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Degradation products of streptozotocin do not induce hyperglycemia in rats

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Abstract—The present study was conducted to determine whether degradation products of streptozotocin formed under various conditions induce hyperglycemia in rats. Streptozotocin is completely degraded in pH 7.4 buffer in 4 hr and even more rapidly in plasma. Streptozotocin degradation products resulting from incubation in pH 7.4 phosphate buffer or in plasma were not diabetogenic in rats.

Streptozotocin is a naturally occurring nitrosamide which is used as a diabetes-inducing agent in animal experiments [1, 2]. Streptozotocin is known to be chemically stable under acidic conditions. However, in aqueous solutions above pH 7, it is readily degraded [3, 4]. As a consequence, when administered *in vivo* to induce diabetes in experimental animals, streptozotocin is usually administered in pH 4.0–4.8 NaCl or citrate buffer solution. This procedure, although successful in inducing diabetes, results in hemolysis of red blood cells and considerable pain and discomfort to the animal [5]. In an effort to eliminate these undesired effects and simultaneously to determine whether streptozotocin's degradation products in buffer or plasma also induce diabetes, the following investigation was undertaken.

Materials and Methods

Animals and treatment groups. Female Sprague–Dawley rats (200–250 g) were obtained from Uhan Research Institute (Seoul, Korea). Animals received food and water *ad libitum* and were housed at 20° with a 12 hr light–dark cycle. Animals were fasted overnight and divided into five treatment groups: pH 7.4 buffer only control, plasma only control, streptozotocin in pH 7.4 buffer, streptozotocin degradation products in pH 7.4 buffer, and streptozotocin degradation products in plasma. Streptozotocin degradation products were prepared by incubating 50 mM streptozotocin in phosphate buffer (pH 7.4) or in plasma at 37° for 24 hr. Animals were administered i.v. 50 mg/kg streptozotocin in pH 7.4 buffer (3.77 mL/kg) or the equivalent degradation products of 50 mg/kg streptozotocin in pH 7.4 phosphate buffer or in plasma (3.77 mL/kg). Control animals were administered 3.77 mL/kg of pH 7.4 phosphate buffer.

Streptozotocin assay. Streptozotocin concentration was determined using a colorimetric method described by Forist [6]. This method depends on the cleavage of the *N*-nitroso group of streptozotocin to yield nitrous acid. This nitrous acid is diazotized with sulfanilic acid and the diazo compound is coupled with *N*-(1-napthyl)-ethylene-diamine to produce a colored azo compound with maximum absorption at 550 nm. A 10 μL sample was obtained at each time pont and diluted with pH 4 acetate buffer to a volume of 5 mL. Volumes of 1 mL 6 N HCl and 5 mL color reagent were then added. This solution was then mixed by vortexing before heating in a boiling water bath. After 5 min heating, the solution was cooled to room temperature, and its optical density at 550 nm was read.

Determination of plasma glucose levels. Blood samples (1 mL) were collected by tail snip and centrifuged to obtain plasma. Plasma glucose levels were determined using a glucose oxidase kit (Young-dong Medical Co., Seoul, Korea).

Results and Discussion

Figure 1 shows the rates of degradation for streptozotocin in rat plasma and in pH 7.4 phosphate buffer. As previous

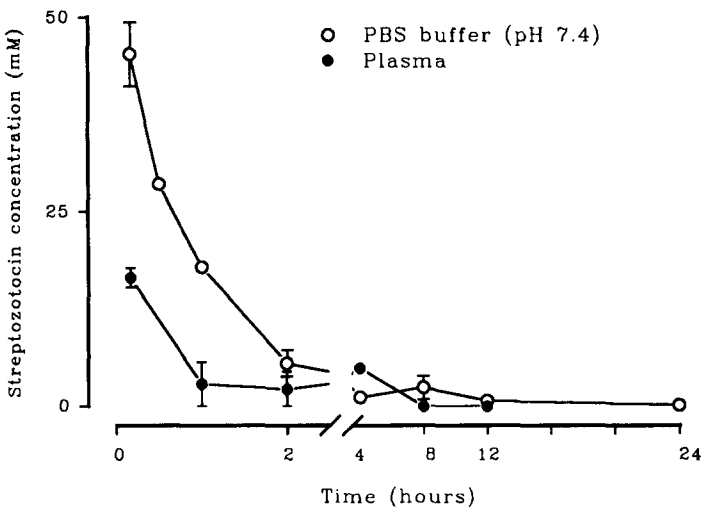

Fig. 1. Degradation of streptozotocin in plasma and phosphate-buffered saline (PBS) (pH 7.4). Data are expressed as the means ± SEM (N = 3).

