Menin and MEN 1 gene: a model of tumour suppressor system

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Key words: hyperparathyroidism; MEN-1; menin, tumour suppressor gene; endocrine tumours

Introduction

Recent progress in the knowledge of the molecular basis of cancer has led to recognition of the existence of two classes of genes whose mutations may be at the origin of malignant transformation. Overexpression of onco-genes on the one hand, and inactivating mutations or deletions of tumour suppressor genes on the other, may cause transformation of specific target cells. At least 10 tumour suppressor genes are known at present, including Wilms’ tumour (WT) suppressor gene, von Hippel–Lindau (VHL) gene, and retinoblastoma (RET) gene, to name but a few of particular interest to the clinical nephrologist. Loss of function of these genes, which usually imposes some constraint on the cell cycle making cells proliferate more rapidly, promotes the occurrence and the progression of various types of cancer, such as Wilms’ tumour [1], renal-cell carcinoma [2,3], and sporadic malignant parathyroid tumour [4].

Among the familial forms of hyperparathyroidism, multiple endocrine neoplasia (MEN) constitutes a fasci-nating group of proliferative multicentric disorders targeting various endocrine glands, dispersed endocrine cells and, in some cases, neurons and their supporting elements [5]. MEN includes three syndromes, namely MEN 1 (Wermer’s syndrome) [6], MEN 2A (Sipple’s syndrome) and MEN 2B. MEN 1 is characterized by the association of endocrine adenomas in the parathyroid, the pituitary, and the pancreas or small intestine (see below). In MEN 2A enlarged parathyroids are associated with thyroid medullary carcinoma and pheochromocytoma, and in MEN 2B [5] medullary thyroid carcinoma is associated with pheochromocytoma and ocular/oral neuromas with gastrointestinal ganglioneuromatosis. They are usually inherited as autosomal dominant traits but may also occur in a sporadic fashion. Interestingly, tumours are preceded in their development by phases of endocrine-cell hyperplasia [5,7–9].

Since the gene of MEN 1 (‘menin’) has been recently cloned [10,11] we will focus here on the role of menin in the hyperparathyroidism (HPTH) of these patients and discuss how this model may allow to improve our understanding of the role of tumour suppressor genes.

The syndrome of multiple endocrine neoplasia of type 1 (MEN 1)

In 1954, Wermer reported for the first time the simultaneous occurrence of adenomas of the anterior pituitary, the parathyroids, and the islets of the pancreas in a man and four women of the same family. He concluded that this adenomatosis was probably of genetic origin [6]. He underlined that ‘in contrast to what is seen in the usual case of 1° HPTH, the parathyroids show involvement of all four glands.’ He hypothesized that the mode of inheritance was probably dominant, due to a single gene, with a high degree of penetrance and expressivity, and postulated that the gene should play a role in the control of normal and pathological growth of endocrine tissue.

MEN 1 is an autosomal dominant familial cancer syndrome characterized by tumours of parathyroids, pancreas, duodenal endocrine, and anteropituitary cells. It is defined as the occurrence of at least two major lesions of classical MEN 1 in the same patient and one lesion in first degree relatives or one lesion either in parathyroids, pancreas, or pituitary in three first-degree relatives [11]. In unselected autopsies, the incidence of MEN 1 seems to be up to 0.25% [12]. HPTH is the most common clinically evident component of MEN 1 and its prevalence is over 90% at the age of 40. Diagnosis of MEN 1-related HPTH can be delayed by several years because hypercalcaemia generally remains asymptomatic in the early stage of the disease [13]. Unlike sporadic HPTH which affects more frequently women, the HPTH of MEN 1 occurs equally in both sexes, and patients with MEN 1 are usually younger at the time of diagnosis [5,7,9]. MEN 1-associated enlargement of the parathyroid is multi-glandular and asymmetric, frequently with one or two glands of normal or minimally enlarged size [14]. From a histopathological point of view, parathyroid lesions correspond to diffuse or nodular chief cell hyperplasia in most cases [9]. In addition, affected patients may

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have evidence of gastrin-producing tumours within the pancreas and the duodenum, carcinoid tumours of the lung, thymus and gastrointestinal tract, adenomas or hyperplastic nodules of the adrenal [15] and thyroid glands, multiple soft tissue lipomas [5], and multiple facial angiofibromas. The latter are also observed in tuberous sclerosis [16]. Except for gastrinomas, most of these tumours are non-metastasizing neoplasias. However, with regard to parathyroid tumours it is of note that although removal of a single enlarged gland may result in the temporary amelioration of the signs and symptoms of HPTH, there is a high frequency of recurrence in patients with MEN 1 who have been thus treated [17,18].

Cloning of menin gene

Initially, comparison of leukocyte-derived and tumour-derived RFLPs showed loss of heterozygosity at chromosome locus 11q13, with either total or subtotal allelic deletions, in parathyroid tumours and malignant insulinomas from patients with MEN 1 [19–21]. The hunt for the MEN 1 gene took another 9 years. It was eventually cloned in 1997 by two independent groups [10,11]. Located in the pericentromeric region on the long arm of chromosome 11, band 11q13, in an interval bounded telomerically by D11S4936 [10] or D11S1783 [11] and centromERICally by PYGM, the MEN 1 gene contains 10 exons and encodes a ubiquitously expressed 2.8-kilobase transcript [10,11]. The predicted protein encoded by MEnI gene, named ‘menin’, contains 610 amino acids, has apparently no similarities with previously described proteins, and is expressed ubiquitously in adult tissues [10]. Over 40 different germ-line mutations distributed throughout all the open reading frame have been found in investigated MEN 1 kindreds, suggesting the absence of founder effect [11]. The sequence abnormalities are either insertional or deletional frameshift, non-sense, or miss-sense mutations which would predict a premature truncation of the menin protein, consistent with its putative role as a tumour suppressor gene. Combined pedigree and tumour studies demonstrated that the tumour-related allelic deletions of chromosome 11 occurred on the chromosome inherited from the normal parent and not from the affected parent. Therefore it is very likely that familial MEN 1 tumours develop according to the two-hit mutation theory of Knudson. Thus far no correlation between genotype and phenotype was found.

Other gene defects and importance of MEN 1 gene in hyperparathyroidism

Recent results of molecular genetic studies in parathyroid tumour biology have provided major insights into primary and secondary HPTH. Using a method based on DNA analysis to demonstrate X-chromosome inactivation, it has been shown that primary parathyroid adenomas are exclusively monoclonal tumours [22]. This is also the case in the majority of parathyroid glands removed from uraemic patients with severe secondary HPTH [23,24].

As to the underlying genetic molecular mechanisms, an activation of the PRAdI cyclin D1 oncogene has been demonstrated to occur in 5–18% of primary parathyroid adenomas [25,26]. The activation is driven by the aberrant placement of this gene in close proximity to enhancer elements of the PTH gene. A number of various somatic gene mutations are probably involved in other primary adenomas as well as in secondary HPTH [26–29]. Loss of activation of the RB gene or of another gene on 13q is an important factor in the genesis of parathyroid carcinoma and aggressively growing benign parathyroid tumours [30–32]. In patients with MEN 2A and MEN 2B, germline mutations at specific sites in the RET proto-oncogene have been shown. In these syndromes, in contrast to what is observed in MEN 1, activating germline mutations are responsible of tumoral proliferation [5]. It is interesting to note that mutations of the RET gene are also responsible for autosomal dominant form of Hirschsprung disease [33]. Moreover, renal agenesis or dysgenesis is observed in mice homologous for a targeted mutation in RET [34]. In hyperparathyroidism–jaw tumour syndrome, a rare autosomal dominant condition characterized by recurrent parathyroid tumours and mandibular or maxillary tumours, parathyroid carcinoma and cystic or tumoral lesions of the kidney are not rare [35,36].

Mutations of the MEN 1 gene in germline DNA are detected not only in MEN 1 families but also in sporadic cases of MEN 1 [37]. A somatic mutation in the MEN 1 gene and an allelic deletion over chromosome 11q13 have been found in 20% of sporadic parathyroid tumours in one recent study [38] and in up to 40% in another [39]. This suggests that somatic point mutations of this gene may be a relatively common event.

