Apoptosis in acute renal failure

Marc E. De Broe

Department of Nephrology, University of Antwerp, Belgium

The landmark publication by Kerr et al. in 1972 [1], describing apoptosis as a new basic phenomenon with wide ranging implications in tissue kinetics, has changed our thinking about cell death (Figure 1). Necrosis and apoptosis differ fundamentally from each other. Unlike necrosis, apoptosis is mediated by an organized, predetermined pathway that can be divided into two stages: the commitment phase and the execution phase. The differences between apoptosis and necrosis are summarized in Table 1. In apoptosis, the most outstanding morphological and biochemical changes occur in the nucleus in which chromatin rapidly forms dense crescent-shaped aggregates lining the nuclear membrane. The plasma membrane becomes convoluted and the cells separate into a cluster of membrane segments after containing normal mitochondria and other cellular organelles. The absence of inflammation in apoptosis permits cell death without damage to surrounding allowing development and homeostasis of organs under physiological and pathological conditions.

In apoptosis, endogenous endonuclease activation has been shown to result in the cleavage of host chromatin into oligonucleosome-length DNA fragments (∼200 bp), considered a characteristic biochemical marker for apoptosis [2].

Ueda and Shah [3–5] correctly pointed out recently that equating DNA fragmentation with apoptosis is problematic: chromatin condensation and DNA fragmentation are regulated by different metabolic pathways, apoptosis can occur without DNA fragmentation and, DNA fragmentation can be seen in necrotic form of cell death.

The most frequently used methodology nowadays to visualize and quantify apoptosis is the in situ end labelling of fragmented DNA following proteinase K digestion (TUNEL). In our hands [6,7] and that of others [8], this TUNEL methodology labels a high proportion of non-apoptotic nuclei and consequently overestimates apoptosis. In addition, apoptosis has been reported to be coupled with the appearance of PCNA as well as significant Brdu incorporation. These results suggest that en route to apoptosis, cells undergo events typical of early cell-cycle traverse by expressing G1 genes and may even experience the late G1/G-phase boundary, as shown by the presence of PCNA before they abort and die [9].

The term apoptosis should only be applied when there are morphological criteria as described in the original paper by Kerr et al. [1].

Apoptosis in ischaemia/reperfusion and toxic renal injury

A major advance in the understanding of cell death is the fact that many features of the cell-signalling process, leading to apoptotic forms of cell death, are shared with those associated with necrotic form of cell death. Lieberthal et al. [10] has shown that the proximal tubule cells subjected to severe ATP depletion die by necrozing whereas moderate ATP depletion results in apoptosis; they also showed that a low dose of cisplatin results in apoptosis whereas high dosage induces necrosis [11].

One of the first in vivo studies demonstrating morphological biochemical and molecular evidence of apoptosis during the reperfusion phase (as early as 12 h) after brief periods of renal ischaemia is from Schumer et al. [12].

Ueda and Shah [13] provided strong evidence for the role of endonuclease activation in DNA damage and cell death in hypoxia/re-oxygenation injury. Of note is the fact that light and electron microscopy did not observe the morphological features of apoptosis, including chromatin condensation. This is consistent with recent studies indicating that chromatin condensation and DNA fragmentation may be triggered through separate metabolic pathways. Kaushal et al. [14,15] demonstrated the participation of caspases in hypoxic injury to the renal tubular cells and ischaemia/reperfusion injury to the kidney.

In several non-ischaemic causes of acute renal failure, such as endotoxaemia, cisplatin, HgCl2, doxorubicin, cyclosporin, radiocontrast media, apoptosis, have been shown [3]. We induced a selective necrosis of the kidney proximal convoluted tubule by injecting...
subcutaneously the aminoglycoside gentamicin to rats
[16]. After injury, the strongly increased cell prolifera-
tion in regenerating necrotic PCT was preceded by an
equally important proliferation in the distal tubules of
the cortex and outer stripe of the outer medulla in the
absence of necrosis but displaying enhanced apoptosis
(day 2 after injury). Yet, epithelial vimentin expression,
a marker of migrating proliferating cells, was restricted

Fig. 1. Apoptosis: a basic biologic phenomenon with wide ranging implications in tissue kinetics. Adapted from [1].

