Clinical value of “zero-hour biopsy”
Clinical and experimental opportunities of qualitative and quantitative improvement of renal transplantation

PhD Thesis

Péter Szakály MD

PhD School Leader:	János Szolcsányi MD, Dsc
Programleader:	Valér Csernus MD, DSC
Mentors:	Örs Péter Horváth MD, Dsc
	Dóra Reglődi MD, PhD

Pécs University
Faculty of Medicine
Center of Medical Studies and Health Services
Department of Surgery

Pécs

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1. INTRODUCTION

Life expectancy of successful renal transplant patients and graft survival were mainly limited by acute rejection processes in the past. Introduction of modern immunosuppressive agents – first of all wide-scale use of cyclosporine from 1980s – led to a dramatic decrease in the acute rejection rate. Due to these agents the rate of functioning kidneys for more than a year exceeded 80% by the end of the 1980s. At the beginning of the new century a wide range of rejection inhibitors are available regarding both initial and long-term immunosuppressive treatments. Today the question to be answered is not whether a kidney can be kept in the body but the functions and the side effects are the main points.

In spite of the improving results the long-term benefits of renal transplantation fell behind the expectable potential possibilities. After the more effective prevention and treatment of acute rejection processes the main cause of kidney graft loss is chronic allograft nephropathy following cardiovascular deaths. This definition replaced the former clinical picture known as chronic rejection. This is such a multiple etiologic clinical picture which may occur at any time. When adequate treatment is not available in time repeated development of chronic renal failure is unavoidable.

Several cost-benefit investigations have already demonstrated that renal transplantation besides providing a better quality of life is a more economical method in treatment of end-stage renal diseases than any other supplementary renal treatment. As a consequence, organ transplantation should be applied in every renal patient where suitable donor organ is available and kidney transplantation is not contraindicated due to physician-professional aspects.

2. OBJECTIVES

Main objectives of my scientific work:

1. Through the literature review of chronic allograft nephropathy (CAN) I focus on the role of factors which have specific role in the long-term function of transplanted kidneys.

2. On the basis of "zero-hour" biopsy findings of our renal transplant patients I analyzed the effects of the original histological quality of transplanted kidneys on early and late outcome of transplantations. During the analysis of our patients I tried to answer the question how the result of "zero-biopsy" influences the development of chronic allograft nephropathy or if it exists whether there is a possibility to maintain renal function.

3. The number of brain-dead donors' kidney is limited, live organ donation fall behind the possibilities in Hungary and the number of patients waiting for kidney is increasing. Due to this, the difference between supply and demand is increasing. The way out of this situation can be to involve non heart beating brain dead donors. Before the change of the legal regulation our clinic performed operations of such kind of donation and later successful renal transplantations. On the basis of our results I offer a survey of potentials to increase the national donor supply.

4. Two important elements of successful transplantation are the prevention and treatment of damage due to ischemia/reperfusion. This process has a more significant clinical role in non-heart beating donors. As the process leads to apoptosis, during my experiments I investigated an anti-apoptotic protein and tried to detect whether the degree of damage is reducible.

3. CHRONIC ALLOGRAFT NEPHROPATHY

Chronic allograft nephropathy seems to be the "cornerstone" of today's transplantation. It may appear at any time. During the development of the disease kidney functions gradually decrease, protein in the urine and high blood pressure appear leading to progressive fibrosis and end-stage renal failure. Both alloantigen-dependent and alloantigen-independent factors have a role in the development.

3.1. Definition of chronic allograft nephropathy

The disease is characterised by gradually decreased renal function of the transplanted kidney, progressive renal fibrosis and the development of end-stage renal failure. Earlier definition was the so-termed chronic rejection as the process was entirely considered to be of immune origin alike acute rejection. In its development the role of several alloantigen factors has recently been revealed so the term 'chronic rejection' may be misleading. Instead, the definitions of 'chronic allograft nephropathy' or 'chronic allograft dysfunction' are advisable to be used as they rather refer to the multicausal nature of the disease.

Exact diagnosis requires histological examination. The picture has to be separated from the recurrent and *de novo* glomerular diseases as well as from immunosuppressive medication-associated renal toxic side effects. In the letter case the histologist may face a really difficult task, to distinguish congenital and required histological changes. The most widely used immunosuppressive medications – calcineurin inhibitors (CNI) – have a well-known side effect, namely renal toxicity with a histological picture highly resembling chronic allograft nephropathy. The picture becomes more complicated when the changes referring to toxicity and chronic allograft nephropathy can be found together.

3.2. Clinical picture of chronic allograft nephropathy

It develops months or rather years after the operation. Clinically the process is characterised by elevated levels of serum creatinine, proteinuria and high blood pressure. Slow elevation in serum creatinine levels is present in 80% of histologically proven cases of chronic allograft nephropathy. The elevating creatinine level refers to a gradual decrease of glomerular filtration rate (GFR). The rate of GFR decrease is about 0.5 ml/min/month which is similar to other progressive renal diseases.