MEN 1 gene, tumour monoclonality and cancer

Several facts argue for a role of the MEN 1 gene in the development of a variety of common tumour types. Allelic loss over chromosome 11q has been found in several malignancies, including breast, kidney, ovary and cervix cancer, as well as in sporadic parathyroid, pituitary and other endocrine tumours, in accordance with the ubiquitous presence of menin in a large number of tissues [40]. Thus MEN-1-associated tumours may be the tip of the iceberg; menin may be a key cell-cycle regulator in many tissues and its inactivation by somatic mutations could be an important step in the development of many common tumour types [41].

However, several physiopathological problems concerning the role of the MEN 1 gene in tumour genesis remain: first, monoclonality of parathyroid nodules associated with MEN 1 does not appear to be necessary to allow autonomous growth [42]. Second, the stimulation of endothelial cell proliferation in endocrine tissue by basic fibroblast growth factor (bFGF) released into the peripheral circulation [43–45] has not been
attributed to the MEN 1 gene up to now. The role of circulating bFGF-like autoantibodies in growth stimulation of endocrine cells of some cases of MEN 1 associated with prolactinoma must be confirmed [46,47]. Finally, chromosomal instability present in tissues from MEN-1 patients suggests that the MEN-1 gene could play a role in cell-cycle and/or DNA repair regulation [48].

Conclusion and future
Tracking the tumour suppressor gene MEN-1 (menin) should soon lead to the discovery of the functional role of the menin protein and an understanding of the place of somatic MEN-1 gene mutations in the pathogenesis of various endocrine tissue tumours. It should also permit the development of an exciting field of applications, extending from presymptomatic diagnosis of MEN-1 disease and perhaps the identification of new pharmacological agents to gene therapy of endocrine deficiency states such as type 1 diabetes mellitus by facilitating pancreatic islet-cell transplantation.

References
Introduction
Arterial hypertension, defined as a blood pressure higher than 160/95 mmHg, is present in more than 70% of the patients with acute stroke [1], and mostly disappears spontaneously in the days following stroke onset without specific therapy [2]. The mechanisms responsible for this blood-pressure elevation are unclear. Central mechanisms, catecholamine and corticosteroid release, mental stress of acute illness, and hospital admission are discussed [3]. A rise in systemic blood pressure in the acute phase of stroke may be a physiological response to a decreased blood flow in the ischaemic penumbra surrounding the core of infarction. A higher initial systemic blood pressure has been found in patients with cerebral haemorrhage compared to those with cerebral infarct, and also in patients with known and treated hypertension [1,2].

The preventive role of antihypertensive treatment in reducing morbidity and mortality from stroke is well established. Treatment that causes a long-term reduction in diastolic blood pressure of 5–6 mmHg results in a 35–40% fall in the risk of stroke [4]. In the setting of acute stroke, blood pressure reduction is one of the most common therapies. Too frequently blood pressure is reduced too rapidly, with little attention to the patient’s prior blood pressure status or to the end organ at greatest risk, the brain. However, no randomized controlled studies of antihypertensive treatment in the acute phase of stroke have been performed. No consensus has been reached concerning indication and mode of blood pressure treatment in acute stroke.

Arguments not to treat hypertension
Justification and rationale for not treating hypertension in acute stroke are based on the fear of worsening the neurological deficit [5,6] as a result of reduced cerebral blood flow (CBF), because autoregulation mechanisms are defective. Physiologically the CBF is kept constant by a mechanism called autoregulation at a level of 55–60 ml/100 g/min in a range of mean systemic arterial pressure between 55 and 125 mmHg. In patients with chronic hypertension, autoregulation starts at a higher systemic pressure. Below this threshold a direct relationship between cerebral blood flow and mean arterial pressure exists [7]. In the acute phase of stroke, a central core of brain infarction is surrounded by the area of ischaemic penumbra, in which the autoregulation of the cerebral blood pressure is impaired (see Figure 1). Perfusion in this area is too low to maintain normal neuronal function, but sufficient to keep the neurons alive for a short period of time. The blood flow in the ischaemic penumbra is pressure dependent [8,9]. A reduction in systemic blood pressure may therefore result in further reduction of the perfusion in the ischaemic penumbra, thereby causing ischaemic

Hypertension in patients with cerebrovascular accident. To treat or not to treat?

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haemorrhagic transformation, especially in anticoagulated patients, are reasons to treat high systolic blood pressures [10,11]. In experimental animals, inducing hypertension may cause worsening of the oedema associated with focal ischaemia, but these findings are inconsistent [9]. It has never been shown that reduction in arterial pressure will lessen oedema formation. Oedema is a result of ischaemia. In addition, reducing blood pressure worsens ischaemia, therefore ischaemia-induced oedema may also be favoured by low blood pressure.

Fig. 1. Regional cerebral blood flow (ml/100 g/min) in the baboon hemisphere immediately after middle cerebral artery occlusion. The central core of severe ischemia (A) is surrounded by a zone with less reduction in blood flow in which flow may be at or just above critical levels for neuronal function or viability (B). There is little or no flow reduction in the remainder of the hemisphere (C) (modified from [9]).

Table 1. Conditions in acute stroke in which acute blood pressure lowering should be instituted

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Practical recommendations</th>
</tr>
</thead>
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<tr>
<td>Acute myocardial infarction</td>
<td>Considering all arguments and the lack of controlled studies, at the moment only pathophysiologically based therapeutic guidelines can be formulated. Currently most authors suggest treating only extremely elevated blood pressure. In the American Heart Association’s Guidelines for the management of patients with acute ischemic stroke [12], in a patient with ischaemic stroke, antihypertensive treatment is recommended if mean arterial blood pressure is &gt;130 mmHg, or if systolic blood pressure is &gt;220 mmHg. Brott and MacCarthy recommend starting antihypertensive treatment if systolic blood pressure exceeds 240 mmHg or diastolic blood pressure 140 mmHg [13]. There are, of course, exceptions to the rule not to treat even moderate hypertension in ischemic stroke (see Table 1). Aortic dissection and also an acute myocardial infarct may require lowering of the systemic blood pressure as a life-saving therapy. Hypertensive stroke patients receiving thrombolytic therapy have a higher rate of intracerebral haemorrhage [14,15].</td>
</tr>
<tr>
<td>Hypertensive encephalopathy/malignant hypertension</td>
<td>Table 2. Suggested algorithms for treatment of acute hypertension from the literature</td>
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### Arguments to treat hypertension

The risk of inducing brain oedema, associated with uncontrolled hypertension, and the risk of secondary tissue to become infarcted, and result in even greater neuronal damage.

In patients with high-grade stenoses of the internal carotid artery, low blood pressure may case haemodynamically induced infarction. In addition partial occlusion of an arterial vessel may create decreased and turbulent flow in the remaining patent portions of the vessel. Decreasing blood flow due to hypotension will facilitate thrombus formation and potentially extend the original occlusion [5].

### Practical recommendations

Considering all arguments and the lack of controlled studies, at the moment only pathophysiologically based therapeutic guidelines can be formulated. Currently most authors suggest treating only extremely elevated blood pressure. In the American Heart Association’s Guidelines for the management of patients with acute ischemic stroke [12], in a patient with ischaemic stroke, antihypertensive treatment is recommended if mean arterial blood pressure is >130 mmHg, or if systolic blood pressure is >220 mmHg. Brott and MacCarthy recommend starting antihypertensive treatment if systolic blood pressure exceeds 240 mmHg or diastolic blood pressure 140 mmHg [13]. There are, of course, exceptions to the rule not to treat even moderate hypertension in ischemic stroke (see Table 1). Aortic dissection and also an acute myocardial infarct may require lowering of the systemic blood pressure as a life-saving therapy. Hypertensive stroke patients receiving thrombolytic therapy have a higher rate of intracerebral haemorrhage [14,15].

