20]. The current case, showing increased VEGF-A expression in affected lymph vessels (Figure 2C), and tumor regression upon bevacizumab treatment (Figure 2B), further supports the mediator role of VEGF-A in lymphangiomatosis.

![Figure 1](image1)

**Figure 2 – VEGF and VEGF inhibition in pulmonary lymphangiomatosis.** A) Treatment effects of bevacizumab on blood hemoglobin levels. Three-week gifts of bevacizumab are indicated with an asterisk. B) The extent of lymphangiomatosis in the left lung as visualized on chest CT 1 day before initiation of bevacizumab treatment (left) and 10 weeks after initiation of bevacizumab treatment (right). C) Immunohistochemical staining of VEGF-A in proliferative lymphatic vessels (top) versus unaffected lymphatic vessels from the same area (bottom) (original magnification, ×5). The brown VEGF-A staining is evident in lymphendothelial cells of proliferative lymphangioma vessels (top, inset) (original magnification, ×40), but almost absent in lymphendothelial cells of the vas afferens in a lymph node (bottom, inset) (original magnification, ×40). In addition, in lymphangioma vessels the density of lymphendothelial cells is higher compared to the vas afferens, suggesting proliferation of lymphendothelial cells. The lymphatic character of the proliferative vessels was confirmed by staining for VEGFR-3 (data not shown). CT = computed tomography; VEGF = vascular endothelial growth factor; VEGF-A = vascular endothelial growth factor A; VEGFR-3 = vascular endothelial growth factor receptor 3.

This case contributes to the understanding of lymphangiomatosis by demonstrating VEGF-A as mediator of lymphangiomatosis. Moreover, it indicates that pulmonary lymphangiomatosis can
be effectively treated by bevacizumab, urging further clinical investigation of bevacizumab in lymphangiomatosis.

**DISCUSSION**

These case-reports present two drugs with previously unidentified beneficial effects in vascular disease. The first report shows that the tyrosine kinase inhibitor imatinib (registered for treatment of chronic myeloid leukemia and gastro-intestinal stromal tumors) attenuates vascular leak. The second shows that the VEGF-blocking antibody bevacizumab (registered as anti-angiogenic therapy in cancer) inhibits pathological outgrowth of lymph vessels.

**Drug repositioning**

Both reports form an illustration of a phenomenon called drug repositioning: ‘finding new uses outside the scope of the original medical indication for existing drugs’ [21]. Drug repositioning flows forth from a late paradigm shift in pharmacological sciences, where the old (simplified) adagium ‘one disease – one target – one drug’ has been replaced for the conception that a disease is the result of multiple derangements in a complex network of multiple signaling pathways, and that drugs are almost immanently promiscuous in that they modulate multiple targets [22]. Given the fact that different diseases share common signaling pathways, and existing drugs modulate targets beyond the ones intended, drug repurposing is merely recombination of drugs and targets. The increasing knowledge on cellular signaling, the integration of these pathways in complex, multi-dimensional signaling networks, together with the good accessibility of compound libraries (commercial and academic) provide limitless opportunities for target/compound recombination. Besides these theoretical advantages, drug repositioning offers a number of practical advantages. Because drug repositioning by definition involves compounds that have been clinically used or have been in a clinical test phase, much is already known about toxicity profiles and pharmacokinetics in humans. In addition, the paths for clinical testing have been paved before, which significantly speeds up clinical implementation [22], (see also blog1). Together, mentioned advantages yield drug repositioning a high reward / low risk strategy [21]. These advantages certainly hold true for imatinib and bevacizumab – the longstanding clinical experience with these drugs have proven the drugs safe, and allow swift implementation in phase I/II trials. Application of imatinib for treatment of sepsis and ARDS may, however, require phase I trials, since its safety in critically ill patients has not been tested before.