Presence of proteinuria may be the first symptom of its development. In most patients daily proteinuria is between 1-2g and only 6-8% have a daily urine protein excretion less than 0.5g. The diagnostic value of high blood pressure onset is low in itself as patients are already hypertensious at the time of the operation and the long term use of rejection inhibitors also

results in high blood pressure. Patients with chronic allograft nephropathy are more likely to develop severe high blood pressure.

3.3 Histological characteristics

Aspecific tissue morphology characterises: glomerulosclerosis, interstitial fibrosis, tubular atrophy and obliterative changes in the blood vessels. The latter accompanies typically extensive, concentric intimal hyperplasia in arteries and arterioles. Intima-proliferation is caused by migration of myofibroblasts from media into the intima, proliferation and synthesis of extracellular matrixprotein. It is accompanied by infiltrating macrophages, T-cells and more rarely foam cells in the veins.

The damaged kidney is infiltrated by mononuclear cells (macrophages and T-lymphocytes). Less significant infiltration degree can be observed then in acute rejection. Inflammatory cells can mainly be found periglomerularly and perivascularly. Both cytotoxic and helper T cell subpopulation appear among T lymphocytes.

Glomerular changes are characterised by the collapse of capillaries, mesangial widening, extensive matrix protein accumulation and development of focal segmental glomerulosclerosis. Podocyte cell bodies become elongated, leg extensions thin and they fuse. Later, seriously damaged cells detach from basement membrane surrounding the capillaries. Appearance of glomerular hypertrophy is frequent but less significantly correlates with CAN progression.

4. CLINICAL SIGNIFICANCE OF “ZERO-HOUR” BIOPSIES

The growing demand for transplantation resulted in easing kidney donor criteria. As a consequence, the number of marginal donors and non-viable grafts increased. I wanted to answer the question, how much the pathological findings of “zero-hour” biopsy determine the early postoperative period and how much it can be considered as an alloantigen-independent factor in the development of chronic allograft nephropathy in a big patient group.

Conflicting opinions were voiced on its predictive value. Some experts assess it inadequate to determine early graft rejection, early and late graft function while others take into consideration its predictive feature. Representatives of different viewpoints agreed that “zero-hour” biopsy may be the basis for comparison in analysis of subsequent rebiopsies.

4.1. Materials and methods

Between 13 May 1994 and 25 September 2007, 502 pretransplant “zero-hour” biopsies were performed just before transplantation. Grafts came from cadaver donors (2 non heart beating donors) in 481 cases while in 21 cases grafts were transplanted from living donors. In 58 cases both kidneys were transplanted in our clinic. Combined transplantation of the kidney and pancreas was performed in 53 patients. One patient received the third, 33 patients the second while the others the first transplant.

"Zero-hour" biopsy was performed from the upper pole of the kidney just before the transplantation after the kidney had been dissected in icy bath. After the resection of about a rice size tissue, the wound was closed by atraumatic continuous suture. Histological changes noticed during process developed either in the donor or during storage. Side effects due to the performance of "zero-hour" biopsy were not observed.

Later subsequent biopsies were performed when:
• more than 7 days delay occurred in the onset of graft function,
• clinical suspect of acute rejection arised,
• steroid-resistant acute rejection occurred,
• after immunotherapy,
• Se creatinine increase was not clinically justified.

Morphological analysis of histological sections was performed by light microscope, immunofluorescence microscopy (IgG, IgM, IgA, C3, MAC), transmission electron microscopy according to standard techniques. Banff criteria were essential to determine histological severity.

4.2. Results

Grafts of donors did not show any macroscopic changes from normal kidney, laboratory findings and vital parameters met the international recommendation/criteria. In spite this fact hardly more than one-third of grafts had normal histological pictures. In the remaining cases arteriosclerosis (AS), acute tubular necrosis (ATN), tubulointerstitial nephritis (TIN) or glomerulonephritis (GN) were found in the pathological findings.

Not significant differences were found in individual groups concerning basic disease, duration of supplementary kidney treatment prior to transplantation, mean age of recipients and HLA, B and DR mismatches. During the follow-up period (on the average 1492 days) 387 biopsies were performed. Massive hematuria resulting bladder tamponade occurred only once during rebiopsies. In spite of conservative treatment it reoccurred every 3-4 days. With the help of image forming processes the location of bleeding could not be detected so we had no other choice than to remove the graft. Since we changed to ultrasound guided biopsy for histological diagnosis we have not experienced any side effects demanding intervention.

Comparable aspects of different groups are summed up in Table 1. Comparison of groups was based on such aspects which had an influence on both the short- and long-term results and showed significant changes among the groups. The examination of significance was performed by means of t-probe and difference was considered to be significant in case of $p<0{,}001$.