Table 2. Suggested algorithms for treatment of acute hypertension from the literature

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>Brott et al. [13]</td>
<td>BP&gt;140 mmHg</td>
<td></td>
<td>Nitroprusside 0.5–10 µg/kg/min i.v.</td>
</tr>
<tr>
<td></td>
<td>BP&gt;230 and</td>
<td></td>
<td>Labetalol 20 mg i.v.</td>
</tr>
<tr>
<td></td>
<td>BP 121–140 mmHg</td>
<td></td>
<td>Labetalol 200–300 mg p.o.</td>
</tr>
<tr>
<td>Krieger et al. [18]</td>
<td>BP 180–230 and/or BP&lt;120 mmHg</td>
<td></td>
<td>Do not treat (unless patient is a candidate for thrombolytic therapy)</td>
</tr>
<tr>
<td></td>
<td>BP&gt;230 and</td>
<td></td>
<td>Labetalol 10 mg i.v.</td>
</tr>
<tr>
<td></td>
<td>BP 120–140 mmHg</td>
<td></td>
<td>Nifedipine 10 mg s.i.</td>
</tr>
<tr>
<td></td>
<td>BP&gt;140 mmHg</td>
<td></td>
<td>Urapidil 12.5 mg i.v.</td>
</tr>
<tr>
<td>Wijdicks [19]</td>
<td>Mean arterial pressure</td>
<td></td>
<td>Nitroprusside 0.5–10 µg/kg/min i.v.</td>
</tr>
<tr>
<td></td>
<td>&gt;130 mmHg</td>
<td></td>
<td>Labetalol 20 mg i.v.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Esmolol 500 µg/kg/min i.v.</td>
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<td></td>
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<td></td>
<td>Enalaprilat 1 mg i.v.</td>
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BP, blood pressure; p.o., orally; s.l., sublingually; i.v., intravenously.
thrombolytic therapy the blood pressure should not exceed 185 mmHg systolic and 110 mmHg diastolic to avoid intracerebral hemorrhage [16]. Hypertensive encephalopathy does require immediate therapy, but it can be difficult to distinguish this syndrome from stroke associated with severe hypertension.

A variety of antihypertensive drugs are available for the acute management of hypertension. Preferred drugs for parenteral administration are the alpha-antagonists urapidil and clonidine, the vasodilators nitroprusside and hydralazine and the sympatholytic agent labetalol. Preferred drugs for oral administration are the calcium-channel antagonist nifedipine, the sympatholytic agent labetalol, and the angiotensin-converting enzyme inhibitor captopril. Factors to be considered in selecting the most appropriate drug for the management of hypertension in acute stroke patients include severity and lability of the hypertension, preferred route of drug administration, aetiology of the hypertension, and concurrent disorders. In cases of intracerebral bleeding, drugs with ICP-elevating side-effect like nifedipine, nitroprusside, and hydralazine should be avoided. Some treatment strategies collected from the literature and an algorithm used in our department are shown in Tables 2 and 3.

Patients with acute stroke respond sensitively to hypotensive treatment, especially if they are elderly or have a pre-existing hypertension [17]. Short-acting substances with moderate efficacy should be used to avoid hypotension. Rapid changes in blood pressure may compromise the brain. The target blood pressure should be achieved over the initial 12–24 h. The initial rate of reduction should be no more than 5–10 mmHg/h for the first 4 h. Thereafter, a rate of no more than 5–10 mmHg/h should be sufficient without posing added risk [13].

References


The renal risks of high-dose intravenous immunoglobulin treatment

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Introduction

The polyvalent intravenous immunoglobulin preparations (IvIg) were introduced in the seventies for replacement therapy in patient with primary immunoglobulin deficiency. An ingenious observation made in 1980 suggested that the administration of high-doses IvIg may have immunomodulatory functions [1]. Idiopathic thrombocytopenic purpura was the first disease in which the positive effects could be demonstrated [2]. Since then IvIg has been tried in almost every possible autoimmune or inflammatory disease, with clear benefit at least in some situations. There have been many investigations addressing the issue how IvIg modulates autoimmunity. Several studies illustrated the role of interactions between idiotypic and antiidiotypic antibodies, others the effects indicated by the Fc or type of the infused immunoglobulins, e.g. inhibition of Fc receptors, or blockade of complement activation. Many additional mechanisms have been explored including the capacity of IvIg to neutralize bacterial superantigens, or to modulate the normal repertoire of B and T lymphocytes in the short-term and long-term [3]. Repeatedly, it has been suggested that the presence of other substances co-purified with IgG and present in IvIg contribute to the modulatory properties of IvIg. Recently, transforming growth factor beta (TGF-β) was found in all batches of various IvIg preparations [4]. Immunoglobulins have been shown to be a carrier for TGF-β, and TGF-β is a potent immunosuppressive agent in many animal studies. From these observations, it does not require an extraordinary leap of imagination to suggest that part of the effects of IvIg is due to the presence of TGF-β.

Despite the vast literature on the subject, the evidence-based indications of IvIg are limited and include only a few autoimmune diseases, e.g. Guillain–Barré syndrome, idiopathic thrombocytopenic purpura, dermatomyositis and Kawasaki syndrome. In many other diseases IvIg have been tried with some or only limited success (see University Hospital Consortium Expert Panel review [5]).

The side-effects of IvIg are diverse, most are due to a too fast infusion rate of the immunoglobulins [6]. The incidence of side-effects of IvIg is reported to be around 5–15%. The majority of these side-effects are minor including headaches and rash, although a recent study has suggested that they were sufficiently serious to lead to the interruption of the treatment in approximately 10% of the patients [7]. Life-threatening side-effects are very rare and have been reported in the form of anaphylaxis (hypotension, bronchospasm), particularly in patients with IgA deficiency. The transmission of viral hepatitis has been a troublesome problem over the years. Despite modern manufacturing practices, the recent transmissions of hepatitis C by a well accepted IvIg preparation [8] should remind us to use blood derived products only for well established indications or in the frame of clinical studies.

IvIg has been tried in several types of renal diseases. To our knowledge, there is no conclusive evidence that IvIg has a significant positive effect in any form of glomerulonephritis. Some uncontrolled trials suggest that IvIg may have modulatory effects in systemic lupus erythematosus (SLE) and systemic vasculitis. Nephrologists are confronted, however, with one of the major side-effects of IvIg, i.e. acute renal failure following the administration of high-doses of IvIg.

The early years

The first report of an acute renal failure after IvIg concerned a patient with cryoglobulinaemia type II (monoclonal IgM rheumatoid factor/polyclonal IgG) who had high levels of the monoclonal rheumatoid factor [9]. This patient developed a lymphoma and hypogammaglobulinaemia (low IgG). She received chemotherapy, but had several episodes of pneumonia. Fifteen grams of gammaglobulin were infused over 4 h. Oliguria with rising serum urea and creatinine developed within 24 h. A renal biopsy performed after 5 days revealed that the glomerular capillaries were occluded with hyaline thrombi surrounded by polymorphonuclear leukocytes. Some arterioles and other small blood vessels were also filled with similar hyaline thrombi. Ultrastructural and immunofluorescence studies showed clearly that the material was cryoglobulin including the rheumatoid factor, with surrounding complement activation. The worsening renal function was relieved only after plasmapheresis. This observation corresponds to rare descriptions made in patients with cryoglobulinaemia in whom the very high levels of cryoglobulins are responsible for capillary obstruction in the glomeruli. Although the binding affinity between the monoclonal rheumatoid factor (antibody) and the polyclonal IgG...
The question as to why IV Ig produces renal failure is unlikely to be the major culprit, since the patients receiving IV Ig for neurological disease [7]. Of 88 patients receiving 1–18 courses of IV Ig for a total of 650 infusions, there was one case of acute reversible renal failure in a 62-year-old diabetic patient who had had a high creatinine concentration (1.9 mg/dl) and proteinuria (761 mg/24 h) before therapy.

**Anecdotal observations of acute renal failure**

After this observation, there was a series of case reports, which all indicated that the administration of high-dose IV Ig can be followed by acute renal failure (see reviews [14,15]). Fifty patients have been included in the review by Cayco et al. [15], and new cases continue to be published [16,17]. This flurry of reports leaves no doubts about a direct causal link. In fact, it is surprising that this side-effect had not been reported during the first 10 years of the use of high-dose IV Ig. The incidence of renal failure is certainly low, however, as suggested by a retrospective analysis of all patients receiving IV Ig for neurological disease [7]. Of 88 patients receiving courses of IV Ig for a total of 650 infusions, there was one case of acute reversible renal failure in a 62-year-old diabetic patient who had had a high creatinine concentration (1.9 mg/dl) and proteinuria (761 mg/24 h) before therapy.

