Aging and Urinary Excretion of Epidermal Growth Factor*

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ABSTRACT

The aging process in man leads to some loss of kidney mass and function. The kidney produces epidermal growth factor (EGF), a polypeptide involved in the repairing process of epithelial cells. Human urine contains high concentrations of EGF, derived from its production in the kidney. It is not known if aging alters urinary EGF production in humans. This study investigates the possibility of decreased urinary EGF in elderly people. Urine samples were collected from 70 healthy subjects of various ages and measured for EGF by the technique of radioimmunoassay. The studied urine samples were divided into five age groups (3 to 10, 11 to 20, 21 to 60, 61 to 70, and 71 to 80 years). Urinary EGF (corrected for the urine creatinine concentration and measured as ng per mg creatinine) was highest in the two youngest groups, 78.5 ± 14.3 and 76.2 ± 18.8 (mean ± standard error of the mean), respectively, and decreased with age so that the lowest urinary EGF was observed in the oldest group (27.0 ± 8.8 ng per mg creatinine). In addition, a significant inverse relationship exists between urinary EGF in all 70 subjects and their respective age (P < 0.001). These findings show that normal values of urinary EGF should take age into account. The reduced production of EGF by the kidney in the elderly may have functional significance in retarding the repair process in the kidney.

Introduction

It has been shown that some loss of renal mass occurs with the human aging process; in young adulthood, a normal kidney weighs 230 to 270 g and decreases to 180 to 200 g by the 8th decade.1 This decrease in renal mass is associated with alterations in renal function. Both glomerular filtration rate and renal blood flow decrease with age.2,3 Other changes in renal function associated with aging include impairment in urinary concentration and sodium conservation.4,5 These functional alterations in the kidney may place old people at risk for acute renal failure (ARF). In fact, recent studies have reported that the incidence of ARF increases with age.6 In particular, the incidence of ARF in people over 70 is much higher than in the general population.7,8 The
elde rly are susceptible to ARF caused by nephro toxic agents or a decrease in renal blood flow from a variety of causes. Furthermore, elderly patients often do not recover or make a full recovery from ARF, resulting in permanent renal damage.7,8,9 The factors which contribute to incomplete recovery from ARF in the elderly are not known. Recovery from ARF depends on the degree of repair of damaged tubules. Necrotic tubular cells are replaced by remaining viable cells, which, from their resting state, re-enter the cell cycle and proceed through DNA-replication into mitosis. This process requires activation of growth-related genes, including the growth factor genes.10 Epidermal growth factor (EGF) is one of the products of the growth factor genes11 that may be involved in the repair process during recovery from ARF.

Epidermal growth factor was first discovered by Cohen in 1962 in the mouse salivary (submaxillary) gland.12 Human EGF was subsequently isolated by Cohen and Carpenter in 1975.13 Epidermal growth factor is a 6,000 molecular weight polypeptide consisting of 53 amino acids, derived from a precursor (prepro-EGF) of 1,217 amino acids.11 It has subsequently been shown to be a potent mitogen, stimulating messenger ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein synthesis in a variety of epithelial cells.11,14

The kidney synthesizes prepro-EGF.11 Prepro-EGF is anchored to the plasma membrane with its C-terminus near the hydrophobic (transmembrane) section; beyond the hydrophobic domain is the mature EGF with eight regions of partial sequence homology in the extracellular area.15 Mature EGF is cleaved from prepro-EGF by proteolytic enzymes.15 Urine contains high concentrations of EGF, originating from the kidney.16 Immunohistochemical studies have demonstrated the presence of EGF in renal tubules.17 In addition, receptors for EGF are expressed in the renal tubular cells, providing evidence for the kidney as a target organ for EGF.18

Studies in animals with ARF have shown EGF to be a potent growth promoter involved in regenerating renal tubular cells. Administration of EGF enhances tubular regeneration and accelerates the recovery of normal renal function in animals with postischemic ARF.19,20 Administration of EGF also accelerates the repair process in gentamicin or mercuric chloride nephrotoxicity.21,22

Growth-promoting factors and their receptors may play important roles in recovery from renal injury. It is not known why recovery from various forms of renal injury in elderly patients tends to be incomplete or slower when compared with young patients. There are several possibilities for the impaired healing in the elderly, including a reduction of EGF or other growth promoting factors produced by the kidney, or an excess of growth-inhibiting factors. To explore these possibilities, it is reasonable to begin by examining whether or not EGF production by the kidney decreases with aging.

