Cast Nephropathy in Myeloma—Does PACAP38, a New Member of the Vasoactive Intestinal Peptide Family, Open a Therapeutic Window?

Arimura A., Li M., Batuman V.

Multiple myeloma is the sixth most common malignancy and accounts for 2% of all deaths from cancer (1). Renal damage from myeloma is an important cause of renal failure (2) with a poor prognosis. The main renal lesions are cast nephropathy characterized by cast formation in the distal nephron, resulting from coprecipitation of pathologic light chains with Tamm-Horsfall protein and marked interstitial fibrosis (3–6). Casts in the distal nephron cause breaks of the epithelial barrier, thus permitting leakage of Tamm-Horsfall protein into the interstitium (7–9). Tamm-Horsfall protein has recently been recognized as an important trigger involved in the genesis of interstitial inflammation (10,11). In addition, proximal tubular reabsorption of aberrant light chains by endocytosis causes cellular protein overload and activates the central switch for inflammatory processes, activating NFκB (nuclear factor κB) and setting in motion the synthesis of inflammatory cytokines and activating signaling pathways (such as mitogen activated protein kinases [MAPK], extracellular signal regulated kinase 1/2 [ERK 1/2], Jun kinase [JNK], p38MAPK), thus promoting interstitial inflammation and fibrosis. A causal role of cast formation is suggested by the observation that the severity of cast formation as found by renal biopsy is predictive of outcome (12).

Apart from treating the underlying malignancy (13), the results of specific therapies to prevent or reverse cast nephropathy have been disappointing. Plasmapheresis has recently been shown to provide no significant benefit in this condition (14) and the results of steroid therapy remain modest at best.

Against this background new approaches are badly needed and the recent finding of Arimura and Batuman discussed here, if confirmed and proven in interventional trials, may open a new therapeutic window.

The authors investigated pituitary adenylate cyclase activating polypeptide with 38 residues (PACAP38), a neuropeptide member of the vasoactive intestinal peptide (VIP) family. PACAP38 had been isolated originally from hypothalamic tissue and was identified as an agent stimulating adenylate cyclase in pituitary cell cultures (15). This peptide has been shown to exhibit activity as a neurotransmitter, neuromodulator, and neurotrophic factor (16), but also, like other members of the VIP group, it has been shown to modulate the activity of immune cells (17–19) operating through the MAPK cascade and interacting with the central switch NFκB (20,21).

The authors had the courage to look at the kidney as a potential target although the kidney had not been proven before to respond to PACAP38. They chose the myeloma kidney as a model because of the known involvement of MAPK and NFκB in the genesis of the renal disease provoked by light chains.

In their study they used κ-light chains that had been isolated from the urine of a patient with myeloma kidney and had been shown to phosphorylate MAPK and NFκB (22). This κ-light chain was used for studies on immortalized human renal proximal tubular cells as well as for examination of rat kidneys after intravenous infusion of the κ-light chains. Reverse transcription (RT)-PCR was used to demonstrate the presence of a receptor for PACAP38 on renal cells. Cytokine production and NFκB activation were studied by standard techniques.

The main findings were that even subnanomolar concentrations of PACAP38 dose-dependently suppressed the release of proinflammatory cytokines, *i.e.*, IL-6 and TNFα, when human proximal tubular cells were exposed to light chains. The effect was comparable to that of dexamethasone. It was mediated via suppression of the light-chain–induced activation of...
p38MAPK and of the p50 chain of NFκB. PACAP38 receptors were expressed by this human renal tubule cell line. The in vivo relevance of these findings was documented by examining in rat kidneys the expression of TNFα after 3 d infusion of the human light-chains achieving clinically relevant concentrations. TNFα expression was suppressed by intravenous injection of PACAP38.

It was necessary to exclude that PACAP38 might stimulate the growth of myeloma cells. This possibility was worrying, particularly in view of the stimulatory action of PACAP in, among others, human pancreatic (23) or colonic carcinoma (24) cell lines. In vitro the growth of myeloma cells was examined after addition of PACAP38 as well as IL-6 production by bone marrow stromal cells, because IL-6 is known to stimulate the growth of myeloma cells. It is good news that PACAP38 suppressed rather than stimulated myeloma cell growth and at the same time also suppressed indirect stimulation via IL-6.