**Experimental data**

There are only few experimental studies which address the question as to why IV Ig produces renal failure. Initially it had been noticed that a slight, but significant increase in creatinine was seen in all patients; it was not, however, accompanied by a commensurate rise in urea [18]. Three of the patients were studied in more details during the two courses of IV Ig infusions they received. Before infusions, the creatinine clearance was higher than the inulin clearance because of the known tubular secretion of creatinine, particularly in patients with heavy proteinuria. After IV Ig infusions on five occasions a marked decrease of creatinine clearance, but not of inulin clearance, was noted. Only once did creatinine and inulin clearance decrease simultaneously. These observations suggested that the IV Ig preparation used interfered with the secretion of creatinine, i.e. produced a tubular dysfunction. In nine patients treated for glomerulonephritis Rostoker et al. [19] monitored the urinary excretion of tubular enzymes during IV Ig infusions. They found a significant rise in urinary alanine aminotranspeptidase after the treatment, and noted a small increase in creatinine in three patients, again without a concomitant increase in urea. Taken together these data favoured the hypothesis that IV Ig induces proximal tubular dysfunction. A more severe form might lead to transient renal failure in a time frame corresponding to the recovery of tubular function after an acute injury, i.e. some weeks.

**The sucrose hypothesis**

The histological findings in renal biopsies provided further arguments against the notion that the mechanism of renal failure after IV Ig was glomerular deposition of immune reactants. Except for the first patient described above, none of the biopsies showed evidence for an immunological reaction. The lesions mostly concerned the proximal tubules with marked swelling of the cells, cytoplasmic vacuolization and degeneration of proximal tubular epithelial cells. The lumina were occluded by the swollen cells. The histology corresponded to what is known to pathologists as ‘osmotic nephrosis’. Sucrose was rapidly considered a candidate for such histological lesions, since most patients with transient renal failure after IV Ig had received sucrose-containing preparations. Sucrose is a disaccharide which is filtered by the glomerulus and reabsorbed by tubular cells. It is remarkable that the brush borders of the tubular cells do not contain disaccharidase so that the sucrose cannot be degraded within the cells. In 1942 it was described for the first time that sucrose caused renal lesions [20]. The ‘sucrose nephrosis’ was characterized by marked swelling and vacuolization of proximal tubular cells [21,22]. Several experimental studies analysed the ultrastructural features of sucrose toxicity, but the mechanisms underlying sucrose toxicity were not clearly understood. Other hyperosmotic solutions, such as mannitol and dextran, are known to cause renal failure which is characterized by similar renal lesions. However, the hyperosmolality of the IV Ig per se is unlikely to be the major culprit, since the patients
do not present a true hyperoncotic syndrome. The hypertonic IvIg solutions are quickly diluted in blood. Furthermore, if it was hypertonicity causing tubular damage, one would expect to see the most severe lesions in the distal proximal tubule, where the osmolality due to reabsorption of water and sodium is highest. But histological examinations indicated that the proximal segments of the proximal tubule were most severely damaged [23]. In animal studies, when parenteral carbohydrate solutions were infused the tubular cells contained the infused substances within vacuoles and these were shown to be of lysosomal origin. The proposed sequence was uptake of sucrose by tubular cells through pinocytosis, followed by incorporation into lysosomes. Sucrose is not quickly degraded. Consequently, it will accumulate and cause lysosomes to enlarge, rupture and to fuse, finally forming vacuoles [23]. However, in patients with acute renal failure after IvIg therapy sucrose or more simply carbohydrates have not been shown to be present in the vacuoles of the swollen cells. In conclusion, it is more likely that the swollen cells are the result of a toxic damage to the cells induced by sucrose, rather than the consequence of accumulation of sucrose.

Another series of observations favours the sucrose hypothesis. Some patients have transient renal failure following sucrose containing preparations, but had no renal side-effects following maltose or glycerine containing IvIg [24].

Based on the foregoing we propose the following hypothesis. Sucrose produces some tubular toxicity in all patients who receive it. Clinically manifest renal failure requires the presence of additional factors which include the rate of infusion, the amount of infused material, pre-existing renal disease or tubular dysfunction, dysfunction of other organs, malnutrition, etc. A detailed analysis of the possible risk factors has indicated pre-existing renal failure and age over 65 as the two most important predictors of acute renal failure after high-dose IvIg [15].

Alternatives to the sucrose hypothesis

Immune-mediated renal damage after IvIg might be of pathogenic relevance on some occasions, as suggested by the initial cases reported. The other substances found in IvIg which systematically co-purify with Ig may be detrimental as well. TGF-β is known to increase fibrosis in many renal pathologies, but there are to date no reports of ‘osmotic nephrosis’ induced by TGF-β. Still, the question remains open, whether so far unidentified substances are also nephrotoxic.

Conclusion

If our hypothesis is correct and if sucrose is the major substance responsible for acute tubular dysfunction, use of new preparations of IvIg containing no sucrose may well stop the ‘endemic’ renal failure after IvIg. Until then, beware of the sugar!

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Polymorphonuclear neutrophils in acute renal failure: new insights

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Introduction

There is still no specific therapy for acute renal failure (ARF) despite the multiple investigative efforts to clarify its complex pathophysiology. ARF occurring during kidney ischaemia/reperfusion is the classical pathological entity in which polymorphonuclear neutrophil (PMN) recruitment during reperfusion appears to be a significant mediator of kidney injury.

Evidence for the role of PMN in acute renal failure

Even though diverse communications have shown that PMN can be increased and activated in renal ischaemia/reperfusion [1,2], definitive evidence which demonstrates that PMN have a necessary role of their own in the outcome of ARF is still lacking. As a more general interpretation, it is not yet clear whether the mechanisms involved in the ischaemia/reperfusion response are actually different, in the early phase, from those triggered by infectious agents or trauma. Furthermore, the participation, relative importance and sequence of interactions of the individual agents potentially involved in the activation of PMN in the setting of ischaemia/reperfusion and ARF need to be clarified. The issue of the importance of PMN activation is not without therapeutic consequences. For example, the use of biocompatible membranes has been encouraged for patients with ARF requiring haemodialysis, due to their lower PMN-activating properties, which might result in improved recovery of renal function [3].

The following are elementary questions pertinent to the issue of PMN accumulation in ARF. (i) What are the mechanisms responsible for triggering, maintaining and relieving PMN accumulation? (ii) What are the conditions which determine the injuring potential of these PMN? (iii) What is the actual pathophysiological importance of PMN in the evolution and outcome of ARF? (iv) What therapeutic interventions can be carried out to limit the deleterious effects of PMN accumulation? In order to ascertain the actual importance of PMN in ARF, the real magnitude of PMN accumulation in renal ischaemia/reperfusion processes must be determined.

Conventional histological techniques may not be sufficient to demonstrate increased amounts of PMN, and more specific techniques, such as measurements of specific enzymatic activities, e.g. myeloperoxidase [4,5], or histochemical stainings, e.g. naphthol AS-D chloroacetate esterase [2], may be required to detect PMN infiltration.

In our experience, myeloperoxidase activity determination is significantly more sensitive than optical microscopy [4,5] for studying PMN activation during renal ischaemia/reperfusion. The possibility exists that myeloperoxidase released by activated PMN becomes attached to cell membranes and, therefore, may be increased even in the absence of histological evidence of the presence of PMN.

