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Commentary
Streptozotocin is not a Spontaneous NO Donor

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The reaction of streptozotocin with oxymyoglobin was analyzed and compared with results using various compounds that spontaneously generate nitric oxide in solution.

Key words: diabetes, FK409, nitric oxide, oxymyoglobin

INTRODUCTION

Streptozotocin (SZ), a 2-deoxy-glucose substituted at C-2 by N-methyl-N-nitroso-urea[1] and synthesized by *Streptomycetes achromogenes*, is the most widely used inducing agent in animal models of experimental diabetes (for review see Kolb and Kröncke[2]) and is also used as anti-insulinoma therapeutic agent in humans. Among several effects it was found that SZ activates guanylate cyclase in cells and acts as a vasodilator which both has been suggested to be due to NO formation.[2] Indeed, SZ has been shown to generate low amounts of nitrite when incubated in the presence of pancreatic islets[3] and we recently showed that SZ is metabolized in hepatocytes and islet cells to yield nitric oxide (NO) which contributes to islet cell DNA damage.[4] In neutral aqueous solution, SZ spontaneously decomposes in a complex way not yet completely understood yielding different products not identified to date.[5] Recently, it has been suggested that SZ produces NO spontaneously during its decomposition in solution,[6] but NO generation from SZ in the absence of cells has only been demonstrated following irradiation with light of 250–450 nm.[7,8] We now investigated whether SZ spontaneously generates NO by measuring NO-induced spectral changes of oxymyoglobin in the presence of decomposing SZ in comparison to well characterized spontaneous NO donors of various half-lives.

L-cysteine-HCl, horse skeletal myoglobin and spermine were purchased from Sigma (Deisenhofen, FRG), $NaNO_2$, CH_3CN and $Na_2S_2O_4$ from Merck (Darmstadt, FRG), streptozotocin (SZ) from Boehringer Mannheim (Mannheim, FRG) and FK409 ((±)-(E)-ethyl-2-[(E)-hydroxyimino] – 5-nitro-3-hexeneamide), isolated from the

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fermentation products of *Streptomyces griseosporus*, from Alexis (Läufelfingen, Switzerland). SIN-1 was kindly provided by Cassella AG (Frankfurt, FRG). A 100 mM stock solution of S-nitrosocysteine (SNOC) was freshly prepared prior to use as follows: 1.76 mg cysteine-HCl and 0.69 mg sodium nitrite were dissolved in 48 µl H_2O each and mixed; the slightly red solution was acidified by 2 µl 1 M HCl to achieve pH 2 for complete S-nitrosylation and neutralized after 2 min with 1.5–2 µl 1 M NaOH. The spermine/NO adduct (Spe/NO) was synthesized as described.[9,10] Briefly, NO gas (Messer Griessheim, Frankfurt, FRG), purified by bubbling through a solution of 10 M potassium hydroxide and dried by passage through potassium hydroxide pellets, was bubbled through a solution of 10 g spermine dissolved in 100 ml dry CH_3CN for 2 h under anaerobic conditions. The colourless amorphous reaction product was isolated by filtration, washed with CH_3CN and diethylether and dried in vacuo (yield: 731 mg = 6.4%). Spe/NO exhibited a broad adsorption at 230–270 nm with an adsorption maximum $\varepsilon_{252nm} = 7.74\ mM^{-1}\ cm^{-1}$ as described.[11] Oxymyoglobin was prepared by reduction of myoglobin with excess sodium dithionite[12] and purified from low-molecular compounds using a PD-10 column (Pharmacia, Freiburg, FRG).

Half-lives were determined photometrically with a Beckmann DU 640 spectrophotometer using a temperature controlled auto 6-sampler with PBS as blank in position 1 to minimize light exposure of the specimen during decomposition (average reading time: 0.5 sec). The following wavelengths were used: SNOC, 340 nm; Spe/NO, 250 nm; SIN-1, 300 nm; SZ, 390 nm. Oxymyoglobin spectral changes (450–700 nm) were recorded with a scan time of 30 sec at the time intervals indicated.