The results are encouraging, particularly because at least short-term administration of effective doses of PACAP38 to inhibit release of adenohypophyseal hormones has not shown any side effects (25). Further studies will be required to document the in vivo relevance of the above findings in patients and to show whether the compound does not only prevent but is also able to reverse myeloma-associated renal damage. Whether renal tubulointerstitial fibrosis of etiologies other than light chain-mediated injury is beneficially affected by PACAP38 also deserves further study.

In general, PACAP38 has a fascinating spectrum of actions spanning the range from neuroprotection (26–28) and neuropsychiatric disease (29) to β cell protection (30). There is currently intense research going on in this field. The mode of pharmacologically modulating PACAP38, either by inhibiting the breakdown of endogenous PACAP38 (31) or finding novel modes of chronically administering it (32), is currently under investigation as well.

References


Plasma Exchange for Acute Renal Failure of Myeloma—Logical, Yet Ineffective

Plasma Exchange When Myeloma Presents as Acute Renal Failure. A Randomized, Controlled Trial. *Ann Int Medicine* 143: 777–784, 2005

It has been estimated that in the US multiple myeloma will be diagnosed in 15,980 persons and that 11,300 persons will die from it, constituting 2% of all deaths from cancer (1,2). At the time of diagnosis only 52% of patients have normal serum creatinine concentrations (3) and in different series 12 to 20% were found to present with acute renal failure (4–6).

Plasmapheresis has been shown to remove light chains, but the information on its effect on recovery of renal function had remained controversial. An uncontrolled observational series of 50 patients with myeloma and acute renal failure had initially shown that more patients who had chemotherapy plus plasma exchange recovered renal function (61% of cases) than patients who had been treated only with chemotherapy (27%) (7). A further report had stated that all 3 patients with multiple myeloma and severe acute renal failure recovered renal function after plasmapheresis (8).

These encouraging early observations prompted Zucchelli (9) to perform a small, controlled, randomized trial on a sample of 29 patients with multiple myeloma and Bence-Jones proteinuria >1 g/d who presented with acute renal failure. Dialysis was required in 24 cases and 5 cases had serum creatinine concentrations in excess of 5 mg/dl. Patients were randomly allocated to plasma exchange plus corticosteroids, with cytotoxic drugs and hemodialysis when required (n=15), or to peritoneal dialysis together with corticosteroids and cytotoxic drugs (n=14). As anticipated, patients treated with plasmapheresis, but not the patients without, had a dramatic reduction of Bence-Jones proteinuria. More importantly, 13 of the 15 patients with plasmapheresis, but only 2 of the patients without, recovered renal function defined as serum creatinine <2.5 mg/dl. In this underpowered study with different renal replacement therapies in the two arms of the study, the 1-yr survival was significantly higher in patients with plasmapheresis (66%) than in patients without (28%).

In contrast, however, a subsequent controlled randomized study by the Mayo clinic failed to show a benefit of plasmapheresis (10). A total of 21 patients with incident or prevalent active myeloma and progressive renal failure, a somewhat different and also small group, was randomized to either forced diuresis, chemotherapy, and plasmapheresis or to forced diuresis and chemotherapy: Of the 5 patients already on dialysis who received plasmapheresis, only 3 recovered, and of the polyuric patients the number of recoveries was similar in the two groups (i.e., 7 versus 5 patients). The 6-mo mortality was 20% in either group and rose to 60 to 80% at 1 yr. This study is somewhat difficult to interpret because it studies patients presenting with renal failure due to myeloma (incident patients) as well as patients with established renal failure due to myeloma (prevalent patients).

Despite these less than convincing results, several authors (11–13) as well as guidelines (14,15) recommended plasmapheresis in such patients. Such recommendations were based on uncontrolled clinical observations, which admittedly were occasionally impressive—however, plasmapheresis by necessity was never used as a treatment modality in isolation, but was always combined with cytotoxic therapy so that it was impossible to find out which did what. In this murky situation, controlled, randomized, prospective information was most welcome, particularly because it has been well documented that reversal of renal failure was a more important prognostic factor than response to chemotherapy (4,16,17). Renal dysfunction impacted on outcome even after bone marrow transplantation (2). Consequently, if effective, plasmapheresis might confer substantial clinical benefit.

This randomized, open, controlled, Canadian study (18) was conducted between 1998 and 2004. Patients were stratified by chemotherapy and dialysis dependence and subsequently
randomized to receive either 5 to 7 plasma exchanges in addition to conventional therapy (50 ml/kg body weight with acid citrate as anticoagulant and 5% human albumin and normal saline as replacement fluid) within the first 10 d of study entry or conventional treatment. The primary endpoint was a composite outcome comprising death, dialysis dependence, and GFR <30 ml/min per 1.73 m² at 6 mo.