Activation of PMN is a critical component of several processes causing organ damage. In fact, PMN accumulation is a common finding, not only in ARF but also in other acute renal diseases. In this regard, PMN activation and/or accumulation is an important feature in entities as different as post-streptococcal glomerulonephritis [6], Puumala hantavirus-induced nephropathia epidemica [7] or haemolytic uraemic syndrome [8]. In acute glomerulonephritis, adhesion molecules, granule enzymes, reactive oxygen radicals, lipid metabolites and cytokines of activated neutrophils and monocytes mediate glomerular capillary constriction and occlusion, with different degrees of tubular damage and subsequent ARF [6]. In haemolytic–uraemic syndrome, verotoxin-1 modulates leukocyte–endothelium interaction, thus increasing leucocyte adhesion and up-regulating adhesive proteins on endothelial surface membranes [8]. It is difficult, however, to ascertain whether PMN accumulation is or is not mandatory for the complete development of these pathologies and to identify the putative common points and differences between the effects of PMN infiltration in ARF and other conditions.
Mechanisms of neutrophil activation in ischaemia/reperfusion

The precise mechanisms leading to the activation of PMN in ischaemia/reperfusion are insufficiently characterized. During tissue reperfusion, a host of oxygen free radicals, autacoids and autocrine, paracrine and endocrine mediators and cytokines are released, which result in changes in the adhesive properties of the endothelium, in the chemotactic attraction of cells from the blood and in localized and selective changes in vessel permeability. Leucocyte adhesion molecules appear to facilitate PMN recruitment in this setting. Studies using monoclonal antibody, antisense oligonucleotide and gene ‘knock-out’ techniques indicate that blockade of CD11/CD18 integrins and intercellular adhesion molecule-1 (ICAM-1) attenuates ARF in some experimental models of renal ischaemia [9,10]. Linas et al. have shown that neutrophil retention, activation and renal injury can be completely prevented with anti-ICAM-1 [11]. These authors also demonstrated that previous activation of PMN is an important condition to provoke renal PMN accumulation during ischaemia/reperfusion. This phenomenon might be of particular importance in the case of ARF in patients with sepsis, who might present both renal hypoperfusion and PMN activation [11,12]. In fact, PMN obtained from ischaemic kidneys were able to induce ARF when perfused in isolated kidneys, suggesting that the role of PMN in the whole phenomenology of ARF is remarkably important. The fact that fully developed ARF occurs in neutropenic subjects raises a caveat in attributing an important role to PMN.

Agents potentially involved in the accumulation of neutrophils in acute renal failure: endothelin 1 (ET-1) and nitric oxide (NO)

The release of endothelin-1 is increased in ischaemia/reperfusion conditions. The simultaneous occurrence of increased concentrations of ET-1 and PMN infiltration after ischaemia/reperfusion suggests the possibility that both are related phenomena. In fact, the administration of ET-1 antisera or ET-1 antagonist has been shown to ameliorate the functional deficits resulting from ischaemic ARF [13,14]. The observations of Morita et al. [15], who have shown that untreated and activated PMNs led to a 1.4- and 6.3-fold increase in ET-1 mRNA levels in endothelial cells, respectively, suggest that in conditions of direct PMN-endothelial cell contact, an intensified expression of the ET-1 gene might occur. The first hint regarding a possible effect of ET-1 on PMN was provided by Ishida et al. [16] who communicated that ET-1 stimulates the production of superoxide anion by the chemotactic peptide, N-formylmethionyl-leucyl-phenylalanine (fMLP). Other authors found that ET-1 provoked a [Ca^{2+}] transient on PMN [17]. The ET-1-induced [Ca^{2+}] transient was blunted when the PMN were pre-treated with the NO precursor, l-arginine, suggesting the inhibition of ET-1’s effect by NO. The role of ET-1 as a priming factor for PMN was illustrated further by Hafstrom et al. [18], who demonstrated that both ET-1 and neuropeptide Y are able to prime PMN oxidative metabolism and the fMLP-induced rise in [Ca^{2+}]. It is of interest that ET-1 alone failed to stimulate reactive oxygen species generation [16], favouring the possibility of a limited, selective degree of stimulation, instead of a massive PMN activation.

Additional studies have provided further information to clarify the mechanisms by which ET-1 acts on PMN. ET-1 induces dose-dependent PMN aggregation starting 1 min after ET-1 exposure [19]. This aggregation is accompanied by a significant increase in the production of platelet-activating factor (PAF) by PMN. In this regard, PAF receptor antagonists significantly blunted the ET-1-induced [Ca^{2+}], peak and aggregation [19]. In addition, ET-1 stimulates PMN adhesion to cultured endothelial cells (increase of 1×10^4 ± 1×10^4 neutrophils per well) [20]. The ET-induced adhesion was blocked (83±6%) by the anti-CD18 antibody TS1/18 and by several anti-β-subunit antibodies. The expression of CD18 and CD11b on the neutrophil surface was also increased by ET-1, suggesting another putative functional effect of the ET-1-mediated activation of the neutrophils. The effect of ET-1 on PMN adherence was confirmed in studies from other groups [21]. More evidence was needed to determine whether the findings with cultured endothelial cells were also valid in conditions closer to those in vivo. Experiments with the isolated rabbit heart model provided this evidence, by showing that the increased neutrophil adhesion to the endothelium in the presence of ET-1 also occurred in a whole organ model. Neutrophil accumulation was also inhibited by an anti-TS1/18 antibody [4]. Wright et al. [22] found that the potency of PMN chemotactic activities of the endothelin peptides are ET-1 = ET-2 > ET-3. They also found that ET-1 fails to stimulate PMN respiratory burst, degranulation or arachidonic acid metabolism [22].

Further data pursuing the functional implications of ET-1 activation of PMN demonstrated an effect on enzyme release. Immunostaining of human PMN incubated with ET-1 showed an intense, spreading pattern of anti-human granulocyte elastase within the cytosol [23]. This reflects PMN activation by ET-1, followed by the release of granule contents. ET-1 caused a dose- and time-dependent increase in PMN intracellular calcium and elastase release into the medium. Moreover, ET-1-activated neutrophils were shown to cause massive destruction in umbilical cords [23]. These results suggest the existence of a previously undescribed pathway to produce endothelial injury and tissue damage in conditions with high blood levels of endothelin. However, as occurred with other effects of ET-1 on PMN, the data on exocytotic enzyme release were not uniformly positive in experiments by different authors [24].

The interactions between the effects of ET-1 and those of l-arginine are critical in different tissues; data
concerning these interactions were, however, not available in neutrophils. A separate study examined such interactions in human PMN [25]. Some of the experimental findings relevant to this review showed that in human PMN: (i) ET-1 and the chemoattractant peptide fMLP induce both the metabolism of \( \text{L-arginine to L-citrulline and cyclic GMP (cGMP) formation} \); (ii) the ET-1-induced cGMP production is inhibited by the L-arginine antagonist, NG-monomethyl-L-arginine, suggesting the involvement of NO; and (iii) the ET-1- or fMLP-induced NO/cGMP stimulation is critically dependent on the availability of L-arginine [25].

ET-3 activates PMN migration at concentrations \( \leq 10 \text{nM} \). The stimulating effect of ET-3 appears to be mainly chemotactic, in contrast with that of ET-1, which is mainly chemokinetic [24]. Extracellular calcium and protein kinase C, protein tyrosine kinase, and phosphatase activity are involved in the ET-1-induced activation of neutrophil migration. The ETA receptor antagonist, BQ123, the ET receptor agonist, sarafotoxin S6c, and the ETB receptor antagonist, IRL 1038, inhibit ET-3-activated migration, suggesting that the ETA and the ETB receptor are both involved in the effects of ET-3 on PMN [24].

Recent studies have shown that the effect of ischaemia/reperfusion on PMN accumulation could be blocked by anti-ET-1 antiserum. Subsequent \textit{in vitro} studies demonstrated that ET-1 facilitates the accumulation of PMN on isolated rabbit kidneys by a CD18 antigen-related mechanism [4]. It is of further interest that an increased accumulation of PMN was detected in the hearts of rabbits with ischaemia/reperfusion injury or following intrarenal ET-1 infusion. These effects were both blocked by a specific anti-ET-1 antibody [4]. The latter results favour the existence of systemic effects of renal ischaemia mediated by ET-1.