	Normal	AS	ATN	TIN	GN
Number of patients / frequency	203 / 40,4%	107 / 21,3%	139 / 27,7%	21 / 4,2%	32 / 6,2%
Age of donor (year)	33,4	54,2	36,1	51,8	45,6
Start of kidney function (day)	0,16	2,2	6,1	4,3	3,2
Number of DGF / frequency	21 / 10,35%	29 / 27,1%	**65 / 46,76%**	**9 / 42,856%**	7 / 21,88%
Number of NVG / frequency	1 / 0,05%	3 / 2,8%	5 / 3,6 %	1 / 4,76%	0
Se creatinine after charge (µmol/L)	158,7	258,3	**343,3**	**306,5**	216,1
Se creatinine 3 months later (µmol/L)	119,5	204,2	162,7	**285,5**	171,8
Se creatinine last (µmol/L)	114,2	217,5	119,7	**319,6**	121,4
Number of acute rejection / frequency	157 / 77,34%	94 / 87,85%	126 / 90,65%	18 / 85,71%	25 / 78,13%
Steroid resistant AR / rate*	11 / 5,42% / 7%	10 / 9,35% / 10,64%	14 / 10,07% / 11,1%	**4 / 19,05% / 22,2%**	2 / 6,25% / 8%
Number of CAN / frequency	41 / 20,2%	**46 / 43%**	30 / 21,6%	**16 / 76,2%**	7 / 21,9%
Development of CAN (day)	1074,6	**513,6**	987,3	**227,8**	962,7
Return to dialysis / rate	23 / 11,3% / 56,1%	**42 / 39,25% 91,3 %**	21 / 15,1% / 70%	**16 / 76,2% / 100%**	5 / 15, 65 % 71,4%
Start of Re-dialysis (day)	1149,2	672,4	1324,1	**255,9**	1311,2
Number of functioning graft / rate	153 / 75,4%	**42 / 39,3%**	95 / 68,35%	**2 / 9,5%**	23 / 71,88%

Table 1: Contrasting of different type of histological group (bold: significant different to the normal histological group, $p<0,001$)

* first value show correlate the rate of the percentaged to the all patients of gruop, the second value to the number of cases above

4.3. Discussion

Normal histological group

Best results can be expected in this group both on short- and long-term if serious side effects do not develop during the early postoperative period. The risk of development of severe rejection process is low. When adequate attention is paid to follow up and patient compliance is satisfactory the treatment of CAN detected at early stages can be successful as well as effective graft function period can significantly be improved.

Grafts with arteriosclerosis

Aging increases the probability of arteriosclerosis in donors. These grafts are characterised by acceptable early function but later they lack the necessary reserves for long term adequate function. Patients having this kind of kidney form a potential risk group for CAN as the developed histological changes are practically identical with those demonstrated by grafts with arteriosclerosis. Long term function of these grafts can be worsened by prolonged cyclosporine therapy and are negatively influenced by many of chronic renal failure end-stage complications such as hypertension, hyperlipidaemia and diabetes mellitus. In the absence of "zero-hour" biopsy it is difficult to diagnose cyclosporine nephrotoxity as cyclosporine similarly to arteriolosclerosis forms a nodular picture.

Acute tubular necrosis

As graft tubular damage mostly occurs in the same age group where grafts have normal histological pictures it is to be expected that these grafts would have belonged to the normal group but during the treatment of the patient, operation, perfusion or storage they suffered damages to a lesser or more extent.

In acute tubular necrosis of grafts the result of "zero-hour" biopsy may predict delayed onset of graft function. These grafts are advisable to be followed by biopsy once a week until the onset of the function. The degree of regeneration can be assessed in this way. In non-functioning grafts due to ATN the development of acute rejection can be more often observed which may remain undiagnosed and untreated in absence of biopsy during the follow-up.

Furthermore, characteristic features are poorer graft survival and function as well as higher rate of nonviable grafts. The need for supportive haemodialysis is the highest in this group. Later no significant differences can be observed in functioning grafts compared to the normal group.

Tubulointerstitial nephritis

In spite of the small number of cases it is obvious that the tubulointerstitial group has the most unfavourable prognosis. They have higher Se creatinine level, elevated acute rejection rate and the highest graft loss rate. The most important characteristics of this group are the poor early and late graft functions. Due to this fact CAN develops significantly earlier and we are helpless in these cases. In spite of our therapeutic efforts we could not achieve essential changes. Prognostically, this group is considered to be the most unfavourable. If histological findings were available before the operation then transplantation of kidneys in this group could be avoided.

Group of glomerulonephritis

IgA nephropathy was the observed lesion in every case, donor's anamnesis involved alcoholism. Renal function measured before donation proved to be normal. Significant difference from the normal group was only detectable in their mean age. Their functions approached the findings of the group with normal histological results both in a short- and long term. The results of the subsequent re-biopsies were unexpected as the original histological changes were totally eliminated. An interesting aspect of IgA glomerulonephritis is that it causes chronic renal failure then, after the transplantation it may appear in the transplanted graft but at the same time IgA nephropathy in the transplanted graft terminates due to immunosuppression. It is thought-provoking. It does not have a disadvantageous effect on the outcome of the transplantation so no further examinations were carried out in this direction.

4.4. Conclusion

On the basis of the long-term follow up of 502 kidney or combined kidney and pancreas transplants' we can establish that the findings of "zero-hour" biopsies have an effect on the assess of both the early postoperative period and the subsequent term. They may help in clinical judgement of postoperative anuria, influence therapeutic decisions in case of a severe, acute rejection or they can have a prognostic feature in the development of chronic allograft nephropathy.