What were the results? In the control group 69.2% and in the plasmapheresis group 57.9% of patients had an event (composite outcome). The unadjusted odds ratio (OR) for the primary composite outcome was even higher in the plasmapheresis group (OR 1.71), but it decreased to 1.20 when adjusted for baseline treatment with the VAD scheme (vincristin, doxorubicin, methylprednisolone), for staging of myeloma (according to Durie-Salmon), dialysis, age, serum albumin concentration, and urine protein excretion; the 95% confidence intervals were very large, however (0.73 to 4.01 and 0.42 to 3.44, respectively). The cumulative 6-mo survival was also similar in the two groups, 66.7% versus 67.2% in the control versus the plasmapheresis group, and the same was true for several other secondary outcomes.

What can be concluded from the results? The authors are presumably correct in stating that it is unlikely that plasmapheresis yields a clinically meaningful outcome in patients with acute renal failure at the onset of myeloma. So the guidelines were dead wrong. It is highly unlikely that we shall ever get a better and more penetrating study, so in the future decisions on patient management must be based on the above study results. Nevertheless, it is useful to point to some of its shortcomings.

First, the huge confidence intervals illustrate that the study was still underpowered, although it exceeded the previous studies by a wide margin. As a result it cannot be totally excluded that plasmapheresis might be beneficial in specific subgroups.

Second, the authors failed to monitor urinary excretion, or better plasma concentrations, of light chains. This resembles antihypertensive treatment without measuring BP.

Third, despite concealed central randomization there was a substantial imbalance in treatment allocation, i.e., 39 patients were evaluable in the control and 58 patients in the plasmapheresis group.

Fourth, the authors did not perform renal biopsies. It is not likely, but cannot be excluded, that the study included patients who failed to have cast nephropathy, particularly since renal biopsy studies showed considerable heterogeneity of renal findings in myeloma patients (19–21).

What is remarkable is that the proportion of these incident patients who became independent of dialysis was large, i.e., 39.5% overall, 7/19 in the control and 10/24 in the plasmapheresis group. By contrast, previous series had reported that no more than 3 to 12% recovered renal function (4,22 to 25). The most plausible, but not the only, interpretation is that modern treatment has become much more effective than what had been available in the past (26). This may possibly also have contributed to the observation that today plasmapheresis on top of modern cytotoxic treatment no longer improves outcome.

References
Insulin Therapy and Improved Outcome in the Intensive Care Unit—Beyond Lowering of Glycemia

Intensive Insulin Therapy Protects the Endothelium of Critically Ill Patients.
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In uncontrolled observations it had been shown that hyperglycemia increases morbidity and mortality in critically ill patients even in the absence of diabetes (1–3). Furthermore, mainly in diabetic patients, the Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) study had shown improved short-term and long-term outcome from a regimen comprising strict glycemia control using infusion of glucose and insulin in patients with acute myocardial infarction as compared with conventional glycemia control (4,5). The same protocol has been tried in stroke, so far without definite results (6). It is true that the beneficial effect on outcome after myocardial infarction could not be reproduced in the DIGAMI-2 study (7), presumably because coronary care has improved in the meantime and because the study may have been underpowered.

Five years ago van den Berghe *et al.* (8) reported the results of a prospective, randomized, controlled study of 1548 patients in a surgical intensive care unit (ICU) to address the hypothesis that hyperglycemia or relative insulin deficiency (or both) during critical illness predisposed patients to complications such as severe infections, polyneuropathy, multiple organ failure including acute renal failure (9), and death. Patients were randomly assigned to intensive or conventional insulin therapy. In the intensive treatment group patients received an insulin infusion once the blood glucose concentration exceeded 110 mg/dl to maintain blood glucose within a range of 80 to 110 mg/dl, the maximal dose of insulin being 50 IU/h. In the conventional treatment group patients were started on insulin treatment once blood glucose concentration exceeded 215 mg/dl. This regimen achieved morning blood glucose concentrations of 103 ± 19 mg/dl in the intensive treatment group and 153 ± 33 mg/dl in the conventional treatment group. Death rate during intensive care was 4.6% in the intensive treatment versus 8.0% in the conventional treatment groups and in-hospital death for patients receiving intensive care for >5 d was 16.6% with intensive versus 26.3% in conventional treatment. The main features were less multiorgan failure with sepsis as well as—of particular interest to the nephrologists—less acute renal failure in the intensive treatment group (dialysis 4.8% in the intensive versus 8.2% in the conventional treatment group).