The contribution of NO to ischaemic ARF is unclear. \textit{In vivo} experiments on renal ischaemia (60 min) suggest that the administration of the NO precursor, L-arginine, might be beneficial for both improving renal function and reducing PMN accumulation, but only if accompanied by the inhibition of superoxide accumulation by superoxide dismutase. The NO donor, sodium nitroprusside, appeared to be as effective as L-arginine + superoxide dismutase. These results differ markedly from the findings by Yu \textit{et al.} using preparations of renal tubules submitted to 15 min of hypoxia followed by 35 min of reoxygenation [26]. In fact, these authors found that NO might have deleterious effects on ischaemic tubular cells; however, these experiments were done \textit{in vitro}, and the role of PMN could not be assessed. In the same line of evidence, studies by Chao \textit{et al.} [27] have recently demonstrated that \( \zeta \)-melanocyte-stimulating hormone, which is a potent anti-inflammatory agent that inhibits neutrophil migration and production of neutrophil chemokines and NO, inhibits renal injury in a model of bilateral renal ischaemia. Under these conditions, \( \zeta \)-melanocyte-stimulating hormone significantly decreased tubule necrosis, PMN plugging and capillary congestion, and prevented the induction of the inducible isozyme of NO synthase-II, suggesting that renal failure, inducible NO formation and PMN accumulation are related phenomena. In a recent study in isolated perfused rat kidneys, Linas \textit{et al.} [28] provided more data on this issue. Their experiments disclosed that after 20 min of ischaemia/60 min reperfusion, NO worsens ischaemic injury in the absence of PMN, but NO prevents the PMN component of ischaemic renal injury by blocking PMN retention and the deleterious effects of activated PMN on glomerular and tubular function [28].

The heterogeneity of the available information on the subject of vasoactive mediators, PMN and ARF highlights the fact that outcome of ARF depends on the individually variable extent of damage and efficiency of the repair. In this setting, it is rather naïve to consider PMN only as potentially injuring agents. In fact, it must be remembered that recruitment or activation of macrophages and PMN in the area of injury results in scavenging of cellular debris, control of coagulation phenomena and local release of growth factors to promote regenerative repair [29]. This positive view of some aspects of PMN action will have to be considered in order to complete the picture of the role of PMN in ARF.

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Angiodysplasia in the renal patient: how to diagnose and how to treat?

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Definition and pathology

Angiodysplasia, also referred to as vascular ectasia or arteriovenous malformation, is a distinct pathological and clinical entity. It is the most common vascular abnormality of the gastrointestinal tract and probably the most frequent cause of recurrent lower-intestinal bleeding in otherwise healthy elderly patients. Angiodysplasia is likely a degenerative lesion associated with ageing and (other than hereditary haemorrhagic telangiectasia, Osler–Weber–Rendu) not associated with angiomatous lesions of other viscera or the skin.

In people with normal renal function lesions are most often found in the caecum or ascending colon, usually multiple, and smaller than 10 mm in diameter. In large series of colonoscopies angiodysplasias have been detected in 0.2–2.9% of ‘non-bleeding patients’ and in 2.6–6.2% of patients who underwent endoscopy for anaemia, faecal occult blood loss or intestinal haemorrhage [1–3]. In general, bleeding from angiodysplasia is recurrent and ‘low grade’; approximately 15% of patients, however, present with massive haemorrhage. In at least 90% of episodes bleeding stops spontaneously [4].

Microscopically angiodysplasia consists of dilated, thin-walled, distorted vessels lined by endothelium and, infrequently, by a thin layer of smooth muscle. Submucosal veins and venules are primarily affected. It is presumed that some of these lesions become more extensive over time; when capillaries dilate, precapillary arteriovenous fistula is produced. Associated with angioma
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The cause for angiodysplasia is unknown. It has been suggested that repeated intermittent low-grade obstruction of submucosal veins at the point where they cross the muscular layers of the colon leads to development of the lesion [5]. Since increased wall tension is presumed to result in obstruction of submucosal veins, according to Laplace’s principle the caecum and right colon, having a large diameter, appear to be a preferred site for development of angiodysplasia. It is unlikely, however, that this mechanism plays an important role in the development of angiodysplasia elsewhere in the gastrointestinal tract. It is likely that, at least in younger patients, some or
even the majority of these vascular lesions, especially those in the stomach, the duodenum, and the small intestine are congenital in origin [6,7].

Clinical presentation and diagnosis

Modes of presentation can vary for individual patients. Multiple bleeding episodes are common. Patients may present with either iron-deficiency anaemia, haemocult positive stool, haematochezia, or melena. In all cases bleeding from angiodysplasia is painless.

Diagnosis of angiodysplasia can be difficult. Because the lesions do not distort the mucosa or submucosa, in principle they cannot be detected by barium contrast studies. Colonoscopy and angiography are the only effective means for diagnosis. Because of its favourable safety profile, diagnostic efficacy and therapeutic capability colonoscopy (or upper gastrointestinal endoscopy) has emerged as the method of choice for evaluating patients suspected of having bleeding angiodysplasia [8]. Endoscopically, lesions appear flat or slightly raised above the mucosal surface, cherry red in colour, and usually 2–10 mm in size. Multiple angiodysplasias are present in a high percentage of patients (40–75% [7]).

The lesions may be round in contour, stellate, or having sharply circumscribed fern-like margins. False-negative endoscopic examinations may occur in severely anaemic or volume-depleted patients because the distinctive red colour of angiodysplasia may be absent [9]. Therefore those patients should receive transfusions prior to the endoscopic examination. When performing the procedure, one must differentiate angiodysplasia from suction marks and other artefacts which may be induced by minor trauma during the examination. Careful inspection on insertion of the instrument, rather than relying on assessments made during the withdrawal phase of the procedure can minimize this problem. Active bleeding from angiodysplasia and the presence of an adherent clot on a lesion are definite signs. Presence of fresh blood in a segment of the gastrointestinal tract in the absence of concomitant pathological findings or recurrent episodes of haemorrhage and no possible source detected other than angiodysplasia, is also persuasive evidence.

The most common angiographic sign is an early and prolonged opacification of a draining vein, recognized in 85–90% of visualized lesions [10,11], which is indicative of an arteriovenous communication. Other angiographic criteria include clusters of small arteries and a vascular tuft. However, definite proof that a visualized lesion is the cause for bleeding requires observation of extravasation of contrast material in the gastrointestinal lumen. This is an uncommon finding, seen in only 20% or less of patients [10], since angiographic visualization requires a bleeding activity of at least 1 ml/min. It should be noted that the angiographic criteria mentioned above are not specific for angiodysplasia but can be found in patients with Crohn’s disease, polyps, and cancer as well.

Sensitivity for endoscopy and angiography for diagnosis of angiodysplasia is not exactly known. Based on small series, however, sensitivity of endoscopy is much higher compared to angiography (approximately 80% vs 20% [12–14]). Nevertheless, since endoscopy fails to detect a number of lesions, angiography should be regarded as complementary test when colonoscopy, oesophagogastroduodenoscopy and, if available, enteroscopy of the small bowel is unrevealing. For examination of the small bowel one can use either a properly disinfected colonoscope which is passed per os in the distal duodenum and the proximal jejunum, detecting approximately one-third of angiodysplastic lesions in the small intestine [15], or a special longer push enteroscope. In problem patients with ongoing bleeding undiagnosed by conventional methods both jejunum and ileum can be inspected by using a normal colonoscope intraoperatively.

Biopsy of angiodysplastic lesions should be avoided, since endoscopically derived biopsies containing only mucosal tissue are usually non-diagnostic but may provoke haemorrhage. Radionuclide scans can be used in patients actively bleeding (especially from the small bowel) to localize the bleeding site. However, scans are unable to identify the cause of bleeding.

Angiodysplasia and renal failure

Angiodysplasia is an important cause of haemorrhage in chronic renal failure observed in up to 19–32% of patients in retrospective reports [16–19]. Lesions are usually multiple, often located in the stomach and duodenum, but can affect the colon and the jejunum as well, and have a high tendency of rebleeding (25–47% [17,18]). It is unknown whether the incidence of angiodysplasia or its tendency to bleed is increased in chronic renal failure and whether formation of angiodysplastic lesions precedes or follows onset of renal failure. One study, including 59 patients with chronic renal failure and upper gastrointestinal haemorrhage identified angiodysplasia as the most frequent cause of recurrent bleeding in patients with renal failure (53% [17]). Interestingly in this study, 39% of patients on dialysis and 70% of renal transplant recipients associated clotting disorders. Other comorbid conditions often found in renal patients as congestive heart failure, diabetes mellitus, coronary artery disease, and hypertension may increase the risk of bleeding [20].