‘Serendipity’ versus ‘informed insight’

Actual drug repurposing, i.e. the discovery that a certain drug has a beneficial effect on a disease other than that for which the drug was originally developed, either results from pre-existing insight into disease/drug interaction, or from incidental combination of drug and disease. The clinical cases described in this chapter represent one of each. By the time pulmonary lymphangiomatosis was diagnosed in our patient, preclinical studies had indicated the putative role of VEGF in the proliferation of lymph vessels [16,17]. Informed insight in this mechanism combined with the VEGF-blocking effects of bevacizumab, brought about the idea to treat the patient with bevacizumab. The efficacy of bevacizumab in this case also supports the mediator role of VEGF in human lymphangiomatosis, confirming preclinical studies (bench-to-bedside and back).

In contrast, the original discovery of imatinib as possible therapeutic strategy for vascular leak [23] can be fully described as serendipitous. Here, imatinib treatment was initiated because of pulmonary hypertension, but followed by fast (and unexpected) resolution of pulmonary edema. Although the exact mechanism of edema resolution (changes in hydrostatic pressure versus changes in vascular permeability) remained unclear, the case-report described in the current chapter provides evidence for a direct protective effect on the endothelial barrier. Further evaluation of this barrier-protective effect of imatinib effect in experimental models (in vitro and in vivo), have yielded new pathophysiological insights in endothelial barrier function and identification of ARG as novel mediator of barrier disruption (bedside-to-bench) [Chapter 3 and 4].

Together two opposing directions of drug discovery are illustrated here: 1) a well-informed application of preclinical findings in a clinical settings, and 2) serendipitous clinical observations, yielding novel targets.

Forced serendipity

In the pursue for additional pharmacological agents for vascular leak, both serendipity and informed insight may be forged into a single drug development program. One way to achieve this ‘forced serendipity’ is extensive compound screening based on phenotypic read-outs [5]. Phenotype-based instead of target-based screening enhances the possibility of finding clinically relevant compounds, even though underlying molecular mechanisms remain unclear. For vascular leak the Electric Cell-substrate Impedance Sensing (ECIS) assay provides a number of characteristics that enable compound screening. First and most important, the ECIS assay provides a phenotypic read-out (endothelial permeability) directly relevant for vascular leak [24]. Second, its multi-well culturing system (96-well) allows for simultaneous testing of several compounds. The selection of compounds for this phenotype-based screening involves either random screening of existing drugs or target-specific compounds (e.g. ARG inhibitors), depending on the degree
of serendipity that is allowed. Compound libraries of academic institutions or pharmaceutical companies may provide access to existing compounds/drugs. Screening of existing drugs offers advantages mentioned before (availability of some clinical experience, toxicity screenings have been performed, clinical scale-up has often been initiated). In addition, random screening of compounds may reveal novel molecular mechanisms of vascular leak [5]. Drawbacks of random drug screening include its random character (where to start?), associated with the time required to test large amounts of compounds. Target-specific compounds are identified by \textit{in silico} comparison of target/compound structure or can be designed on the basis of crystal structure of the target. Recently, the crystal structure of ARG/Abl2 has been revealed [25]. Advantages of target-based selection include a clear definition of the compounds to be screened. This is, however, compensated by the time required for the \textit{in silico} screening and compound design. Once the ECIS-based screening has identified compounds that effectively attenuate endothelial barrier dysfunction, the barrier-protective effect of these compounds need to be established in other \textit{in vitro} assays (macromolecule passage), \textit{in vivo} models of vascular leak (Miles assay) and relevant disease models.

\textbf{Conclusion}

Altogether, the case-reports presented in this chapter illustrate two ways of drug discovery – development of pharmaceutical agents based on the molecular mechanisms of disease, and clinical observation of drug effects outside the scope of their original use. Current assays for endothelial barrier function and vascular leak may combine these two ways by screening large numbers of existing drugs or novel compounds. This drug and compound screening may well contribute to development of novel agents for vascular leak.

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REFERENCES


