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Peri-Transplant Apoptosis in Renal Allografts*

Apoptoza w tkance nerkowej w czasie transplantacji

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Abstract

Background. Apoptosis is one of the central mechanisms leading to organ damage in the course of renal ischemia-reperfusion.
Objectives. The aim of the study was to investigate the extent of apoptosis in 53 renal allograft biopsies.
Material and Methods. Twenty-two specimens were taken from deceased donors during organ procurement, 18 were taken after cold ischemia, and 13 were obtained 30 minutes after reperfusion. Apoptosis in kidney tissue was examined by TUNEL staining. Expression of the apoptotic mediator genes for Bcl-2 and iNOS were assessed using semiquantitative evaluation of the RT-PCR *in situ*.
Results. Intense TUNEL staining as well as *bcl-2* and *iNOS* gene expressions were found in the specimens taken from the deceased donors compared with those of a living donor. No significant differences between the three examined groups were noted. There was no effect of duration of brain death on gene expression and TUNEL staining. Cold ischemia time influenced the expression of the gene for tubular iNOS analyzed after cold ischemia ($r = 0.98$, $p < 0.0001$). There was no significant correlation between the level of apoptosis and the extent of delayed graft function. Early allograft function (at days 14 and 30) positively correlated with the expression of *bcl-2* as well as TUNEL staining in tubules after cold ischemia and negatively correlated with tubular TUNEL staining and expression of *bcl-2* and *iNOS* in biopsies obtained during procurement ($p < 0.05$).
Conclusions. Extensive apoptotic cell death was observed in the specimens taken directly after brain death without a further increase during cold ischemia and reperfusion. Surprisingly, only the presence of the apoptotic process in tubular cells exerted a negative effect on early allograft function (**Adv Clin Exp Med 2008, 17, 2, 197–200**).

Key words: apoptosis, kidney transplantation, brain death, ischemia-reperfusion injury.

Streszczenie

Wprowadzenie. Apoptoza jest jednym z głównych mechanizmów prowadzących do uszkodzenia tkanki nerkowej w przebiegu niedokrwienia i reperfuzji.
Cel pracy. Zbadanie natężenia apoptozy w 53 wycinkach kory nerek podczas transplantacji.
Materiał i metody. 22 wycinki pobrano od zmarłych dawców podczas pobierania narządu, 18 wycinków pobrano po okresie zimnego niedokrwienia, a 13 po 30 min reperfuzji. Apoptozę w tkance nerkowej oceniano metodą TUNEL. Dodatkowo będzie zbadana ekspresja *in situ* enzymów biorących czynny udział w indukcji apoptozy (bcl-2, iNOS).
Wyniki. Znaczne natężenie apoptozy podobnie jak ekspresję genów dla Bcl-2 and i-NOS wykazano w wycinkach pobranych od zmarłych dawców w porównaniu z normalną tkanką nerkową. Nie wykazano różnicy między trzema badanymi grupami. Nie wykazano wpływu długości śmierci mózgu dawcy na indukcję apoptozy. Długość czasu zimnego niedokrwienia wpływała na cewkową ekspresję genu dla iNOS ($r = 0,98$; $p < 0,0001$). Nie wykazano korelacji między natężeniem apoptozy a opóźnioną czynnością przeszczepu. Obserwowano dodatnią korelację między czynnością przeszczepu w 14. i 30. dobie a ekspresją genów dla Bcl-2 i barwieniem metodą TUNEL w cewkach po

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okresie zimnego niedokrwienia oraz ujemną korelację między indukcją apoptozy w cewkach i ekspresją genów dla Bcl-2 i iNOS w wycinkach pobranych od zmarłych dawców ($p < 0.05$).
Wnioski. Znaczne natężenie apoptozy obserwowano po śmierci mózgu dawców. Okres zimnego niedokrwienia oraz reperfuzja nie wpływały na dalsze zwiększenie intensywności apoptozy (**Adv Clin Exp Med 2008, 17, 2, 197–200**).

Słowa kluczowe: apoptoza, przeszczepianie nerek, śmierć mózgu, uszkodzenie niedokrwienno-reperfuzyjne.

Events which take place after donor brain death and ischemia-reperfusion are essential for kidney allograft survival. Apoptosis is one of the central mechanisms leading to organ damage in the course of renal ischemia-reperfusion (I/R). Programmed cell death usually leads to phagocytosis of the apoptotic body without inducing inflammation. However, under some specific conditions, both clinical and experimental apoptosis may lead to an inflammatory process, which is considered to be a major cause of tissue injury. Inhibition of apoptosis in a murine model contributed to the inflammatory response during renal I/R [1].

A large variety of signals which modulate apoptosis have been described. Among these, nitric oxide (NO) is established as one of the key pro-apoptotic mediators. Under inflammatory conditions, NO is produced by inducible NO synthase (iNOS), which is widely expressed in many cell types, including macrophages, neutrophils, and renal mesangial cells; the precise role of iNOS in renal I/R, however, remains controversial. Bcl-2 protein is known to exert an anti-apoptotic effect. In this study we examined the extent of apoptosis in renal tissue as well as the *in situ* gene expressions of the apoptosis-modulating genes for Bcl-2 and iNOS after donor brain death and during I/R.