Treatment

Since the natural history of angiodysplasia in renal failure is unknown, lesions found incidentally in patients who have never bled before should be left untreated. On the other hand taking into account the acquired platelet defect that may arise in chronic renal failure, patients should avoid taking aspirin or non-steroidal anti-inflammatory agents.

In patients with active bleeding or those who have bled and do not show another identifiable cause
endoscopic haemostatic therapy is generally the initial form of treatment. Different endoscopic devices have been used and described in non-randomized reports. Injection sclerotherapy is widely available, cheap, and relatively simple to perform. To avoid deep ulceration only small amounts of the sclerosing agent (e.g. sodium tetradecyl sulphate, 0.5 ml) should be used. The full spectrum of complications of this method have not been defined so far. Concerns have been raised regarding possible consequences of injection of sclerosant through the bowel wall into the peritoneal cavity [9,21].

Monopolar electrocoagulation using hot biopsy forceps may cause serious complications in up to 9% of patients, including perforation of the bowel in approximately 3% [2,22,23]. Therefore, other devices should be preferred. Bipolar contact probes have several advantages. Published experience with these devices in the treatment of angiodysplasia is, however, limited so far [9].

The majority of treatment trials have been performed with lasers, mostly with Nd:YAG (neodymium, yttrium, aluminium, garnet) [7]. Complications have been observed in 4–10% of patients, including bowel perforation. Thus, when using Nd:YAG lasers less than 200 J of total energy should be delivered, especially in the caecum and right colon [24]. The argon laser has the advantage of causing less depth of penetration and tissue injury. The argon laser (with moderate power settings of 5–7.5 W) has been used successfully for treatment of angiodysplasia, one caecal perforation being the only major complication observed among 38 patients treated [25]. Obviously the efficacy and complication rate of endoscopic treatment depend largely on the skill of the endoscopist and his personal experience with the particular endoscopic device.

For those patients whose bleeding persists despite endoscopic therapy surgical resection has to be considered if persistent or recurring bleeding necessitates repeated transfusions. If either surgery appears too risky in an individual patient or the bleeding site cannot be identified exactly due to multiple angiodysplasias in different parts of the gastrointestinal tract, a trial of oestrogen–progesterone therapy may be undertaken.

The mechanisms by which oestrogens might work in reducing bleeding activity of angiodysplasia are unknown. Speculations include improved endothelial integrity, promotion of clotting activity, and retardation of the mesenteric microcirculation [7]. Results of trials of oestrogen therapy in patients with angiodysplasia and normal renal function are conflicting [26,27] and suggest that lesions in the small bowel may be less affected by hormonal therapy than lesions in the stomach or colon. Two uncontrolled studies described successful application of hormonal therapy for bleeding angiodysplasias in chronic renal failure. In the first trial seven patients with bleeding angiodysplastic lesions in the stomach or colon received norethynodrel (2.5–9.85 mg/day) and mestranol (0.075–0.15 mg/day) for a mean of 12 months [28]. Bleeding was controlled in all patients over the follow-up period of 4–28 months without significant side-effects. In the second study four patients with recurrent bleeding from angiodysplasia received 50 or 100 μg 17β-oestradiol transdermally twice a week for a period of 2 months [29]. Bleeding activity in all patients either ceased or improved as shown by a reduced number of transfusions needed. Again no adverse reactions were observed over 2 months of therapy. However, it has to be stressed that controlled trials on the use of hormonal therapy for bleeding angiodysplasia in renal patients are lacking and the well-known side-effects of oestrogens including gynaecomastia, fluid retention, vaginal bleeding, thrombosis, and stroke should not be overlooked.

Other attempts to stop major bleeding from angiodysplasias include transcatheter embolization and intraarterial or intravenous infusion of vasopressin, which may cause serious side-effects such as ischaemia of mesenteric, coronary, and peripheral vessels. Patients with renal failure appear especially threatened by these complications.

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Regurgitation, a seemingly simple concept

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Introduction

Recirculation, a condition observed during haemodialysis which diminishes the efficiency of extracorporeal clearance is a measure for ‘access’ (in a broader sense) of the extracorporeal system to the patient. Under certain circumstances, recirculation is a measure for vascular access function. Now that new techniques which are easily performed during haemodialysis and which yield instant bedside information on access function are available, the value of recirculation measurements is in debate. Why is this the case?

In theory, recirculation can be due to several conditions:

- insufficient access blood flow,
- close position of arterial and venous blood lines,
- reversed placement of blood lines,
- cardiopulmonary recirculation, and
- compartment effects of indicators.

To complicate the subject, recirculation often relates to a combination of these effects. All these effects impact on treatment efficiency and need to be considered for the delivery of adequate dose of dialysis. However, because of the high rate of complications with vascular accesses and especially with PTFE-grafts, the current interest in recirculation has shifted from concerns delivering reduced dose of dialysis to monitoring vascular access function which is only one component contributing to recirculation.

A discussion of recirculation has to identify the errors (type-I and type-II errors) in relating access problems to the presence of recirculation.

False positive recirculation with regard to access function

The origin for false positive detection of access complications is related to the circumstance that the access is only one of the possible components contributing to recirculation. Indeed, access recirculation caused by insufficient access blood flow (so-called ‘true’ access recirculation) is rare, at least in PTFE-grafts. A recirculation in the range of 20–30% more often than anticipated is due to reversed placement of blood lines. In a recent study with 72 patients recirculation was present in 18 patients because of inadvertent reversal of blood lines. When 13 of these patients were studied at a later time, recirculation was again detected in five patients because misplacement of blood lines had again occurred [1].

If recirculation is in the range of 5–15%, with techniques such as thermodilution and urea recirculation, more often than not this is due to recirculation of indicator through the heart and lungs [2,3]. The indicator reappears in the extracorporeal circulation after bypassing systemic tissue compartments because of cardiopulmonary recirculation. Separation of the cardiopulmonary transient from a ‘true’ access recirculation transient requires exact timing of automatic techniques such as ultrasound and conductivity dilution [4,5], and meticulous sampling with manual techniques. A revised ‘low-stop flow’ sampling technique for measurement of urea recirculation yielded 100%

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sensitivity and specificity when the threshold for recirculation was set to 10% [6].

In any case, false positive recirculation can be identified by a second measurement switching the placement of extracorporeal blood lines. The value of a second measurement with switched blood lines is given by the possibility to identify the correct position of blood lines, where the correct position of blood lines is determined by the smaller recirculation value, and, most importantly, to calculate access blood flow.

**Access blood flow**

Reversing the connection of blood lines and performing recirculation measurements has been used in experimental situations [7] but the outstanding value of this approach to measure access blood flow was only recognized by Krivitski [8]. There is increasing evidence that low access blood flow is a sensitive predictor for future access thrombosis. In a recent study by May et al. mean access blood flow calculated from recirculation of injected saline with reversed placement of blood lines was significantly lower in accesses that thrombosed (875 ± 426 ml/min) versus accesses that did not thrombose (1193 ± 677 ml/min), whereas urea recirculation by slow flow technique performed with correct placement of blood lines was not predictive of early graft thrombosis [9]. However, the result also can be interpreted in favour of recirculation: since calculation of access blood flow is based on forced recirculation (with reversed placement of blood lines), and if measurements were performed at the same extracorporeal blood flow, the lower access blood flow in grafts that thrombosed must have been related to a higher recirculation measured with reversed placement of blood lines.

**False negative recirculation with regard to access function**

Absence of ‘true’ access recirculation measured with correct placement of blood lines is not an indicator for absence of access problems.