With full knowledge of results, those in TIN group, 4,2% out of transplanted grafts should have been disregarded. Not even the possessed outcome or the retrospectively analysed data could prove a parameter which could have served as a basis for selection before donation. The unfavourable outcome of Grade III AS was unforeseen as changes of blood vessels in the kidney were not proportioned with sclerosis of big vessels.

It is true that donors in both groups were aged over 50yr or in most of the cases they were defined as marginal donors. One of the main characteristics of marginal grafts is that damages due to ischemia/reperfusion, length of cold ischemia and haemodynamical stability of recipient are highly emphasised. In the light of this fact some principles of the national transplantation program should be revised. In case of marginal donors taking histological sample could be introduced during donation and HLA determination would not be necessary but two blood group compatibles recipients – in good health condition - from the region on the basis of negative cross matching would get the kidneys. On the basis of histological findings a considerable proportion of NVG could be screened. Ischemic period would be within 12 hours reducing damages due to ischemia/reperfusion. Neglecting marginal donors in transplantation is unfeasible because of the increasing demand. If we had the chance to increase the supply of donor organs then the severity of the acceptance criteria of marginal donors' could be increased. Presently, the definition of marginal donor is not yet classified in Hungary rather it is specified on different basis in different countries or during clinical examinations. The Monitoring Board for supervision of transplantations urges the creation of classification and rethinking of allocation.

I believe the clinical value of "zero-hour" biopsy is expressed:
1. In the judgement of primary non-functioning grafts.
2. In the influence on treatment strategy for severe rejection processes.
3. In its predictive value in both the short- and long- term results.
4. When supports the pathologist to evaluate rebiopsies.
5. In determination of effective treatment for chronic allograft nephropathy.
6. In cases of marginal donors the findings of biopsy taken during the donation could be conclusive.

5. NON-HEART-BEATING DONATION

There is a special possibility to use cadaver donors the so-called non-heart-beating donors. In these dead individuals the irreversible circulatory failure precedes the diagnosis of brain death or follows it soon. This is still an unutilized possibility in Hungary which could highly increase the number of suitable kidneys for transplantation.

5.1. Method

In NHBD classification the location and circumstances of death are taken into consideration. On this basis warm ischemic damages are considered to be either controlled or uncontrolled. This classification determines whether it is accepted for organ donation by the transplant team or is declared to be only a tissue donor (Table 2).

	Circumstances	Location	Transzplant team	Warm ischemia
I.	Dead on arrival	Outside the hospital	Tissue donor	Uncontrolled
II.	Unsuccesful resuscitation	Emergancy department	Tissue donor	Uncontrolled
III.	Threatening circulatory arrest (artificial respiration is stopped)	Intensive Care Unit	Organ donor	Controlled
IV.	When brain death is diagnosed or subsequent circulatory arrest	Intensive Care Unit	Organ donor	Controlled
V.	Unsuccessful resuscitation after sudden circulatory arrest	Intensive Care Unit	For consideration	Controlled

Table 2: Maastricht classification of non-heart-beating donors

When organ donation is determined perfusion has to be started without delay to avoid further damages. The fastest method is to insert a double-balloon 3 lumen catheter through the femoral artery still in the intensive care unit. After leading up the distal balloon it is filled up with air and at the same time it is positioned in the aortic bifurcation. With the inflation of the proximal balloon the parts of abdominal aorta where kidney vessels may derive/originate are excluded. With the onset of perfusion the period of warm ischemia is over. Through Foley catheter - led up in vena femoralis communis - blood and preservative fluid from the kidney can be drained. The donor should be transferred to an operating theatre - under constant perfusion - where the donated organ can be transplanted.

5.2. Case report

On 25 June, 1995 a male donor was reported from the Neurology Clinic of our University who suffered an irreversible brain damage. His renal function was in the normal range. Neither legal nor medical objections were found to organ donation. After brain death was confirmation of brain death the patient was transferred to our clinic. However, before the donation the patient suffered a circulatory arrest. During an unsuccessful resuscitation with the surgical preparation of a. femoralis communis a double ballooned, 3-lumen intraluminal catheter was placed into the aorta. After adequate positioning in situ perfusion was initiated with Histidine-Tryptophan-Ketoglutarat solution. Both kidneys were removed after perfusion with 5000ml solution. During the process a 16-minute warm ischemic period was registered.

We performed the transplantation of one of the kidneys on 26 June at our clinic. A 54-year-old male patient was the recipient who had received haemodialysis due to chronic glomerulonephritis since December 1992. His past medical history besides the kidney disease included hepatitis C positivity.

During the operation the renal artery was anastomosed end to side to the arteria iliaca communis and v. renalis was anastomosed end to side to v. iliaca externa. The ureter was sutured into the bladder using the antireflux technique of Lich-Gregoire.

Histological findings of “zero-hour” biopsy: marked epithelial cell vacuolisation in tubules but in half of the tubules retained morphology, scarred enlargement in the tubulointerstitium, minimal inflammatory cells infiltration and no pathological changes were observed in the blood vessels. The histological findings complied with the origin of the donor and on the basis of the findings we could look forward to the postoperative period with optimism.

