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Clinical experience with human anodal trypsinogen (HAT) for detection of pancreatic allograft rejection

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Abstract To date one of the major dilemmas in clinical pancreas transplantation is the lack of a reliable indicator for pancreas rejection. In a consecutive series of 52 patients undergoing simultaneous pancreas and kidney (SPK) transplantation with bladder drainage technique between October 1991 and December 1992 a new test using serial levels of serum human anodal trypsinogen (HAT) was evaluated for its efficacy to detect pancreas rejection. Postoperative baseline levels of HAT were compared to peak HAT values at time of rejection. HAT profiles at time of rejection were calculated and compared to profiles of urinary amylase, serum amylase, fasting blood sugar and serum creatinine. In this series one year patient survival was 97%, graft survival of the pancreas 86% and of the kidney 90%. In 71% of the patients at least one rejection episode occurred. At time of kidney-biopsy proven rejection with a concurrent serum creatinine rise a significant HAT level rise to more than 1000 ng/ml was observed from baseline levels of 200 ng/ml ($P < 0.001$) indicating kidney and pancreas rejection (73%). Urinary amylase levels decreased in the majority of rejection episodes at the same time from baseline levels to less than 20000 U/l. In 25% of the rejection episodes a significant serum creatinine rise was observed without a HAT rise or urinary amylase decrease indicating kidney-only rejection, while in 2% a urinary amylase decrease and simultaneous HAT also was observed with a negative kidney biopsy indicating pancreas-only rejection. We feel that increase in HAT levels significantly correlates with pancreas rejection. After SPK, determination of HAT is an additional helpful non-invasive test. In pancreas transplantation alone HAT can be a useful indicator to detect rejection and facilitate timing of a pancreas biopsy and initiation of antirejection treatment.

Key words Pancreatic transplantation · Rejection
Anodal trypsinogen

Introduction

One of the major dilemmas that has persisted in pancreatic transplantation is the lack of an early and reliable marker for rejection. This marker should be simple, sensitive, serological, and preferably noninvasive. Currently, the kidney is used as a marker for rejection when pancreas and kidney are transplanted simultaneously (SPK). By using the kidney as an indicator for pancreatic rejection, simultaneous rejection of both organs is assumed and treated, possibly overtreating the pancreas. This regimen, however, seems to be justified by the excellent results that have been reported after SPK transplantation [1, 2]. The question remains what to do when the pancreas is transplanted alone or pancreas and kidney are derived from two different donors. In recent years, a substantial number of parameters have been studied with variable success in the pursuit of a specific biochemical marker to detect pancreatic rejection [3–6]. In the absence of a convincing indicator, many centers have continued to use urinary amylase excretion in SPK recipients with bladder drainage as their next best option in clinical practice [7]. In 1986, a potentially useful new marker to determine pancreatic rejection was described by Borgstroem and Marks [8]. They reported a significant correlation between elevated serial anodal trypsinogen levels and rejection after whole organ pancreatic allograft transplantation in pigs. Rejection episodes were confirmed by pancreatic histology. The concept of human anodal trypsinogen (HAT) is that the majority of the pancreatic allograft consists of exocrine tissue and during an episode of rejection, this will be severly damaged causing a release of the protein. Extensive protein release will cause changes in the concentration in the patient's serum. Thus, increased serum HAT levels should be compatible with rejection, graft inflammation, as well as direct surgical trauma. After development of an enzyme immunoassay kit, clinical studies were initiated and the first results have been reported [9, 10].

This study reports our clinical experience at the University of Wisconsin with HAT after SPK transplantation with bladder drainage as an indicator for pancreatic allograft rejection. The aim of this study was to observe changes in HAT profile at the time of kidney biopsy proven rejection. HAT profiles correlated with the profiles of serum creatinine, urinary amylase, and other serological parameters during the initiation of antirejection treatment in SPK recipients.