**Intra-access strictures**

The presence of substantial intra-access strictures is not detected by conventional recirculation measurements. Intra-access strictures are not uncommon. Besarab et al. detected 42 intra-access strictures in 182 accesses during an 18 month study period [10]. With intra-access strictures producing a significant resistance to access blood flow between arterial and venous needle puncture sites, blood flow evades intra-access resistance during haemodialysis and takes a functional bypass through the extracorporeal circulation. Even though the graft is under high risk for future thrombosis because of the intra-access stricture, there is sufficient blood supply to the inlet of the arterial blood line and there is no recirculation. Again, the situation is clarified by reversing blood lines and returning extracorporeal blood to the upstream section of the access. As a consequence of high intra-access resistance, and because extracorporeal blood flow is set by the machine, access inflow will adjust according to the resistance given by the stricture. This explains the paradoxical observation where access inflow measured with reversed placement of blood lines is considerably smaller than extracorporeal blood flow with correct placement of blood lines. In theory, access inflow is not independent of the extracorporeal circulation [11]. In general, the effect is small with correct placement but may be substantial with reversed placement of blood lines. However, the sensitivity of tests to detect access problems using reversed placement of blood lines increases because of this effect: recirculation will be higher and calculated access flow will be lower than with undisturbed access flow.

**Development of recirculation**

The frequency of access recirculation is higher in fistulae than in grafts [5,6,12]. Conversely, the failure rate is higher in grafts than in fistulae. One could conclude that a graft fails before access flow drops to a level where access recirculation develops during haemodialysis. It also could be concluded that the decrease in access flow occurs very rapidly and that the presence of access recirculation is missed with long screening intervals.

The relative risk of thrombosis within a subsequent observation phase of 2–3 months significantly increased with graft blood flows below 800 ml/min which were sufficient to deliver adequate amounts of blood to the extracorporeal circulation without occurrence of access recirculation [9,12]. The rate of access flow reduction was also determined as a predictor of access failure. The situation was different in native fistulae which remained patent with blood flows in the range of 250–500 ml/min [12]. In a study of 114 PTFE-grafts in 100 patients and 58 native fistulae in 57 patients for a period of 13 months and a screening interval of 3 months only one of four thrombotic events was preceded by a detection of access recirculation. Albeit, the predictive value of access recirculation was better in fistulae at 6 of 13 events than in grafts at 10 of 67 events. An increase in measuring frequency has the potential to improve the predictive value both for fistulae and grafts. However, the technology needed to measure the vascular access will have to become more automated before it can be used on a routine basis.

**Conclusion**

Recirculation has become a confusing subject because different effects have been described by the same term. With the help of new bed-side techniques it has become possible to focus on selected aspects of recirculation such as access function. However, recent studies show that the predictive power of access recirculation with regard to future access thrombosis is poor. This could be due to the prevalence of PTFE-grafts in these studies and to the screening interval which might be too long in order to detect access recirculation, the last stage of the failing access. On the other hand, the measurement
of access recirculation which does not require manual switching of blood lines is easy to perform in routine dialysis. Therefore, recirculation should not be dismissed unless future studies show that thrombotic events in grafts and fistulae occur so rapidly that they are not preceded by access recirculation.

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What place diuretics in long-term CAPD?

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The role of residual renal function

It is generally accepted that residual renal function provides a major contribution to the well-being of patients with end-stage renal failure (ESRF) on dialysis. It is of greater importance for patients on continuous ambulatory peritoneal dialysis (CAPD) than those on haemodialysis. This is because renal removal of solutes continues to constitute a significant proportion of the total body solute clearance even when patients are well established on CAPD. Decline or loss of this residual renal function can make adequate dialysis by CAPD difficult or sometimes impossible. As a consequence these patients are at risk of underdialysis and may be forced to change dialysis modalities with clear medical, social and cost implications.

Preservation of residual renal function is therefore of paramount importance. In addition to solute clearance residual renal function also provides a means of controlling body water. Although an individual patients’ urine volume tends to remain constant and is largely unresponsive to changes in fluid status, this nevertheless provides several benefits. It allows patients more freedom in their fluid intake, and allows physicians to achieve better control of the patients’ fluid balance. This is important as the acceleration in age related cardiovascular deterioration amongst patients on dialysis, although multifactorial in nature, is thought to be hastened by a state of persistent volume overload. In addition it could be speculated that greater renal water clearance would reduce a patients’ need for hyperosmolar dialysis solutions, and hence result in better preservation of peritoneal membrane function.

Once a patient is established on either haemodialysis or CAPD, residual renal function has been shown to progressively decline [1]. There is good evidence that this progression is slower in patients on CAPD than on haemodialysis [2]; the explanation for this is multifactorial and will not be discussed here. The rate of decline of residual renal function is variably reported, but has been estimated to be approximately 3% per month [2]. However, whilst some authors conclude that after a period of approximately 36 months on CAPD the contribution of residual renal function to dialysis adequacy is negligible [3], others feel that it makes a useful contribution for much longer [4].

The use of diuretics

There remains considerable controversy over the role of loop diuretics in long-term CAPD, and as a consequence clinical practice varies widely between units and countries. A few studies, much correspondence,
and many anecdotal reports have considered this issue. Proponents of the use of diuretics believe that diuretics can stimulate an effective diuresis, even at the low GFRs seen in patients on CAPD. Others believe they have actions beyond a simple nature and diuresis, and may preserve residual renal function over the longer term. Disappointingly there are very few clinical studies of sufficient design and size to guide evidence-based medicine. The existing studies can be divided into either short-term use of diuretics over a few days given to patients with or without volume overload, or studies in which diuretics are given regularly to CAPD patients over several months.

There are a relatively greater number of studies investigating the short-term effects of diuretics. The principle diuretics considered are frusemide and muzolimine. Large doses of frusemide (500–2000 mg/day) have been shown to increase urine volume, sodium and potassium excretion in patients on CAPD [5, 6]. Predictably the authors found that there was no effect from diuretics if the patient passed less than 100 ml of urine per day. The scale of nature was also found to be proportional to residual GFR, with greater efficiency of naturesis in the patients with greater preserved GFR. As these studies only lasted for 1–3 days they cannot be used as evidence for any long-term effects of frusemide. As neither study showed any change in urinary creatinine clearance they also cannot be used to support any putative protective properties of diuretics in preserving residual renal function.

The effect of long-term diuretics on residual renal function is less well understood. No large prospective study has addressed this question, although conclusions have been drawn from small, often retrospective studies of dialysis adequacy. In the only prospective study, Bandiani et al. [7] studied six CAPD patients along with 10 HD patients taking muzolimine for 1 year, and compared them to matched controls. Despite small numbers of patients they were able to show a slower decline in diuresis and greater residual renal function at 1 year in the diuretic-treated group. This is consistent with previous studies which have also suggested that diuretics may perhaps maintain the volume of urine [1], but there is debate about whether they also preserve renal solute clearance [8].

In addition to the potential benefits of diuretics, consideration must be given to the potential adverse effects of their use in high doses. Given orally the total body clearance of frusemide is significantly lower in patients on CAPD than in healthy controls (62 vs 140 ml/min) [9]. Despite this, however, in a recent study using a single dose of frusemide 2000 mg, none of the eight patients studied had a plasma frusemide level above the theoretical ototoxic threshold (50 mg/l) [6]. Korytowska and Grzegorzewska [10] have shown that frusemide can be given at least in an intraperitoneal dose of 2000 mg/week for 3 months without audiometric changes.

In pre-dialysis patients, diuretics are known to inhibit urinary urate excretion, and hence precipitate symptomatic gout. Interestingly, in patients on CAPD, frusemide given by the intraperitoneal route has been shown to increase rather than decrease urinary uric acid excretion [11]. Thiazide diuretics, and also to a lesser extent loop diuretics, are also known to adversely affect plasma lipid profiles. Serum cholesterol rises in most patients during the first years on CAPD [12], but there is no comparative data on any intercurrent effect on lipid profile by diuretics once patients are established on dialysis.

Conclusions

In summary, the role of diuretics in the management of patients on long-term CAPD remains unknown because there is insufficient evidence. Diuretics may be helpful in controlling acute fluid overload in long-term CAPD patients, although not all patients will respond [13]. There is insufficient evidence, however, to recommend their routine use in the long-term CAPD patient because, ironically, despite 20 years of experience with CAPD, and 100,000 patients using the technique worldwide, no controlled evidence is available.

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