Renal function started to improve gradually 4 hours after the operation. Diuresis was subsequently stabilized. Differently from usual the serum creatinine level decreased only slowly, on the day of the patient’s discharge it was still 275 μmol/L.

According to the recommendation of those days we started induction treatment with ATG and it was continued up to the fifth postoperative day. At the same time methylprednisolon and azathioprint were administered. From the fourth day on 4mg/kg/day and from the fifth day on 8mg/kg/day cyclosporine was orally administered. The dosage was subsequently controlled due to regular blood tests. Postoperative period was eventless. On the 14^{th} postoperative day the patient was discharged home with stable renal function and good general status.

A few days later the patient was readmitted with fever and decreased urine volume. In the background of the complaints lymphocele resulting ureteral compression was proved.

Applying percutaneous drainage and antibiotics the complaints and perirenal fluid accumulation disappeared. On leaving the hospital serum creatinine level was already 124 μmol/L.

In 1996 the patient was readmitted with fever, fatigue and elevated liver function values. Serological test proved flare-up of Hepatitis C virus. After stopping the administration of azathioprin and giving a daily dose of 20mg methylprednisolon liver function showed a slow improvement. At the same time, due to the steroid treatment continuous and high dose (60U/day) of insulin was necessary. After a temporary feverless condition the patient again developed fever with mycotic pneumonia in the background. During targeted treatment fever subsided and chest X-ray picture showed improvement. When steroid doses were gradually decreased the need for insulin administration also decreased and later even ceased. Subsequently, antidiabetic treatment was not required. The patient was discharged after a 57-day hospitalization with a creatinine level at 88 μmol/L.

The next readmission occurred in April 1997 due to a decrease in daily urine output, fatigue and elevated temperature. Histological findings confirmed clinical signs of rejection. Patient's condition became normal after parenteral administration of methylprednisolon at the dose of 500mg/day for three days. Serum creatinine level decreased to 82 μmol/L.

The summer of 2007 showed a slow deterioration of renal function therefore ultrasound guided renal biopsy was performed. The findings verified chronic allograft nephropathy, cyclosporine was replaced with sirolimus. Currently, renal function in the patient is stable, about 217 μmol/L.

The other kidney transplant was performed in Szeged. The recipient – two years after the operation – suffered a stroke leaving the local pub. He died with a normally functioning kidney.

The case of these two patients was a milestone in the history of national transplantation because their donor was the first non-heart beating donor in Hungary.

In 1998 another NHBD was operated on under similar circumstances. The transplanted and functioning kidney was lost due to Grade III/B acute rejection more than 2 months after the operation. Unfortunately, the other kidney was not transplanted as it was refused by other centres due to its origin…

Due to changes of legal provisions regarding determination of death and brain death no further donation occurred.

5.3. Discussion

More active European countries than Hungary refuse to give up the use of them, even if they have a well operating living kidney donor programme. Besides Europe, countries where NHBD use is legally accepted and the number of cases is revealed are as follows: USA, Australia, Guatemala, Hong-Kong, Israel, Japan, Canada and Malaysia. Among these countries the highest number of cases occurs in the United States and Japan. In the former between 1996 and 2006 with an increasing tendency 71-647 transplants were performed while in the latter between 2001 and 2006 this number was 59-102.

As the date of introduction differed the number of performed transplants shows also a wide range. Their rate versus beating heart donors may represent a considerable part. Considering the non negligible fact that mean age of NHBD is lower despite of circulatory stop besides the kidneys other organs are also utilized (Table 3).

	HBD	LD	NHBD	HBD/NHBD	Since	Total No. of NHBD	Kidney	Liver	Lung	Pancreas
Austria	202	59	3	1 %	1994.	26	+			
Belgium	273	60	28	11%	1994.	73	+	+		
Czech Republic	193	33	2	1%	1972.	12*	+			
France	1441	282	1	<1 %	2006.	1	+			
The Netherlands	200	277	90	45%	1981.	674	+	+	+	+
Coatia	60	24	0	<1 %	2004.	2	+			
Latvia	43	0	12	28 %	1992.	86	+			
Italy	1239	223	0	<1 %	2005.	1	+	+		
Spain	1509	120	76	5 %	1994.	582	+	+	+	
Switzerland	80	125	0	<1 %	2002.	19	+			
UK	633	683	146	19 %	1989.	928	+	+	+	+

* since 2001.

Table 3: Europian data of organ donation in 2006, when has non heart beating donor program

5.4 Feasibility in Hungary

The above mentioned two examples clearly show that successful renal transplantations from NHBDs can be performed under national circumstances. Cadaver donation activity fails to come follow the leading European countries. In 2006 Hungary was among the average range. The problem originates from the fact that this low activity can not meet the requirements. Unfortunately, the current reorganization in the Hungarian health care has a negative effect on donor application. Living donor transplants are well behind expectancy.

Donor reports of intensive care units are basically determined by the management of the institute or ward and their attitude. Due to this fact significant activity differences can be seen in different parts of the country. It is worth mentioning that effectiveness of intensive care units is not classified by the high number of donors. A fair comparison could be made if we knew how many patients became donors out of those who were reported to have received artificial respiration.