Materials and Methods

Collection of Samples

This study was approved by the research and clinical projects committee at the Brookdale University Hospital and Medical Center. Morning urine samples (about 20 ml) were obtained from a total of 70 healthy subjects and arbitrarily divided into five age groups (3 to 10, 11 to 20, 21 to 60, 61 to 70, and 71 to 80 year olds, respectively). There were 37 male and 33 female subjects. The purpose of the study was explained to each individual or his/her parents before the collection of the urine samples and permission obtained. All urine samples were tested first with Labstix* for blood, protein, and glucose. A portion of each urine sample was also tested for protein with sulfosalicylic acid. Only urine samples which were negative for blood, protein, or glu-

* Miles Inc., Diagnostics Division, Elkhart, IN.
Figure 1. Radioimmunoassay standard curve. B/Bo denotes percent bound labeled-epidermal growth factor (EGF) and is plotted against EGF concentration.

Assays

Epidermal growth factor was measured by radioimmunoassay, performed with the Human EGF Radioimmunoassay Kit. Two tubes were set up for total counts and received only radioactive $^{125}$EGF, while two additional tubes were designated for nonspecific binding and received 200 µl of Tris-saline buffer. Two separate tubes were used for maximum binding and received 100 µl of the buffer. In order to generate a standard curve (figure 1), seven tubes were filled with 100 µl of solutions containing varying amounts of EGF ranging from 0.25 to 50 ng per ml. From each urine sample, 100 µl of urine were pipetted into a tube reserved for measurement of EGF. All tubes then received 100 µl of tracer, containing $^{125}$-human EGF, buffer salts, bovine serum albumin, protease inhibitors, and sodium azide. All tubes, except the total counts and nonspecific binding tubes, received 100 µl of antiserum, which contained high-titered, specific, rabbit anti-human EGF, as well as buffer salts, bovine serum albumin, and sodium azide.

The solutions were mixed gently and incubated at 4°C for approximately 20 hours. The total count tubes were put aside for counting, while 100 µl of GAR-IgG (goat anti-rabbit IgG, mixed with sodium azide) and 100 µl of PEG (polyethylene glycol mixed with sodium azide) were added to all other tubes. The solutions were mixed and stored for 15 minutes at 4°C. To all tubes except the total count tubes, 1.5 ml of chilled Tris-saline buffer were added. The solutions were then vortexed and centrifuged at 2000 g for 15 minutes at 4°C. The supernatant was decanted, and the tubes were counted in a gamma spectrometer for at least one minute. All samples were measured in duplicate.

Creatinine concentration in urine was measured on a Creatinine Analyzer 2. The EGF concentration in urine was normalized by cre-

† Biomedical Technologies Inc., Stoughton MA.
‡ Beckman Instruments Inc., Brea, CA.
TABLE I
Demographic Distribution and Urinary Findings in Five Age Groups

<table>
<thead>
<tr>
<th>Age Groups (years)</th>
<th>1–10</th>
<th>11–20</th>
<th>21–60</th>
<th>61–70</th>
<th>71–80</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>8</td>
<td>12</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>6.47</td>
<td>13.38</td>
<td>42.67</td>
<td>66.52</td>
<td>76.88</td>
</tr>
<tr>
<td>±0.72</td>
<td>±0.96</td>
<td>±1.29</td>
<td>±0.60</td>
<td>±1.04</td>
<td></td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>5/10</td>
<td>4/4</td>
<td>6/6</td>
<td>16/11</td>
<td>6/2</td>
</tr>
<tr>
<td>U_{\text{osm}} (mOsm per Kg H_2O)</td>
<td>760</td>
<td>736</td>
<td>754</td>
<td>615</td>
<td>595</td>
</tr>
<tr>
<td>±47</td>
<td>±113</td>
<td>±61</td>
<td>±43</td>
<td>±48</td>
<td></td>
</tr>
<tr>
<td>Urine sodium (meq per liter)</td>
<td>162</td>
<td>137</td>
<td>153</td>
<td>108</td>
<td>87</td>
</tr>
<tr>
<td>±16</td>
<td>±19</td>
<td>±22</td>
<td>±12</td>
<td>±20</td>
<td></td>
</tr>
<tr>
<td>Urine creatinine (mg per 100 ml)</td>
<td>89</td>
<td>125</td>
<td>134</td>
<td>121</td>
<td>127</td>
</tr>
<tr>
<td>±11</td>
<td>±24</td>
<td>±19</td>
<td>±14</td>
<td>±21</td>
<td></td>
</tr>
</tbody>
</table>

n = Number of subjects. U_{\text{osm}} = Urine osmolality. All values are expressed as mean ± SE.

