In Vitro and in Vivo Evaluation of Native Glucagon and Glucagon Analog (MAR-D28) during Aging: Lack of Cytotoxicity and Preservation of Hyperglycemic Effect


Abstract

Background:
For automated prevention of hypoglycemia, there is a need for glucagon (or an analog) to be sufficiently stable so that it can be indwelled in a portable pump for at least 3 days. However, under some conditions, solutions of glucagon can form amyloid fibrils. Currently, the usage instructions for commercially available glucagon allow only for its immediate use.

Methods:
In NIH 3T3 fibroblasts, we tested amyloid formation and cytotoxicity of solutions of native glucagon and the glucagon analog MAR-D28 after aging under different conditions for 5 days. In addition, aged native glucagon was subjected to size-exclusion chromatography (SEC). We also studied whether subcutaneous aged Novo Nordisk GlucaGen® would have normal bioactivity in octreotide-treated, anesthetized, nondiabetic pigs.

Results:
We found no evidence of cytotoxicity from native glucagon or MAR-D28 (up to 2.5 mg/ml) at a pH of 10 in a glycine solvent. We found a mild cytotoxicity for both compounds in Tris buffer at pH 8.5. A high concentration of the commercial glucagon preparation (GlucaGen) caused marked cytotoxicity, but low pH and/or a high osmolarity probably accounted primarily for this effect. With SEC, the decline in monomeric glucagon over time was much lower when aged in glycine (pH 10) than when aged in Tris (pH 8.5) or in citrate (pH 3). Congo red staining for amyloid was very low with the glycine preparation (pH 10). In the pig studies, the hyperglycemic effect of commercially available glucagon was preserved despite aging conditions associated with marked amyloid formation.

continued
Abstract cont.

Conclusions:
Under certain conditions, aqueous solutions of glucagon and MAR-D28 are stable for at least 5 days and are thus very likely to be safe in mammals. Glycine buffer at a pH of 10 appears to be optimal for avoiding cytotoxicity and amyloid fibril formation.