Hospital wards having reported donors so far probably would not refuse a NHBD program. The wards "arguing" with the fact of brain-death may take a role in this program. A national coordination network has been operating for some years under the authority of National Blood Supply Service as Organ Coordination Office helping our work. In organization of NHBD program their network could have a significant role.

Regarding the investment the launch costs are not expected to be significant. Intensive care units should have a special catheter and perfusion solvent. Bigger clinics usually have vascular surgeons but it does not cause any difficulties for general surgeons to lead up the catheter through the prepared femoral vessels and start perfusion until the arrival of the transplant team.

5.5 Conclusion

The number of non heart beating donors in Hungary can only be guessed. Taking advantage of the potentially involved possibilities the number of transplanted organs could significantly be increased. Taking into consideration that mostly younger donors are involved in spite of the initial poor results the long-term results do not suggest any drawback to HBD. Comparing to the non-negligible number of marginal donors long-term results in NHBD kidney transplants are significantly better.

The growing demand for transplants requires the national transplant program to use non heart beating donors.

6. EXPERIMENTAL POSSIBILITIES TO IMPROVE TRANSPLANTABLE KIDNEY QUALITY

During transplantation damage due to ischemia/reperfusion, its prevention and treatment possibilities have significant clinical importance. These damages essentially determine both early and late renal functions. It follows from this that knowledge and influence on apoptosis may have a positive effect on grafts. In the previous chapter I introduced the potential clinical possibility of donor pool increase. Opponents to non heart beating donation focus on higher rate of late function due to incalculable ischemic damage

and the higher number of nonviable grafts. If damage due to ischemia/reperfusion could significantly be decreased a remarkable increase in the number of donors could be experienced.

I wanted to prevent the development of apoptosis so my aim was the experimental investigation of an anti-apoptotic protein. I panned to prove the effect during investigation of pituitary adenylate cyclase activating polypeptide (PACAP).

6.1. Pituitary Adenylate Cyclase Activating Polypeptide

Pituitary adenylate cyclase activating polypeptide was first discovered in 1989 as a hypothalamic peptide. PACAP exerts a variety of biological functions in the nervous system and in several peripheral organs. Diverse effects have been described in the cardiovascular, respiratory, immune and gastrointestinal systems and in endocrine

PACAP also occurs in the urogenital system and several effects on smooth muscle contraction, inflammation and sensory innervation. However, relatively little is known about the distribution and functions of PACAP in the kidney. Although early studies showed no binding sites for PACAP in the kidney, later studies demonstrated binding of PACAP and presence of the specific PAC1 and both VPAC1 and VPAC2 receptors in renal tissues. It has been shown that PACAP stimulates renin secretion in the kidney via activation of PAC1 receptors and that PACAP increases renal blood flow.

PACAP is anti-apoptotic, anti-inflammatory, protects neurons against toxic agents, and has survival-promoting effects in animal models of neurodegenerative diseases, traumatic and ischemic conditions. The anti-inflammatory effects of PACAP have been shown to lead to the alleviation of several in vivo models of inflammatory diseases. Although the most well-studied cytoprotective effects of PACAP are its neuroprotective effects, the peptide exerts protective actions also in several peripheral systems. It has been shown to be protective against oxidative stress-induced apoptosis of cardiomyocytes and endothelial cells. PACAP also reduces the apoptotic death of ovarian follicular cells and of prostate cancer cells. Apoptosis of immune cells can also be prevented by PACAP: spontaneous and glucocorticoid-induced apoptosis of $CD4^+CD8^+$ thymocytes and activation-induced apoptosis of T lymphocytes through reduction of FasL expression. These observations show that PACAP promotes survival of most studied neuronal and non-neuronal cultures, however, this effect may be cell-and tissue-specific, since no survival promoting effect can be shown in adult rat myenteric neurons.

Although neuronal and renal cells are different in nature, their responses to ischemia share several mechanisms. Apoptotic and inflammatory pathways play important roles in both types of tissue injury. The neuroprotective effects of the peptide have already been demonstrated in several models of neuronal ischemia, including global and focal ischemia in rats and mice and also in retinal ischemia. In a pioneer study, PACAP has been shown to be protective against renal ischemia via minimizing renal inflammatory cell infiltration. Most recent studies have shown that PACAP is also protective against myeloma kidney injury via suppressing cytokine production and inhibiting the upregulated apoptotic pathways. In both in vivo studies, PACAP was used as a continuous infusion. Its efficacy has also been proven in a human study. The aim of the present study was to examine the effects of a single dose PACAP administered after varying times of renal ischemia on renal morphology and survival.

6.2. Materials and Methods

Male Wistar rats weighing 300-350 gr were used. Animals were maintained under 12-hour light/dark cycle with free access to food and water. Animal housing, care and application of experimental procedures were in accordance with institutional guidelines under approved protocols (No: BA02/2000-20/2006, University of Pecs).

