

# Nephrotoxicity of Bence-Jones proteins: interference in renal epithelial cell acidification

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## Abstract

The aim of the present study was to evaluate the acidification of the endosome-lysosome system of renal epithelial cells after endocytosis of two human immunoglobulin lambda light chains (Bence-Jones proteins, BJP) obtained from patients with multiple myeloma. Renal epithelial cell handling of two BJP (neutral and acidic BJP) was evaluated by rhodamine fluorescence. Renal cells (MDCK) were maintained in culture and, when confluent, were incubated with rhodamine-labeled BJP for different periods of time. Photos were obtained with a fluorescence microscope (Axiolab-Zeiss). Labeling density was determined on slides with a densitometer (Shimadzu Dual-Wavelength Flying-Spot Scanner CS9000). Endocytosis of neutral and acidic BJP was correlated with acidic intracellular compartment distribution using acridine orange labeling. We compared the pattern of distribution after incubation of native neutral and acidic BJP and after complete deglycosylation of BJP by periodate oxidation. The subsequent alteration of pI converted neutral BJP to acidic BJP. There was a significant accumulation of neutral BJP in endocytic structures, reduced lysosomal acidification, and a diffuse pattern of acidification. This pattern was reversed after total deglycosylation and subsequent alteration of the pI to an acidic BJP. We conclude that the physicochemical characteristics of BJP interfere with intracellular acidification, possibly explaining the strong nephrotoxicity of neutral BJP. Lysosomal acidification is fundamental for adequate protein processing and catabolism.

## Key words

- Bence-Jones proteins
- Renal cell acidification
- Endocytosis
- Lambda light chain protein

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Renal handling of monoclonal immunoglobulin light chains, Bence-Jones proteins (BJP), is an important aspect of multiple myeloma. Clinical and experimental studies have been conducted to elucidate the mechanisms related to protein overload (1) that lead to aminoaciduria and glycosuria (2,3), acidification defects and tubular cast formation (4).

The main organs involved in the removal of BJP from the circulation are liver and kidney (5). Many investigators have studied

how protein handling by renal cells leads to disease but few studies are available about the sugar content and other physicochemical characteristics of these proteins.

The aim of the present study was to correlate sugar content and BJP isoelectric point (pI) to endocytosis and intracellular acidification. Some studies have indicated the importance of the pI of light chains, since a pI higher than 6.0 is more frequently associated with renal damage (6).

BJP was labeled with rhodamine by the method of Maxfield et al. (7) and sugar content was removed by the method of Spiro (8). After deglycosylation, the samples were submitted to SDS polyacrylamide electrophoresis, isoelectric focusing and quantification by amino acid analysis.

MDCK cells were grown on coverslips on Petri dishes containing Dulbecco's modified Eagle's medium (DMEM) (9). Twenty-four hours before the experiment, confluent cells were incubated with DMEM without calf serum.

Cells were incubated with 40 µg of BJP dissolved in 1 ml DMEM without calf serum for 1, 30 and 60 min. The medium was then discarded, and cells were washed with 0.9% saline solution and fixed with 1% glutaraldehyde solution in phosphate buffer, pH 7.2, for 20 min. Slides were covered with coverslips and observed under the microscope.

Endocytosis was quantified by densitometry (525 nm) of the film and area units were integrated by the densitometer (Shimadzu Dual-Wavelength Flying-Spot Scanner CS9000, Tokyo, Japan). The cellular area was measured on photographs using an image analysis processor system (MINIMOP, Kontron Bildanalyser, Kontron Elektronik Group, Eching, Munich, Germany). Intracellular uptake of BJP is reported as densitometer unit per cellular area.

Acidic compartments were identified after endocytosis of native and deglycosylated BJP by incubation with acridine orange for 60 min. Cells not incubated with BJP were used as controls.

Cells were observed without fixation in glutaraldehyde. Coverslips were mounted on glass slides and observed by fluorescence microscopy (Axiophot, Zeiss, Oberkochen, Germany). The intensity and range of orange color are proportional to acidity, and neutral structures are green.

Living cells took up acridine orange readily. In all cells, the nuclei were stained bright green, yellow-green or yellow (de-

pending on the concentration of the dye).

Results were analyzed with the Sigma Plot 5.0 software (Sigma-Stat Statistical Analysis System version 1.01, Scientific Graph System, Jandel Scientific, Chicago, IL, USA) using the nonparametric Kruskal-Wallis and Mann-Whitney tests. Results are reported as median and 25-75% percentiles. The level of significance adopted was 0.05.

Labeling of neutral BJP chains was significantly more extensive with rhodamine than labeling of acidic BJP. At 1, 30 and 60 min, the absorbance values [median and percentiles (25-75%)] were 794 (752-1159), 1055 (957-1135) and 1444 (1067-1082) for neutral BJP and 479 (248-652), 681 (584-830) and 655 (432-783) for acidic BJP, respectively.

Marked vacuolation was found after 30 min of incubation with neutral BJP, but not after incubation with acidic BJP. After 60 min of incubation with neutral BJP we could observe a reduction of cellular volume and a significant retention of protein (Figure 1A) in comparison with acidic BJP (Figure 1B).

Living cells incubated with acridine orange showed a modified pattern of acidic lysosome-endosome structures after incubation with neutral BJP (Figure 1C). This pattern was reversed after pI alteration of neutral BJP. On the other hand, a normal pattern of acidification was observed after incubation with acidic BJP, with perinuclear localization of lysosomes. Figure 2 illustrates the catabolism of acidic BJP in spite of a progressive accumulation of neutral BJP.

