Meeting Report

STREPTOZOTOCIN: MECHANISMS OF ACTION

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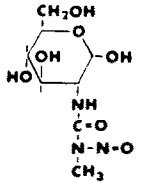
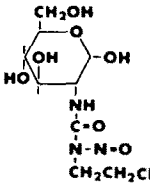
Streptozotocin (2-deoxy-2-(3-methyl-3-nitrosourea)1-d-glucopyranose) ($\alpha + \beta$) is an antibiotic isolated in 1959 from *Streptomyces achromogenes* var. *Streptozoticus* found in a soil sample collected at Blue Rapids, Kansas. The wide range of biological effects of this material were discussed as follows: history, diabetogenesis and the underlying mechanisms, tumorigenesis, clinical applications.

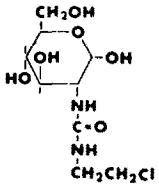
The complete structural determination was reported in 1979 although chemical synthesis was accomplished as early as 1967. Well over a hundred analogues are actually known. In the natural state, streptozotocin exists as a 50–50 mixture of α and β anomers. In about one third of the analogues, the basic streptozotocin structure has been retained but modifications include acetylations, alkylations, replacement of the nitroso group. The acetyl derivatives are as active as the parent molecule. The alkyl glycoside analogues exhibit substantially reduced antibacterial property but retain antitumour activity. Replacement of the methyl group by 2-chloroethyl results in chlorzotocin that seems superior to streptozotocin as a clinically useful anti-tumour agent. The usefulness of other chemical modifications is less certain (Wiley).



STREPTOZOTOCIN



CHLOROZOTOCIN (ANALOGUE I)



ANALOGUE II

Experimental diabetes can be produced transiently by hormones (glucagon, somatotropin, glucocorticoids, adrenaline, sex steroids), cyproheptadine, L-asparaginase, quinolines and related compounds, benzothiodiazine diuretics, hypotensives. Permanent diabetes by alloxan or streptozotocin revealed that both produced irreversible damage to pancreatic β-cells; streptozotocin can also destroy the α-cells and the D-cells in the Chinese hamster. Both chemicals produce an initial rise in blood sugar in the first 4 h post injection followed by hypoglycemia between 6–12 h, and finally permanent hyperglycemia 24 h post treatment. These are due, respectively, to an initial inhibition of insulin release, feedback increase in plasma insulin, and insulin deficiency due to β-cell destruction.

The two compounds differ in many respects. The half life of streptozotocin is 10–15 min and that of alloxan 1 min, after intravenous administration. Liver and islet NAD levels decrease after streptozotocin but not after alloxan. α-Glucose inhibits the action of alloxan but not that of streptozotocin. Nicotinamide, but not nicotonic acid, protects against streptozotocin but both antagonise alloxan mediated diabetogenesis. Nicotinamide will protect if given even 2 h after streptozotocin but it must be given simultaneously or before alloxan. Pyrazinamide, diphenylhydantoin, and 2-deoxyglucose will also antagonise streptozotocin action.

As to the mechanisms of diabetogenesis, the glucose moiety of the α-anomer is believed to act as a carrier for the *N*-nitroso-*N*-methyl-urea portion. Intracellular entry of streptozotocin leads to hypertrophy of β-cell Golgi apparatus within 1 h, and cell pycnosis follows shortly. Alloxan is believed to react with the extracellular receptor for glucose on the β-cell surface

leading to increased permeability and, within 5 min, cytoplasmic fragmentation and chromatin clumping. Morphological changes of cytolysis are similar 4–5 h after either chemical.

Reversion to the normal state is more frequent with alloxan than with streptozotocin. Indeed, diabetes induced by streptozotocin is more akin to juvenile onset diabetes in the human, than alloxan induced diabetes. Both the early and the late changes in renal complications, following natural human diabetes and after streptozotocin in the rat, are very comparable (Rasch).

There are great species specific differences in diabetogenesis and the underlying changes in the enzymes of the carbohydrate pathway in response to streptozotocin administration (Chang). The sensitivity to streptozotocin diabetes may be established as rat > mouse > dog > guinea pig. Other aspects of streptozotocin action include hypertriglyceridemia (Reaven), muscle protease activity (Dahlman), altered lipid metabolism (Stearns), microvascular disease (Reddi), collagen metabolism (Schnier), immunosuppression (Nichols).

The general consensus was to accept the view that streptozotocin is a particularly appropriate experimental tool for studying the pathology of natural diabetes in the human (Rasch) and in this respect several injections of smaller doses of streptozotocin appeared to be more effective than a single large bolus (Agarwal).