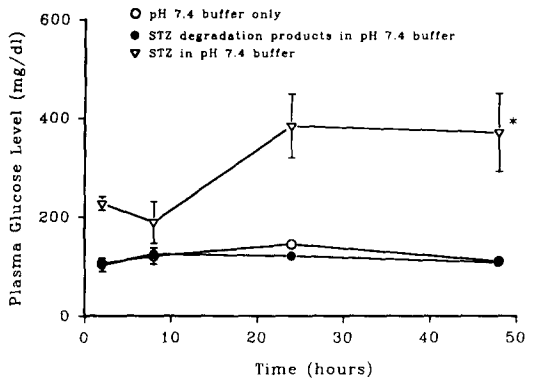

Fig. 2. Effect of streptozotocin (STZ) in pH 7.4 buffer on plasma glucose levels. Data are expressed as the means ± SEM (N = 4–6). *P < 0.05 relative to pH 7.4 buffer only control (two-way ANOVA followed by Duncan's Multiple Range test).

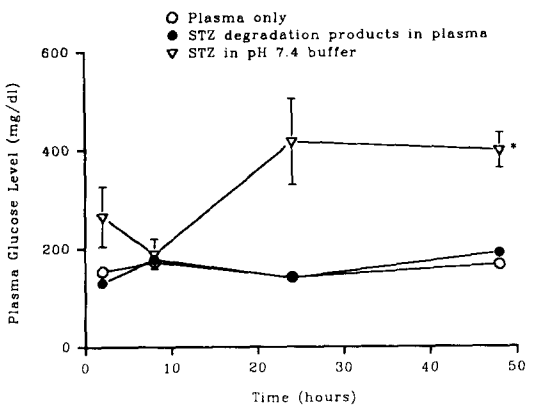

Fig. 3. Effect of streptozotocin (STZ) in plasma on plasma glucose levels. Data are expressed as the means ± SEM (N = 4). *P < 0.05 relative to plasma only control (two-way ANOVA followed by Duncan's Multiple Range test).

investigations have shown [7], streptozotocin completely degrades in pH 7.4 buffer within 4 hr. Surprisingly, streptozotocin degrades even more rapidly in plasma. Within 10 min in plasma, streptozotocin has decreased by more than 60%. It is not clear why streptozotocin was more rapidly degraded in plasma than in pH 7.4 phosphate buffer. Presumably, various plasma proteins or other materials could accelerate the degradation. In any case, our *in vitro* results are similar to those reported by Karunanayake *et al.* [7, 8], who showed *in vivo* that streptozotocin was completely degraded in blood within 4 hr. Although the parent substrate is completely degraded, streptozotocin degradation products in plasma have been shown by Bhuyan *et al.* to be biologically active [9], with at least two degradation products demonstrating antibacterial capacity similar to that of streptozotocin itself. It was speculated that the diabetes-inducing capacity of streptozotocin might also be demonstrated by one or more degradation products.

Figure 2 shows the effect on plasma glucose of streptozotocin incubated in pH 7.4 buffer. As expected, streptozotocin administered right after its preparation in pH 7.4 buffer significantly increased plasma glucose levels in rats. However, streptozotocin degradation products prepared by incubation in pH 7.4 buffer for 24 hr did not. These data clearly show that the degradation products of streptozotocin resulting from incubation in pH 7.4 buffer are not diabetogenic. Similarly, Fig. 3 shows that streptozotocin degradation products resulting from incubation in plasma also did not induce hyperglycemia in rats. This work suggests: (1) unless streptozotocin can be administered immediately in pH 7.4 buffer, streptozotocin should continue to be administered in pH 4.0–4.8 buffer to be diabetogenic, and (2) that only a small portion of the 40 mg/kg *in vivo* diabetic dose is actually responsible for damaging the pancreas, since most of the diabetic dose will be rapidly degraded in an intact animal at 37°. In addition, it is known that the methyl nitroso moiety of streptozotocin exerts its cytotoxic effect on pancreatic islet beta cells, whereas the deoxyglucose moiety facilitates the entry of streptozotocin into the beta cells [10]. Since the degradation products are not toxic to the pancreas, this work suggests that the degradation of streptozotocin under normal physiological conditions involves the methyl nitroso moiety. Alternatively, it is possible that the deoxyglucose moiety of streptozotocin was affected and/or cleaved from the methylating moiety, so that much higher doses of streptozotocin were needed to induce diabetes. However, since it is known that under normal physiological conditions (i.e. pH 7.4), the deoxyglucose moiety is much more stable than the electrophilic methyl nitroso moiety [3], the first explanation appears to be more plausible.

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