Material and Methods

This analysis included 53 renal-core needle biopsies from 12 deceased donor kidney transplants performed before and during transplantation procedures. Among the 12 donors were 7 men and 5 women with a mean age of 50 years (range: 26–66 years). Twenty-two specimens were obtained during organ procurement after perfusion with cold solution, 18 after cold ischemia just before transplantation, and 13 30 minutes after reperfusion. In 11 cases all three consecutive biopsies were harvested from one kidney. A specimen acquired from a living related donor (woman, 62 years old) during organ procurement was used for comparison. The mean duration of brain death was 7 hours (range: 1–14 hrs) and mean cold ischemia time was 29 hrs (range: 16–42 hrs). The postreperfusion biopsies were obtained after a mean warm ischemia time of 24 min (range: 0–35 min).

Paraffin-embedded tissue samples were prepared and a TUNEL assay was carried out to localize DNA fragmentation in the tissue sections. The expressions of the apoptosis-mediating genes for Bcl-2 and iNOS were assessed using semiquantitative evaluation of the RT-PCR *in situ* on the paraffin tissue sections. After RT-PCR amplification and fixation of the reaction products in 2% paraformaldehyde, a mixture of digoxigenine-labeled specific sense and antisense oligonucleotide probes for the *in situ* hybridization reactions was used. After washing, the specimens were incubated with anti-digoxigenine alkaline phosphatase conjugate (Roche) and then with the chromogen mixture NBT/BCIP. The location of mRNA chains, visible under an optical microscope as granular precipitates, were quantified by calculating the mean number of positive points per area unit in all glomeruli and 20 hpf in the tubulointerstitium. The results were corrected by β-actin gene expression.

Results

Apoptotic cells were observed in all the examined kidney tissue biopsies. In normal kidney tissue (obtained from the living donor) only a few apoptotic cell were observed in the tubulointerstium and glomeruli. Intense TUNEL staining was found in the specimens taken from the deceased donors compared with that of the living donor. Only slight *bcl-2* and *iNOS* gene expression was detected in normal kidney tissue whereas the expression was significantly enhanced in the samples taken from deceased donors. No significant differences were noted in terms of TUNEL staining intensity and *iNOS* and *bcl-2* gene expression among the three samples taken after brain death, after cold ischemia, and after reperfusion ($p < 0.05$). No effect of brain death duration on the selected gene expressions and TUNEL staining was observed. Cold ischemia time influenced tubular *iNOS* expression in the samples taken after cold ischemia ($r = 0.98$, $p < 0.0001$).

There was no significant correlation between apoptosis level and extent of delayed graft function. Early allograft function (defined as serum creatinine concentration at 14 days) positively correlated

with the expression of the gene for Bcl-2 as well as TUNEL staining in the tubulointerstitium in the biopsy samples taken after cold ischemia ($p < 0.002$) and negatively correlated with tubulointerstitial *bcl-2* gene expression in biopsies obtained during organ procurement ($p < 0.05$). Serum creatinine concentration at 30 days positively correlated with *bcl-2* gene expression in the tubulointerstitium and glomeruli in the samples harvested after cold ischemia ($p < 0.002$) and with *bcl-2* gene expression in biopsies taken after donor brain death. Negative correlation was established between renal function at 30 days and *bcl-2* and *iNOS* gene expression in the tubulointerstitium of the biopsies obtained after donor brain death ($p < 0.01$).

Discussion

The presence of apoptotic cells has been observed in kidney tissue after donor brain death, I/R connected events, and during various post-transplant conditions, i.e. acute rejection episodes and calcineurin inhibitor toxicity [2–4]. Biopsies of patients with "biopsy-proven" acute tubular damage showed significantly higher counts of apoptotic tubular epithelial cells compared with patients with immediate transplant function or even early rejection [5].

Apoptosis of renal cells may evoke factors produced during ischemia-reperfusion, such as reactive oxygen species (ROS), TNF-α, IL-1β, and TGF-β [6]. It has been demonstrated in animal models that apoptosis in renal tubular cells is induced mainly by the reperfusion process, whereas the cold ischemia period was principally connected with necrosis [7]. In the present study, intense TUNEL staining was observed in biopsies taken directly after donor brain death in comparison with normal kidney tissue. A further impact of ischemia-reperfusion on an increase in apoptotic cell counts was not noted.

Burns et al. reported that programmed cell death occurred in postreperfusion compared with prereperfusion biopsy specimens from deceased donor transplants, but a similar difference was not observed in living related donor renal transplants. Furthermore, significantly more apoptosis was observed in postreperfusion biopsy specimens from deceased compared with living related renal transplants [8]. The number of apoptotic renal tubular epithelial cells in donor biopsies before engraftment was seen to be predictive of the early postoperative course in patients undergoing kidney transplantation [5]. In the present study a positive impact of *bcl-2* gene expression and TUNEL staining was found in samples harvested after cold ischemia and a negative impact of *bcl-2* and *iNOS* gene expression in biopsies obtained after donor brain death on early allograft function.

The authors conclude that extensive apoptotic cell death was observed in specimens taken directly after brain death without a further increase following cold ischemia and reperfusion. Surprisingly, only the presence of apoptotic processes in tubular cells in samples taken after cold ischemia exerted a negative effect on early allograft function.

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