Age and urinary excretion of epidermal growth factor (EGF)

Table I presents the demographic data and urinary findings in five age groups. The table shows a decrease in urine osmolality with age, particularly in the elderly groups. There were no significant differences in urine creatinine concentrations among the different age groups.

The results of urinary EGF/urine creatinine ratios in the five age groups are depicted in figure 2. The highest levels were observed in the two youngest age groups (3 to 10 and 11 to 20-year olds), whereas the lowest level was noted in the oldest age group (71 to 80-year olds). The average urinary EGF/urine creatinine ratios were correlated with the five age groups and a significant linear relationship was seen [correlation coefficient (r) = 0.978; P < 0.01].

In figure 3 are shown the results of individual urinary EGF/urine creatinine ratios for all 70 subjects. The data were examined by regression analysis after a logarithmic transformation was performed for individual urinary EGF/urine creatinine ratios, which were then correlated with age. A significant inverse relationship was noted between the two parameters:

\[
\ln y = -0.0137 x + 4.2727 \quad (r = 0.5082; \quad P < 0.001)
\]

where y is urinary EGF and x is age (figure 3).

Statistical Analysis

All results are expressed as means ± standard error of the mean. Statistical significance among the groups was determined by one-way analysis of variance. Correlation between two variables was determined by the method of least squares; P value of < 0.05 was considered statistically significant.

Results

In Table I are summarized the demographic data and urinary findings in these subjects. Urine osmolality tended to decrease in the elderly groups. There were no significant differences in urine creatinine concentrations among the different age groups.

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§ Precision Systems Inc., Sudbury, MA.
|| NOVA Biomedical, Newton, MA.
Figure 2. Relationship between average urinary epidermal growth factor (EGF) concentrations and age.

Figure 3. Relationship between urinary epidermal growth factor (EGF) after logarithmic transformation and age. The logarithmic transformation of the urinary EGF concentration is represented by the ordinate (y) and plotted against age (represented by x).

\[ \text{Ln } y = -0.0137x + 4.2727 \]

\[ r = 0.5082; P < 0.001 \]
Discussion

Previous studies have demonstrated that urinary EGF measured either in the total amount excreted per 24 hours or in the urinary EGF/urinary creatinine ratio is not affected by diurnal rhythm or meals. In addition, it has been established that an excellent linear correlation exists between the urinary EGF and urinary creatinine concentrations. The urinary EGF excretion can therefore be conveniently measured in a random urine sample and normalized by the urine creatinine concentration, eliminating the need of collecting 24-hour urine specimens.

In the original report of Dailey, Kraus, and Orth, urinary EGF in 34 normal healthy adults ranged between 18 and 52 ng per mg creatinine. Since they collected urine from healthy physicians and laboratory personnel, urinary EGF excretion was not measured in the very young or old. In this report, the urinary EGF values in adults are similar to those reported by Dailey and co-workers; however, in the very young (10 years or younger) the values are substantially higher than the older subjects (over 70 years). In addition, urinary EGF/creatinine is inversely and significantly correlated with age, suggesting that EGF production by the kidney declines with age.

Urine contains high concentrations of EGF. The urinary EGF is thought to originate from renal production and not through glomerular filtration. Circulating blood levels of EGF are approximately 40 to 50 picomole per liter, while urinary EGF concentrations are approximately 1,000-fold higher (50 nanomole per liter). Furthermore, removal of one kidney reduces urinary EGF excretion by half; however, removal of the submandibular gland and duodenal Brunner's glands (organs known to produce EGF) does not reduce urinary EGF excretion. Thus, urinary EGF excretion can be used as a valid index of EGF production by the kidney.

Both kidney mass and function decline with aging. The decrease in urinary EGF excretion in the elderly observed in this study raises a few possibilities as to its role in this aging process. As renal mass is known to be lower in the elderly, this may contribute to a reduction in the EGF production by the kidney. It is also possible, however, that the observed age-related reduction in renal EGF production is important to the aging process of the kidney, particularly in light of the fact that EGF has been shown to be a potent growth promoter in regenerating renal tubular epithelial cells. In addition, it is conceivable that a decreased production of EGF might retard the repair process in the kidney and hamper recovery from ARF in the elderly.

In conclusion, urinary EGF excretion in healthy humans is highest in the young subjects and declines with age; in addition, a significant inverse relationship exists between urinary EGF excretion and age. These findings suggest that age is a variable when evaluating the significance of a urinary EGF level. It remains to be determined whether or not the reduced production of EGF by the kidney in the elderly plays a role in retarding the repair process in the kidney.

Acknowledgments

This work was supported by a research grant from Nephrology Foundation of Brooklyn.

References