GENERAL DISCUSSION

NO gas or NO derived from NO donors is known to induce the oxidation of oxymyoglobin ($MbFe^{II}O_2$) yielding nitrate and ferric metmyoglobin ($MbFe^{III}$) which can be recorded by spectral changes.[13–15] To show whether SZ spontaneously releases NO, we investigated the reaction of SZ with oxymyoglobin and compared the results with compounds that are known to spontaneously generate NO in solution. As spontaneous NO donors we used compounds of three different chemical classes: the S-nitroso-thiol S-nitrosocysteine (SNOC), the polyamine/NO complex spermine/NO (Spe/NO) and 3-morpholino-sydnonimine (SIN-1) (for review of decomposition-mechanisms see Kröncke *et al.*).[16] The half-lives of these NO donors (ranging from several minutes to 2 h) and of SZ at the concentrations used as determined photometrically at 37°C in the dark under neutral conditions are shown in Table 1.

While 0.1 mM MbO_2 autoxidizes very slowly under aerobic conditions, it was oxidized by 0.1 mM nitrite within 75 min. The velocity of MbO_2 oxidation by NO donors depends on the rate of NO generation and thus on the half life of the NO donor. MbO_2 is oxidized by 0.1 mM SNOC, which generates NO very fast, within 15 min and by Spe/NO and SIN-1 (0.1 mM each) within 90 min, which correlates with the half-lives of these NO donors. The microbial products FK409 and SZ differed in their properties concerning MbO_2 oxidation. While 1 mM of FK409, also described as

TABLE 1 Half-lives in the dark of spontaneous NO donors and of SZ at 37°C in PBS, pH 7.2, as measured photometrically (for wavelengths see Materials and Methods) and times of 50% oxidation of 0.1 mM oxymyoglobin to metmyoglobin as measured at 581 nm.

Compound	$t_{1/2}$[min]	50% MbO_2 oxidation [min]
0.1 mM SNOC	6.5±1	5.7±1.2
0.1 mM Spe/NO	40±2	33.5±2.1
0.1 mM SIN-1	124±1.5	43.3±1.5
1 mM FK409	40*	11.6±3.2
10 mM SZ	126±2	–[a]

[a]Not detectable, see Figure 1B.
Values are the means of 3 or more separate determinations.
*Value is taken from reference 17.

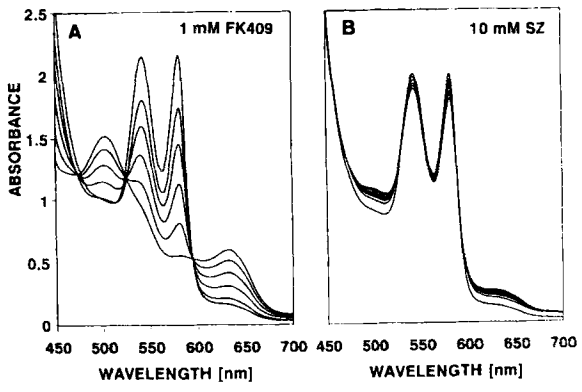

FIGURE 1 Oxymyoglobin (0.1 mM) was incubated in the presence of 1 mM FK409 (A) or 10 mM SZ (B) in PBS, pH 7.2, at 37°C. Spectral changes were recorded at time intervals of 5 min (A) or 15 min (B).

spontaneous NO donor,[17,18] oxidized MbO_2 within 25 min (Figure 1A), SZ at concentrations of up to 10 mM did not result in metmyoglobin formation within 90 min (Figure 1B). These data demonstrate that NO is not spontaneously generated during decomposition of SZ.

CONCLUSION

The results presented here confirm our findings that SZ does not spontaneously generate NO during decomposition but has to be metabolized by cells to generate NO[4] and thus cannot be regarded as a spontaneous NO donor as has been suggested by Tanaka *et al.*[6] Cytotoxicity induced during metabolization of N-methyl-N'-nitro-N-nitrosoguanidine, a compound with a similar chemical moiety as the N-methyl-N-nitroso-urea-derivative SZ, has very recently also been shown to involve NO generation.[19] This strongly suggests that NO generation during cellular metabolization of N-nitroso-ureas and N-nitroso-guanidines within cells contributes to the well known mutagenic/carcinogenic properties of these two classes of compounds besides their alkylating activities.

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