Animals were anesthetized with pentobarbital (35 mg/kg bodyweight). Heparin (100IU/kg bodyweight) and PACAP (100 µg dissolved in 100 µl saline) or vehicle were injected into the jugular vein was injected into the jugular vein before laparotomy. Administration of PACAP and vehicle was performed in a randomized, blinded fashion. Following drug administration total median laparatomy was performed, renal vessels were freed and warm ischemic damage was induced by crossclamping both renal pedicles for 15, 30, 45, 60 or 75 minutes. Renal pedicles of shaminjured animals were clamped for 1 second and clamping was immediately released. The number of animals was 10 in each time-group, half of them were controls and half of them were PACAP-treated. Animals were sacrificed 16 days after operation, which was considered 100% survival compared to rats not surviving until the day of sacrifice. In case of premature death, kidneys were removed and further processed for histological analysis.

Rats were decapitated under pentobarbital anesthesia and kidneys were removed and placed in 4% paraformaldehyde for fixation. Serial, 10 µm thick sections from the kidney were made and stained with routine haematoxylin-eosin staining. Digital photomicrographs were captured with a Nikon FXA photomicroscope attached to a digital camera (Spot RT Color camera). The degree of renal tubular atrophy was determined in the cortex and medulla of the kidney.

6.3. Results

PACAP-treated rats had a significantly higher survival rate than control animals. While all rats with 15 and 30 min renal ischemia survived until sacrifice, premature death among control animals with 45 min ischemic time was 25% in contrast to PACAP-treated animals, which all survived until the day of sacrifice. Most striking difference between the mortality of control and PACAP-treated rats was observed in the 60-min ischemic groups. All control rats with 60 in ischemia died prematurely, between days 1-5 after the operation, while PACAP-treated rats with 60 min ischemia had 95% survival rate. 75-min ischemia was not possible to perform in control animals due to death before termination of ligation, while PACAP-treated rats had an approximately 50% survival time, rats died between 5-10 days after the operation (Fig. 1). These observations clearly show that PACAP treatment increased the survival time after renal ischemia/reperfusion injury.

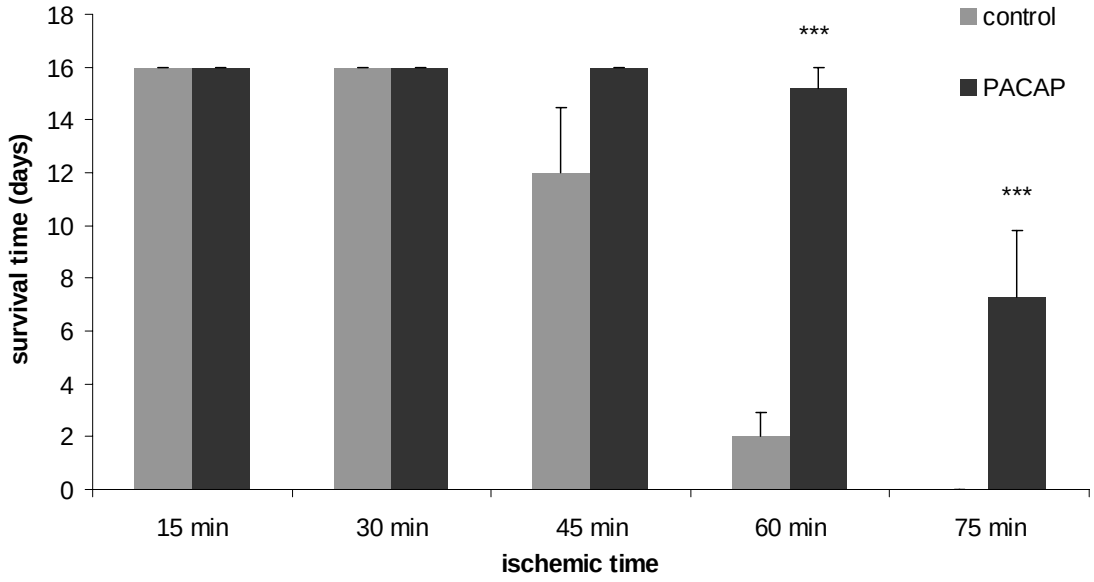

Fig. 1: Survival rate of control and PACAP-treated animals after renal ischemia/reperfusion with varying ischemic times

Histological analysis revealed that kidneys of rats with 15 or 30 min renal ischemia showed normal appearance, with no difference between control and PACAP-treated groups. After 45 min of ischemia, surviving rats displayed multifocal acute tubular atrophy of Grade I. In contrast, PACAP-treated animals survived with normal appearance or very subtle morphological changes in the kidneys. 60 min of ischemia led to severe, multifocal Grade II tubular atrophy in control rats. PACAP-treated animals showed subtle focal tubular alteration after 60 min of ischemic time. No evaluation of control kidneys was done in the 75-min group due to premature deaths. Although PACAP-treated rats survived longer, they also exhibited severe focal tubular atrophy.

6.4. Discussion

In summary, our results clearly show that PACAP is able to prolong the renal ischemic time, it decreases mortality and attenuates tubular degeneration following renal ischemia.

