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AGE ACCELERATING ACCUMULATION ADVANCED GLYCATION END PRODUCTS (AGE) IN HUMAN URINARY BLADDER

Aims of Study

In the Maillard reaction, prolonged incubation of proteins with glucose leads to advaced glycation end products (AGE) through early stage products such as a Schiff base and Amadori rearrangement products. The AGE display a heterogeneous structure characterized by a yellow-brown color, autofluorescence, intra- or intermolecular cross-linking, and interaction with cellular receptors. Since the first discovery of hemoglobin A1c in 1968, the *in vivo* presence of early stage products has been demonstrated for many proteins. So far it was revealed that some kind of AGE-structures existed in human tissues.

Our previous immunohistochemical study using anti-AGE antibodies in several human tissues led to a general contention that AGE may be involved in aging processes and age-accelerated diseases such as diabetic complications, atherosclerosis, and hemodialysis-associated amyloidosis. In the present study, to investigate the possibility that AGE is related to age-accelerated bladder dysfunction, we utilized several kinds of mouse monoclonal anti-AGE antibodies for immunohistochemistry to examine the accumulation of AGE proteins in human bladder.

Methods

Normal bladder tissue samples were obtained from 10 radical cystectomy and one bladder augmentation cases. The bladder tissue specimens were fixed with periodate-lysine-paraformaldehyde(PLP).Anti-AGE monoclonal antibodies, anti

N -(carboxymethyl)lysine(CML), anti-imidazolone and anti-pentosidine antibodies were used for 1^{st} antibody. Bladder tissue sections were incubated with the 1^{st} antibody, washed, and reacted with peroxidase-labeled anti mouse immunoglobulin $F(ab')_2$ as second antibody. After visualization with 3,3'-diaminobenzidine, the sections were counterstained with hematoxylin. Double immunohistochemical staining with anti-CML antibody and anti-human macrophage antibody KP1 (DAKO, Glostrup, Denmark) was performed. In the first step, frozen sections were stained with anti-CML antibody. After visualization of peroxidase activity, the sections rinsed with glycine-HCl buffer (pH 2.2). In the second step, the sections were incubated overnight with KP1. After sections were washed, they were incubated with rabbit anti-mouse immunoglobulin. The sections were then washed and reacted with alkaline phosphatase anti-alkaline phosphatase (Dako), after which they were incubated with a mixture of naphtol AS-MX phosphate, fast BB salt and Tris-HCl buffer (pH 8.7).

Results

In the present study, 9 cases over 50 years and one-half were over 60 years old; only one case was child under 10 years and one case was adolescent. There were no diabetes mellitus cases.

There was no CML deposition in child case. On the contrary, in the over 60 years case there were significant CML deposition, which was observed bladder interstitial of muscle layer . Observing in the high power field, there could detect some kind of large cell body cells which were strongly positive for anti-CML antibody. Similarly, there was no pentosidine deposition in child case. In the over 60 years case there were significant pentosidine deposition, which was observed bladder interstitial of muscle layer. However, there was imidazolone deposition neither in child case nor in adult case.

To detect what type of cell was concentrated AGE deposition, double immunohistochemical staining was performed. Cells were positive for CML in bladder interstitial of muscle layer, were also positive for KP1.

Conclusions

The current study demonstrates that AGE are produced extracellularly in age dependent manner in bladder and ingested by macrophages. From analyses in other tissues and organs, it has been revealed that AGE ingestion by macrophages was endcytosis which was mediated by AGE specific receptors. In addition to mediating endocytosis, AGE receptors are known to possess several cellular functions, for example, stimulating intracellular signaling and growth factor secretion. These notions provided the suggestion that accumulated AGE in bladder endocytosed by tissue macrophages, which also stimulated intracellular signaling and secreted second messengers, subsequently led to bladder dysfunction.