Materials and methods

Patients

Fifty-two patients received an SPK transplant at our institution between October 1991 and December 1992. Our standard surgical technique and posttransplant regimen, including quadruple immunosuppression, have been described previously. Follow-up of the study group ($n = 52$) ranged from 89 to 546 days. Demographics of SPK recipients in the present HAT study were similar to our previously reported series. The mean age of recipients was 34.7 years with a range of 22–51 years. Of the 52 patients, 58% were male and 42% female. The median duration of diabetes mellitus at the time of transplantation was 22 years with a range of 10–33 years. Approximately 73% of the patients received dialysis treatment before SPK transplantation. In only 12% was an O DR mismatch obtained versus one DR mismatch in 48%, and two DR-mismatches in 40%. Preservation times with University of Wisconsin (UW) solution varied from 6 to 29 h with a mean of 15 h.

Sampling and assays

Posttransplant and at the time of readmission, serial samples of serum creatinine, BUN, B-2-microglobulin (B_2M), fasting blood sugars (FBS), serum amylase, urinary amylase, and HAT were collected. HAT levels were analyzed using an ELISA [11]. Samples were analyzed according to the protocl of Borgstroem and Marks in duplicate and with a standard dilution series of the antigen and control human serum. Urinary amylase was calculated daily by either analyzing 24-h collections or determination of amylase in urine aliquots. Other parameters were analyzed by following the standard clinical laboratory procedures.

Rejection

Rejection was treated after establishing the diagnosis of rejection on a clinical basis and confirmation by kidney biopsy in almost all cases (89%). The day of initiation of antirejection treatment was considered the day of the diagnosis of rejection.

Evaluation

HAT in SPK recipients was evaluated by calculating posttransplant profiles of HAT per patient. Values used to determine the postoperative baselines were obtained after day 7 and varied (< 15%) on 3 consecutive days. Postoperative baseline levels were compared to peak HAT values at the time of rejection. HAT profiles were then correlated with profiles of serum creatinine, BUN, B_2M, serum amylase, urinary amylase, and FBS levels. Patients with nonrejection-related HAT rises are described separately.

Statistics

Student's t-test for unpaired data was used to compare baseline data and peak values at time of rejection. P values of less than 0.05 were considered significant.

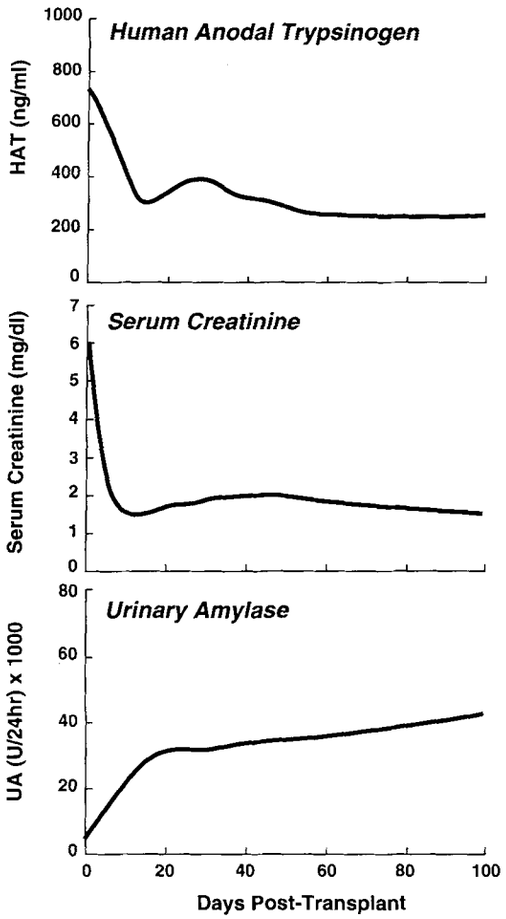


Fig. 1 Posttransplant profiles of human anodal trypsinogen (HAT; ng/ml), serum creatinine (mg/dl), and urinary amylase (U/l) after SPK transplantation

