Pharmacologic Limits of the Protective Effect of Polyaspartic Acid on Experimental Gentamicin Nephrotoxicity

S. K. SWAN,¹ D. N. GILBERT,² S. J. KOHLHEPP,² P. W. KOHNEN,³ AND W. M. BENNETT¹*

Division of Nephrology, Hypertension, and Clinical Pharmacology, Oregon Health Sciences, 3314 SW U.S. Veterans Hospital Road, Portland, Oregon 97201,¹ and Department of Medical Education and Infectious Diseases and Chiles Research Institute² and Department of Pathology,³ Providence Medical Center, Portland, Oregon 97213

Received 14 May 1992/Accepted 15 November 1992

Polyaspartic acid (PAA) ameliorates experimental gentamicin nephrotoxicity despite marked accumulation of gentamicin in the renal cortex. The experiments described here probe the extent of PAA's nephroprotective action when increasing doses of gentamicin, in excess of an established nephrotoxic dose (40 mg/kg of body weight per day), are administered. After 10 days, virtually complete nephroprotection was conferred by PAA coadministered to animals receiving three times the nephrotoxic dose (120 mg/kg/day) of gentamicin.

Polyaspartic acid (PAA) prevents experimental gentamicin nephrotoxicity despite marked accumulation of aminoglycoside in renal cortical tissue (3, 8, 9). In addition to PAA, a number of other polyanionic compounds have been reported to confer nephroprotection (2, 4–7) in aminoglycoside models, but PAA remains the most extensively studied to date. Clinically, it is well established that peak serum aminoglycoside concentrations correlate with bactericidal efficacy, while increased frequency of dosing correlates with nephrotoxicity. Theoretically, a full day's dose of aminoglycoside could be given with PAA to provide maximal antibacterial efficacy while minimizing nephrotoxicity. In patients with preexisting renal dysfunction, nephroprotection rendered by PAA might be particularly advantageous if it allows adequate peak aminoglycoside levels. It has been difficult to characterize PAA's pharmacodynamic and pharmacokinetic properties because no consistent formulation is currently available. The PAA compound used for most experimental studies represents a mixture of variable-length polymers composed of both D and L optical isomers. The following experiments were performed to examine the extent of nephroprotection conferred by PAA when gentamicin was given in multiples of known nephrotoxic doses for 10 days.

Acclimatized male Fischer 344 rats with an initial average weight of 200 g were used in all studies (Charles River Breeding Laboratories, Wilmington, Mass.). The sodium salt of PAA was purchased from Sigma Chemical (St. Louis, Mo.) (lot 80H5521). The mean molecular weight was estimated at 7,540 by a visible light-scattering technique and at 5,100 by a laser technique. No data on the relative amounts of D and L optical isomers present in the PAA preparation were available. Gentamicin was purchased from Schering (Kenilworth, N.J.) for subcutaneous injection.

PAA was administered in a dosage of 320 mg/kg of body weight per day as a single subcutaneous injection. When coadministered, gentamicin and PAA were injected at different sites and in rapid succession. Control animals received phosphate-buffered saline in a volume equivalent to that given animals administered PAA and gentamicin. All animals received equivalent amounts of sodium each day.

Animals were divided into six groups (n = 4 in each) and given one of the following treatments: diluent only, gentamicin at 80 mg/kg/day (G80), G120, G80 plus PAA, G120 plus PAA, and G160 plus PAA. Animals were treated for 3, 5, 7, and 10 days. At sacrifice, blood was obtained for determination of the blood urea nitrogen and serum creatinine levels by standard methods. One kidney from each animal was processed for light microscopy. The second kidney was weighed, homogenized in 1 N NaOH, digested at 80°C for 15 min, allowed to cool to room temperature, and then assayed for gentamicin with a polarized fluorescence immunoassay (Abbott TDX). The assay has a lower limit of sensitivity of 0.1 μg/ml. Recovery studies using known standards showed greater than 90% yield.

The renal histopathologic techniques have been described previously (3). One pathologist evaluated all renal sections in a masked fashion and reported the estimated percentage of necrotic and regenerating cortical tubular cells.

Statistical analysis was performed with the software program Abstat from Anderson-Bell, Parker, Co. The analysis of variance program, which tests for significant differences among the means of four-way unequal n groups of data, was used with Scheffes post hoc test with a significance level of 0.05.

Table 1 summarizes the serum creatinine results. After 7 days of treatment, renal injury was manifested by a signifi-

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Serum creatinine level (mg/dl) on the indicated day of treatmenta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Diluent only</td>
<td>0.3 ± 0</td>
</tr>
<tr>
<td>G80</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>G120</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>G80 + PAA</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>G120 + PAA</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>G160 + PAA</td>
<td>0.4 ± 0</td>
</tr>
</tbody>
</table>

* PAA was given at 320 mg/kg/day.
* Values are means ± standard deviations for four animals per datum point.
* * P < 0.01 versus all other groups, day 7. ** P < 0.001 versus all other groups, day 10. *** P < 0.05 versus G80 plus PAA, G120 plus PAA, and control groups, day 10.

* Corresponding author.
In this study, a dose range of gentamicin was probed to define the limits of PAA's nephroprotective effect in experimental aminoglycoside nephrotoxicity. In our laboratory, G40, given as two equal doses, produces renal dysfunction and tubular necrosis in 10 days (1). PAA produces complete protection from this toxic insult (3). In the experiment described above, gentamicin doses of 80, 120, and 160 mg/kg/day, given as a single daily dose, were coadministered with PAA for 3, 5, 7, and 10 days. When the established daily nephrotoxic dose of gentamicin (40 mg/kg/day) was doubled and tripled, PAA provided nephroprotection after 7 and 10 days as evidenced by normal creatinine values and minimal or absent tubular necrosis and regeneration, in contrast to those of animals treated with gentamicin alone. Although renal dysfunction occurred in animals receiving G160 plus PAA, it was significantly less severe than that in animals receiving lower gentamicin loads (80 and 120 mg/kg/day) without PAA (P < 0.05). These findings reinforce the potential clinical benefit of PAA with regard to nephrotoxicity prevention in the setting of high aminoglycoside loads. Combining large gentamicin doses and concomitant PAA administration with less frequent dosing schedules would, in theory, maximize antibacterial efficacy while minimizing nephrotoxicity.

S.K. Swan is the recipient of a National Kidney Foundation Fellowship award.

REFERENCES