Table 1. Cell death

<table>
<thead>
<tr>
<th>Apoptosis</th>
<th>Necrosis</th>
</tr>
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<tbody>
<tr>
<td>Programmed death of unwanted cells (loss of growth stimuli)</td>
<td>Accidental, unplanned severe injury (toxins, ischaemia, ...)</td>
</tr>
<tr>
<td>Hours (12–18)</td>
<td>Minutes</td>
</tr>
<tr>
<td><strong>Individual cells</strong></td>
<td><strong>Adjacent areas</strong></td>
</tr>
<tr>
<td>• Intact membrane</td>
<td>• Necrosis, plasma membrane disruption</td>
</tr>
<tr>
<td>• Nuclear condensation</td>
<td>• Cytoplasm and cell volume ↑</td>
</tr>
<tr>
<td>• Cell shrinkage</td>
<td></td>
</tr>
<tr>
<td>• Blocking of cell surface</td>
<td></td>
</tr>
<tr>
<td>• Cell fragmentation</td>
<td></td>
</tr>
<tr>
<td>• Organelles retain integrity</td>
<td></td>
</tr>
<tr>
<td><strong>Suicide</strong></td>
<td><strong>Murder</strong></td>
</tr>
<tr>
<td>• Needs energy</td>
<td>• Loss metabolic activity</td>
</tr>
<tr>
<td>• Activation of enzymes (endonucleases)</td>
<td>• Loss of enzyme</td>
</tr>
<tr>
<td>• Save removal by phagocytes</td>
<td>• Leakage-inflammation</td>
</tr>
<tr>
<td>• No inflammation</td>
<td>• Lysis</td>
</tr>
<tr>
<td>• Inconspicuous</td>
<td>• Messy, spectacular</td>
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to regenerating PCT. After full recovery of the PCT, a second burst in proliferative activity occurred in the hypertrophic distal segments most likely in response to functional overload marked by transient PCT necrosis-dysfunction. In the regenerated PCT, an excess cell number was accompanied by increased apoptotic activity (day 10 after injury) (Figure 2).

There was a striking parallelism between the occurrence of the first proliferative wave at days 0–4 and the prevalence of apoptotic nuclei in the distal tubules. Apoptosis of programmed cell death is a process that occurs as part of the normal cell turnover and in tissue remodelling and may be increased by several types of injury. Increased apoptosis and cell proliferation have been observed in distal tubules after brief ischaemia without necrosis, and in scar-adjacent tissue in the remnant kidney. Also, during ureteric obstruction, apoptosis is induced primarily in the distal tubule [17].

Caspases are involved in apoptosis associated with toxic acute renal failure, as was recently shown in cisplatin-induced cell death in mouse proximal tubular cells [18]. Evidence for a role of ceramide in apoptosis comes from the following observation: (i) exogenous ceramide induces apoptosis; (ii) increased ceramide level is seen in apoptotic cells in response to a variety of stimuli; (iii) cells deficient in ceramide or low response of ceramide production are resistant to apoptosis; and (iv) the inhibition of ceramide rescues apoptosis. Ueda et al. [19] showed that ceramide is a regulator of endonuclease in renal tubular epithelial cell injury.

Therapeutic implications

The inhibition of apoptosis may be undesirable such as the apoptotic death of cells by chemotherapeutic agents with irreparable DNA damage. It is not clear that apoptotic death induced in response to mild ischaemic or toxic injury serves any useful ‘homeostatic role’. The observations we made in a toxic model of acute renal injury seem to support a role of apoptosis in the remodelling of the proliferative regenerating proximal and hyperplastic hypertrophic distal tubule [16].

During the commitment phase, the potent pro-survival effect of anti-apoptotic Bcl-2 family member proteins, the activity of the transcription factor NF&B and the upstream signalling kinase pathways represent the three areas for pharmacological intervention [20]. In addition, during the early execution phase the caspase system can be modulated, using cell permanent inhibitors. They have been shown to prevent tubular cell death in response to ischaemic injury [21]. It is clear that more insights into the events involved in apoptosis are needed before therapeutic agents interfering with this system can be used.

References