These data in a surgical ICU might not necessarily apply to a medical ICU. To address this issue the same authors recently studied 1200 patients of a medical ICU. The data showed that intensive insulin therapy failed to reduce in-hospital mortality significantly (37.3% in the intensive treatment group versus 40.0% in the conventional treatment group). Nevertheless, in this study morbidity was again significantly reduced by preventing newly acquired kidney injury—confirming previous results of others (10)—and by accelerating the weaning from mechanical ventilation and discharge from the ICU and the hospital, respectively (11).

These impressive studies left open the question whether it was glycemia control or delivery of insulin that provided the benefit and similarly raised the issue of which target organ function was involved in improving outcome.

In the study discussed here, the same authors now addressed the hypothesis that organ failure and sepsicaemia were the result of an excessive inflammatory response to infection and injury with inappropriate endothelial cell activation playing a central role by compromising the microcirculation, inducing hypoxia and causing organ failure (8). They further hypothesized that during critical illness provision of insulin protected the endothelium. These hypotheses were examined in a preplanned subanalysis of the first of the above studies (8).

What were the effects of intensive insulin therapy? Patients with intensive insulin treatment had lower C-reactive protein concentrations, had a lesser increase of the concentration of...
intercellular adhesion molecule-1 (ICAM-1) but not of E-selectin, had transiently (day 5) lower IL-10 concentrations, and tended to have lower IL-6 concentrations.

To carry this one step further, the authors evaluated the gene and protein expression of the nitric oxide synthase (NOS) family in the livers and muscles of those patients who had died. nNOS gene as well as eNOS gene expression was unaffected by intensive insulin treatment, but iNOS gene expression was significantly lowered by insulin treatment both in the liver and in skeletal muscle.

Furthermore, intensive insulin treatment tended to lower phosphorylated inhibitor of NFκB (IκB) in the liver, pointing to less activation of this central proinflammatory switch.

Finally, serum NO (nitrate plus nitrite) was similarly elevated in the two groups on admission, but decreased on day 7 on intensive insulin treatment. A pathogenic role of NO is suggested by the observation that NO concentrations were higher in patients who did not survive. Interestingly, concentrations were also higher in patients who developed acute renal failure.

The argument can be raised that hyperglycemia is a proinflammatory agent and insulin is an anti-inflammatory agent (12).

The proinflammatory effect of hyperglycemia is linked to increased generation of reactive oxygen species (ROS) (13) among others by inducing mitochondrial abnormalities in the liver, which are prevented by insulin infusion (14). Even an oral glucose load that raises blood glucose but fails to cause hyperglycemia increases generation of ROS (15). This risk may be aggravated if the concentration of the antagonistic agent insulin is low (16). One important consequence of increased ROS generation is scavenging of NO by ROS, thus reducing its availability in the microcirculation and compromising the function of the microcirculation.

The anti-inflammatory actions of insulin are mediated through three different signal pathways: NFκB, activator protein-1 (AP1), and early growth response-1 (EGR-1) (13,17). Genes regulated by these transcription factors code for molecules that include proteins involved in the above inflammatory reaction. The proinflammatory action of hyperglycemia is antagonized by insulin, which suppresses the generation of ROS by interfering with the expression of p47phox, a constituent of NAD(P)H oxidase, one of the pathways of generating ROS (17,18). Further beneficial effects of insulin, important for antagonizing the microcirculatory disturbance and the ensuing predisposition to organ damage, are increased NO release with improved vasodilatation and inhibition of platelet aggregation (19,20).

What are the implications of the study of Langouche et al. (21)? On the one hand, markers of the inflammatory response and predictors of target organ damage, including acute renal failure, may emerge from these results, e.g., plasma NO (NO₂ + NO₃).

Furthermore, the case for using intensive insulin administration in critically ill patients in the medical ICU gets ever stronger (10,11), despite the undoubted serious logistic problems posed by this intervention, which certainly is not without hazard in the absence of rigorous quality control. This study investigating intensive insulin infusion adds to what has already been documented in other settings, such as during cardiac surgery (22), management of the patient with burns (23), or with myocardial infarction (18).

References