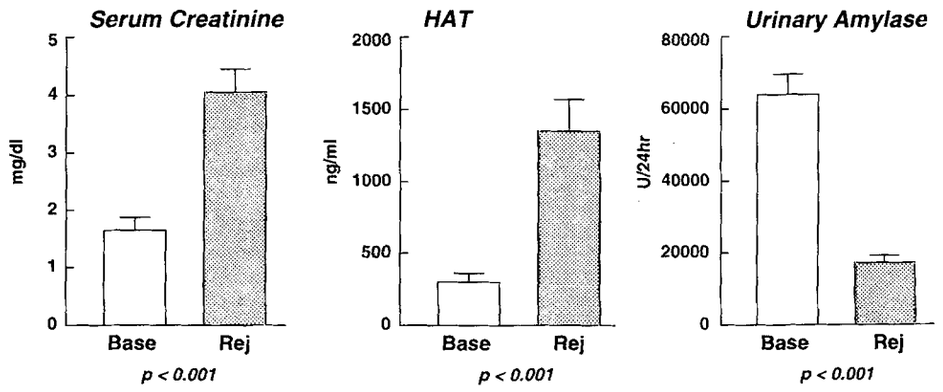
Results

Graft failure and actuarial survival after SPK transplantation were compatible with previous series reported by our group. Kidney failure occurred in only one patient (2%) in the HAT study group of 52 evaluated patients, kidney-and-pancreatic failure occurred in five patients (10%). The actuarial 1-year patient survival was 96.5%. The 1-year graft survival of the kidney was 89.7% and of the pancreas, 87.5%. A total of 49 rejection episodes were seen in this follow-up period. No rejection was seen in 15 patients (29%). One or more rejection episodes occurred in 37 patients (71%). The first, second, and third rejection episodes were documented on (median) days 19, 44, and 52, respectively.

Posttransplant HAT profiles in SPK recipients initially rose to a mean of 720 ng/ml, followed by rapid decrease to baseline HAT levels of 200 ng/ml. This decrease occurred at the same time as serum creatinine leveled off towards normal values of 1.2–1.7 mg/dl and urine amylase started to increase after transplantation, reaching a mean baseline of 35000 U/l (Fig. 1). At the time of rejection, a significant increase in serum creatinine was seen from baseline levels of 1.7 mg/dl to peak values of 4.2 mg/dl ($P < 0.001$). A simultaneous significant rise in HAT levels was noted from baseline levels to more than 1000 ng/ml. Urinary amylase levels decreased significantly at the same time from baseline levels to less than 20000 U/l ($P < 0.001$; Fig. 2).

Figure 3 shows a typical profile of parameters during rejection after SPK. HAT levels increased on the same day as the creatinine rise in 28%, or a few days later in 55% of the rejection episodes. In a smaller percentage, an increase occurred before the creatinine rise (17%). A decrease in urinary amylase levels was found: before the creatinine rise in 36%, on the day of the creatinine rise in 11%, and after the creatinine rise in 53%. In Fig. 4,

Fig. 2 Posttransplant baseline levels and peak values ±SD of serum creatinine (mg/dl), HAT (ng/ml), and urinary amylase (U/l) at time of rejection after SPK transplantation