Our laboratory has reported the nephrotoxicity of BJP in acute and chronic models (10,11). On the basis of the present results, we conclude that pI modulates cytotoxicity by mechanisms related to internalization and intracellular directioning and catabolism. This pattern was reversed after alteration of the physicochemical properties of BJP. These data are in agreement with those reported by others who showed a relation between nephrotoxicity and pI (12,13).

The new contribution of the present study was the correlation between physicochemical characteristics of BJP and nephrotoxicity according to different patterns of uptake and intracellular processing, the direct correla-

tion with lysosomal acidification and consequent intracellular digestion, and the importance of carbohydrate content, amino acid composition and pI before and after meta-periodate oxidation.

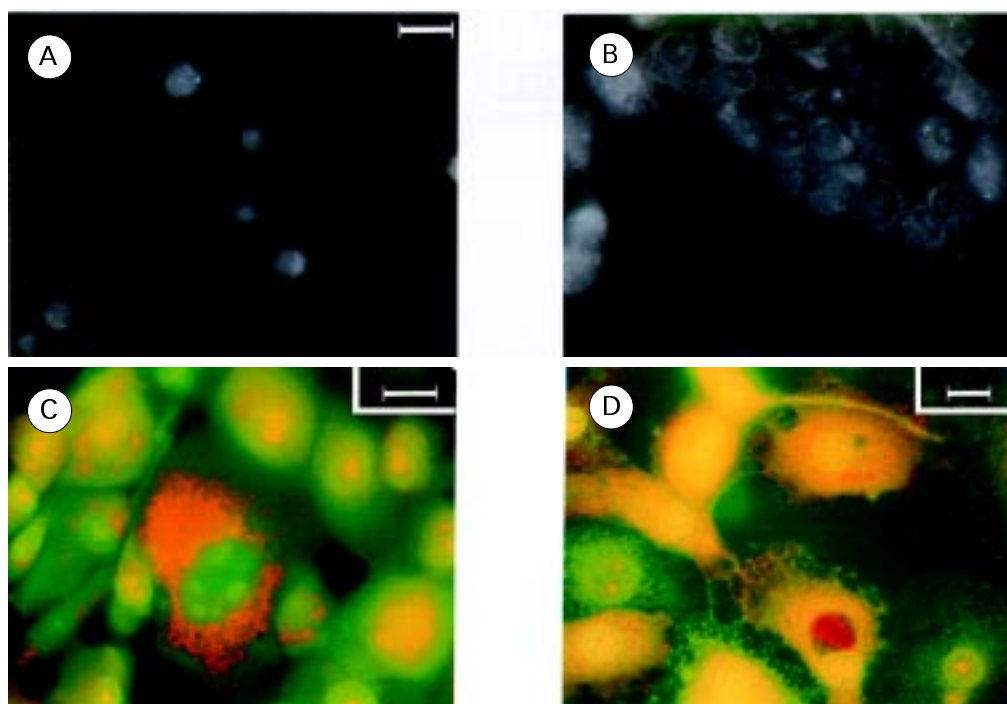


Figure 1. Intracellular uptake of rhodamine-labeled Bence-Jones proteins (BJP) by MDCK cells and patterns of acidification with acridine orange. A, MDCK cells after 60 min of incubation with neutral BJP labeled with rhodamine. B, MDCK cells after 60 min of incubation with acidic BJP labeled with rhodamine. C, MDCK cells after incubation for 30 min with neutral BJP and acridine orange. D, MDCK cells after incubation for 30 min with acidic BJP and acridine orange. Bars = 5  $\mu$ m.

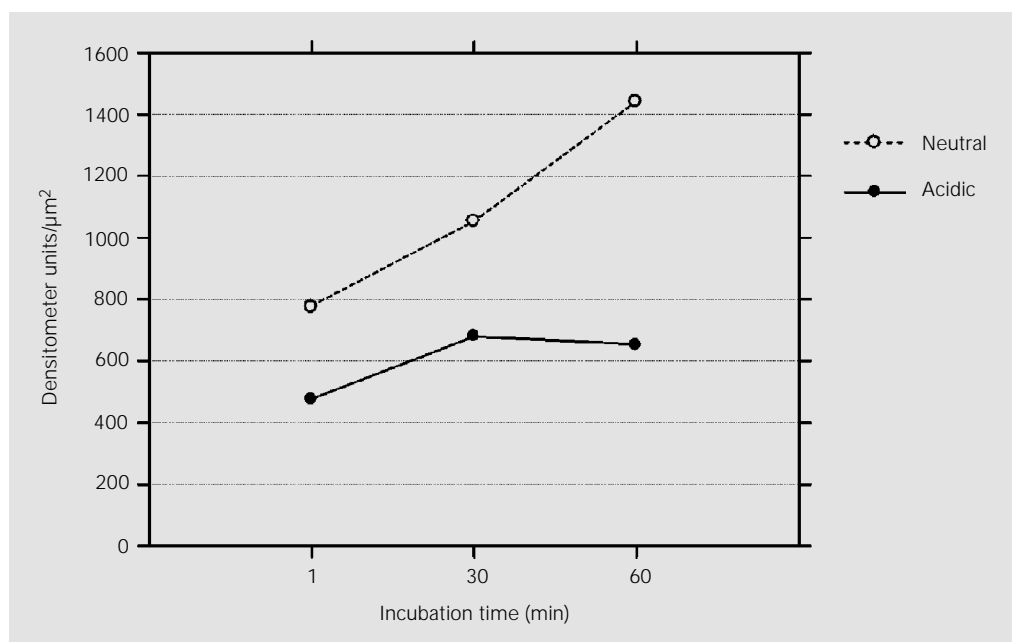


Figure 2. Intracellular uptake of rhodamine-labeled Bence-Jones proteins (BJP). The cellular uptake of rhodamine-labeled BJP was observed after 1, 30 and 60 min of incubation and is reported as densitometer unit per cellular area.

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