The presence and various effects of PACAP under normal and pathological conditions have been described in the urinary system. PACAP receptors occur in normal and cancerous tissues of the urinary. PACAP immunoreactivity has been shown in nerve fibers of the ureter, urinary bladder and urethra, in association with smooth muscle, subepithelial plexus and blood vessels, with PACAP38 being the dominant form. PACAP is suggested to play a role in muscle relaxation, micturition and sensory innervatin of the urinary system.

In spite of the extensive literature on occurrence and effects of PACAP in the urinary system, little is known about the effects of this peptide in the kidney. Although the detailed distribution of PACAP receptors in the kidney is not known, the presence of PAC1 and both VPAC1 and VPAC2 has been shown. Currently available data indicate that PACAP stimulates renin secretion, increases blood flow in the kidney and has renoprotective effects. The focus of our study was to investigate the suggested role of PACAP as a renoprotectant

agent and we provided further evidence to support the general cytoprotective effects of the neuropeptide.

Numerous studies have shown that PACAP has not only protective effects in the nervous system, but similar protective actions are exerted in peripheral, non-neuronal cells and tissues. PACAP increases cell survival and has anti-apoptotic effects in cardiomyocytes, endothelial, immune cells PACAP attenuates ischemic injury in global and focal cerebral ischemia in vivo and in cultured cardiomyocytes in ischemia/reperfusion injury.

A few studies have demonstrated that PACAP has also renoprotective effects in various models of kidney injuries. PACAP38 attenuates myeloma kidney injury. This is accompanied by not increase, but on the other hand, suppression of myeloma cell growth. PACAP inhibits myeloma light chain-induced proinflammatory cytokine expression and attenuates the cellular damage in renal tubular epithelial cells via inhibition of p38 MAPK and nuclear translocation of the p50 subunit of NFkappaB. PACAP is also effective in streptozotocin-induced diabetic nephropathy, where PACAP-treated rats display nearly normal glomerular appearance, no tubular vacuolization and decreased TNFalpha and the profibrotic cytokine, transforming growth factor-beta levels. Similarly, PACAP also reduces TNFalpha levels in the kidneys in gentamicin-induced nephrotoxicity.

In ischemia-reperfusion injury, PACAP administration ameliorated kidney injury in 40-min ischemia followed by reperfusion, minimized renal inflammatory cell infiltration and increased circulating cAMP. The authors showed that the effects of PACAP were dose-dependent and that PACAP was protective even when administration was delayed to 6 hours after ischemia, but further delay led to the failure of this renal protection. However, the effect of PACAP on the possible extension of the ischemic time and survival time has not been known. In the present study we showed that PACAP treatment prolonged the ischemic time, significantly decreased mortality and thus, increased survival rate following renal ischemia/reperfusion injury. These changes were accompanied by ameliorated renal morphological appearance. After 45 min-ischemia, which was a similar ischemic period used by the above-mentioned authors, PACAP treatment increased the survival rate and kidneys showed very subtle changes. A striking difference was observed in the 60-min-ischemic group: all control rats died prematurely, while almost all PACAP-treated rats survived until the time of sacrifice. An ischemia of 75 min was not possible to perform in control rats, while PACAP-treated rats survived for a few days after operation.

In the present study we showed that a single bolus of intravenous PACAP significantly ameliorates the ischemic damage in the kidney. This might be due to the efficacy of PACAP in initiating long-term cell survival cascades, as it has already been shown in other in vitro systems. We have also found in previous studies that a single in vivo injection may lead to long-term protective effects in rat models of cerebral ischemia, retinal degeneration and neurodegenerative disorders.

In summary, the present study further supports the cytoprotective efficacy of PACAP and provides an additional in vivo evidence to the growing number of studies showing the protective actions of this neuropeptide in the kidney. Further studies are required to determine the protective mechanism responsible for the observed renoprotection in ischemia/reperfusion kidney injury.

7. SUMMARY OF NEW RESULTS

1. “Zero-hour” biopsy is a valuable method in renal transplantation as it proves to be an essential basis for comparison in analysis of subsequent histological findings.
2. In case of marginal donors histological sample from the kidney taken during the donation and analysed before implantation could be an obvious demonstration to assess graft capacity.
3. The result of “zero-hour” biopsy is significant in assessment of early graft function and in selection of treatment for acute rejection process.
4. Histological diagnosis of “zero-hour” biopsy has an indicative feature on development of chronic allograft nephropathy, treatment possibility and its efficiency. On this basis congenital histological changes of the kidney have to be considered as alloantigen-independent factors of chronic allograft nephropathy development.
5. Due to the above findings taking pre-transplant histological samples is suggested to be a routine intervention in the practice of organ transplantation.
6. We performed the first non-heart beating donation in Hungary and as a result a successful kidney transplantation.
7. On the basis of our and international findings we can conclude that in case of NHBD early results are poorer but no difference can be observed on long-term compared to HBD. Therefore, their use can be advised in the national transplantation program.
8. I proved in animal experiments that single, intravenous administration of PACAP is also able to decrease renal damage due to ischemia/reperfusion. Due to its effect the degree of histological damage decreases and survival rate increases.

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