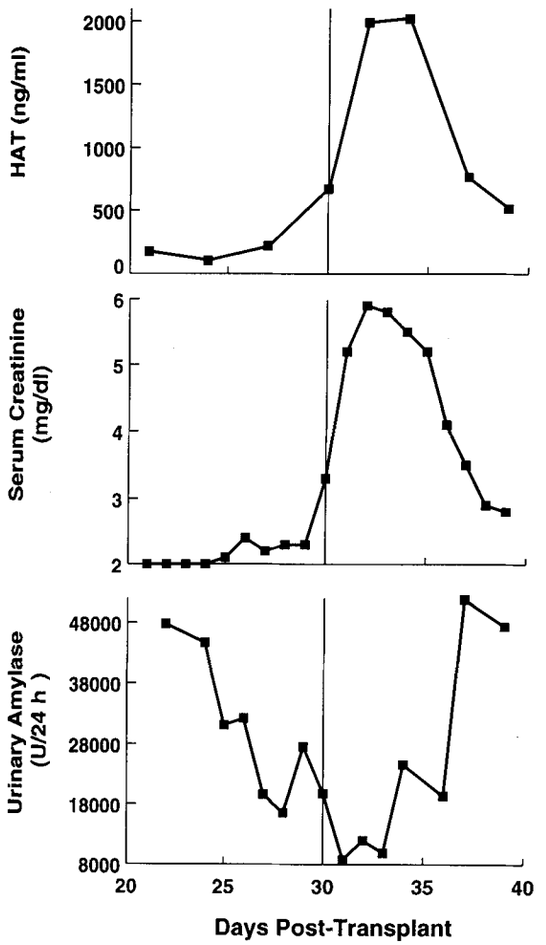


Fig. 3 Typical patient profile after SPK transplantation of HAT (ng/ml), serum creatinine (mg/dl), and urinary amylase (U/l) at time of rejection. The *vertical line* marks the day of initiation of anti-rejection treatment

parameters are shown for the entire study group on days relative to the onset of rejection and start of antirejection treatment.

A HAT rise was observed in seven patients that was due to clinical complications with no evidence of rejection. These clinical problems were hematuria in two patients and graft pancreatitis in two patients. They were treated with Foley placement, fulguration, and conversion of the duodenal segment. Three patients developed a perforation of the duodenal segment due to an ulcer necessitating a conversion operation.

When HAT and urinary amylase correlated after SPK in 34 patients, a decrease in urinary amylase occurred at the same time as the HAT increase (true-positive). Urinary amylase remained unchanged in ten patients in whom HAT increase occurred (false-positive). These ten patients included the seven patients with a clinical problem related to the pancreas without any evidence of rejection. In three other patients, the urinary amylase did not change. Not false-negative cases were observed with a simultaneous urinary amylase decrease and unchanged HAT level. In 27 patients, HAT as well as urinary amylase remained unchanged: these included 15 patients in whom no rejection was documented and 12 patients who were reported to have a kidney rejection with a creatinine rise only. In 36 patients, HAT and creatinine rises were correlated. In eight patients, the creatinine remained unchanged while HAT increased; this included seven patients with documented clinical problems without evidence of rejection and one patient with a presumed pancreatic rejection only with a HAT increase and urinary amylase decrease. This patient was treated for rejection and a kidney biopsy was obtained that revealed no rejection of the kidney. In 12 patients with kidney rejection only, a creatinine increase occurred without any HAT changes. Finally, in 15 patients, no clinical evidence of rejection was found and no serological changes in creatinine or HAT were seen.

Discussion

In this consecutive series of 52 patients with SPK transplantation, an attempt was made to evaluate the relevance of the serological marker HAT for pancreatic allograft rejection. Significant rises in HAT levels were seen during kidney biopsy proven rejection episodes after SPK. There was a good correlation between HAT increase and urinary amylase decrease in most of the patients. HAT rises occurred in 83% on the same day or the day after the creatinine rise. Our data suggested that rejection of both kidney and pancreas occurred simultaneously in majority of cases (73%) while kidney rejection occurred in only 25% and pancreatic rejection occurred in only 2%.

Previously, several parameters have been tested for pancreatic allograft rejection. Increase in serum glucose is a reliable though not very useful marker that occurs too late when destruction of islets is extensive and rejection has become irreversible [3]. Serum amylase has been reported to correlate poorly with rejection [4]. Another marker that has been used is pancreatic trypsin inhibitor, which appears to be too sensitive and has a high percentage of false-positive results [5]. Recently, a new test with a pancreas-specific protein was studied and found to be insensitive [6]. A promising screening could be the use of urine cytology [12]. More conventional is the

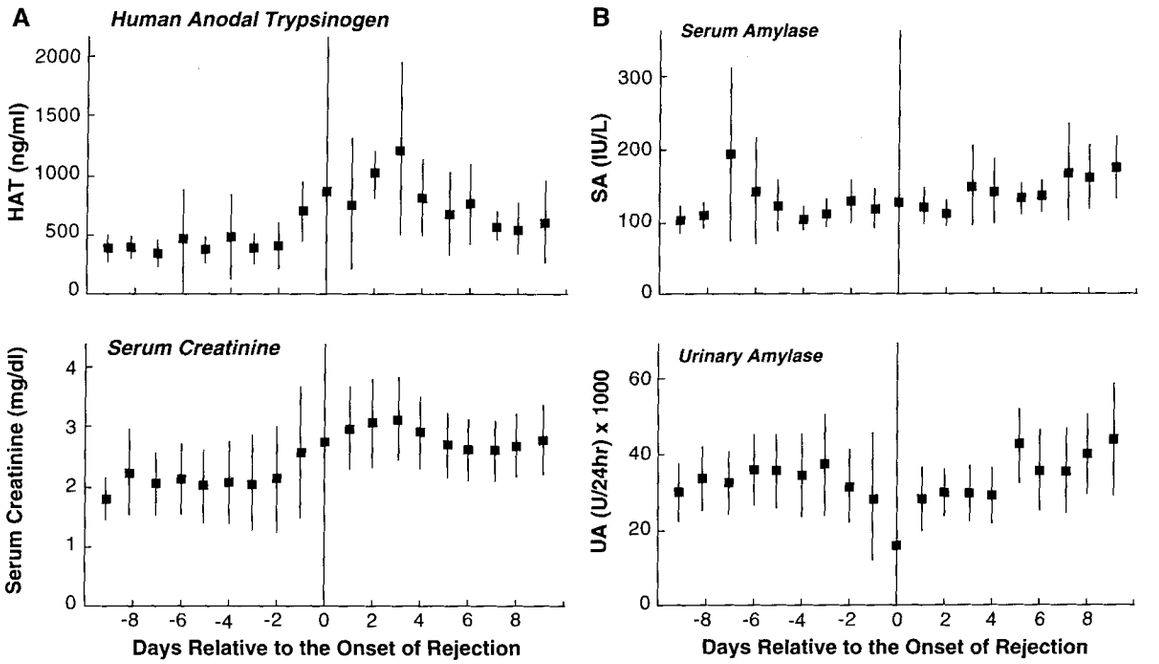


Fig. 4 **A** Profile of HAT (μg/ml) and serum creatinine (mg/dl) and **B** profile of serum amylase (IU/l) and urinary amylase (U/l) on days relative to the onset of rejection after SPK transplantation

determination of urinary amylase, which can be inconsistent and occasionally, difficult to interpret.

To date, this parameter is still used by many centers although opinions differ concerning the usefulness of the urinary amylase test [7]. Finally, the percutaneous pancreatic biopsy has become accepted as a relatively safe procedure to confirm pancreatic rejection, although most authors agree that threre is still need for a reliable serum marker to time an invasive pancreatic biopsy. We suggest that HAT after SPK is an additional helpful noninvasive test. Since the kidney appears to be the most reliable indicator and kidney and pancreas rejection frequently occur at the same time, HAT is not a mandatory additional test after SPK transplantation. The SPK is an ideal setup, however, to gather more information and experience of this test. We suggest that in pancreatic transplantation alone, the HAT test could be a sensitive and useful indicator to detect rejection and facilitate timing of a pancreatic biopsy. One of the drawbacks of this study was that no pancreatic allograft biopsies were performed. Evaluation of the true clinical value of HAT requires a prospective study that will correlate HAT and other noninvasive tests with simultaneously obtained pancreatic histology.

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