In an effort to understand the complications of pregnancy in diabetic women, Dr DeHertogh (Louvain) showed that the early oestrogen action and receptor kinetics in rat uterus are not altered after long term streptozotocin treatment. Similarly, the association of glucocorticoid hormones with their hepatic receptor seems unimpaired in streptozotocin diabetic mice (Agarwal).

The 1-methyl-1-nitrosourea moiety of streptozotocin may be responsible for oncogenesis in the kidney, liver, peritoneum, and pancreas of the rat. Abnormal regeneration of all cell types in the pancreas, possibly aided by hyperglycemia, may lead to β-cell adenoma (Fox). The tumours have very low glucagon levels and large amounts of insulin and are thus multicellular and multihormonal (Baba). Curiously, nicotinamide actually helps streptozotocin mediated oncogenesis and this was confirmed by the group of Professor Baba (Kobe). Thus, diabetogenesis and tumorigenesis are two distinct functions where the latter is essentially a result of long survival after streptozotocin (Baba). However, in other conditions (DeHertogh) long survival did not lead to tumour formation.

Cyclases are currently believed to be related to growth. Dr Vesley (Little Rock) attempted to correlate guanylate cyclase activity with tumour induction by streptozotocin. The activity of this enzyme was increased by streptozotocin in vitro in the order: liver > cerebrum > heart > kidney cerebellum > brain stem > lung > spleen > pancreas. It was pointed out that the smallest increases in the pancreas did not parallel streptozotocin induced pancreatic tumours, and moreover the studies did not show any streptozotocin effect on this enzyme in vivo (Agarwal). Dr Kazumi (Kobe) suggested that the enzyme assays on isolated islets may be more meaningful than on the whole pancreas but Dr Vesley indicated that this was difficult to do and that the activity of this enzyme may have more of a predictive value for streptozotocin therapy than for oncogenic action of the antibiotic.

Pancreatic endocrine tumours and carcinoids, collectively called apudomas, have classically benefitted from surgical resection but many patients exhibit metastases at the time of diagnosis and this led to the search for a specific chemotherapeutic agent. As early as 1969 the affinity of streptozotocin for β-cells was exploited for tumour destruction and more than one hundred patients have been treated thus far. Most tumours are composed of several cell types and secrete many biologically active peptides. These include gastrin, vasoactive-intestinal polypeptide, calcitonin, pancreatic polypeptide, somatostatin all of which originate from the enterochromaffinlike cells in the pancreas and the mid gut. Insulinomas and glucagonomas are strictly pancreatic in origin.

As compared to L-asparaginase, 5-fluorouracil, tubericidin, adriamycin, mithramycin, glucose, and glucocorticoid hormones, streptozotocin is by far the most potent agent if given as 1–2 g/m^2 intravenously per week to a total of 6–8 g with subsequent maintenance in low doses, dissolved in citrated saline pH 4.5. In an earlier study, out of 30 cases, 50% benefitted from a diminished tumoral mass, 17% exhibited complete remission, 64% showed favourable biochemical responses and in 26% insulin levels returned to normal. These were confirmed on 19 cases of pancreatic tumours by Dr Oberg (Stockholm). The drug was of limited value in treating carcinoid

tumours in 12 patients (Oberg). It is less certain whether streptozotocin can be used to treat Hodgkin's disease, lymphocytic lymphoma, and other carcinomas (lung, oral cavity) although such efforts have been made.

The major problem with the drug is its high toxicity for the kidney which can be avoided by monitoring uterine protein; the next dose should not be administered until the proteinuria drops to zero. Other side effects include nausea, vomitting, toxicity on the liver and the hematopoietic system. The affinity of the drug for the β-cell is shown by the fact that insulinomas are effectively destroyed but glucagonomas are far more resistant. Thus, diabetes may eventually become a complication of streptozotocin therapy. Other side effects have not yet been worked out but are evident from the influence of streptozotocin on various types of tumours, especially VIPomas that are very sensitive to the antineoplastic effect.

Agarwal et al. (Paris) presented evidence that streptozotocin may not be as specific a β-cell toxin as hitherto believed. β-cell destruction and hyperglycemia take 24 h to manifest themselves but the drug can sensitize mice to endotoxin lethality in the first few hours after intraperitoneal administration. Dr Vesley suggested that early effects are difficult to tie in with affinity for the β-cell and its eventual destruction. The multiple hit hypothesis is also supported by the activity in the Chinese hamster where all pancreatic cells are attacked, and by effects of streptozotocin on various types of tumours indicated above (Agarwal).

In conclusion, streptozotocin is unusually effective in simulating human juvenile onset diabetes in experimental animals. It may also be a good model for understanding host regulation at the level of immunology, physiology, pharmacology and biochemistry. Although it may have more than one site of action in the beginning, the long term effects can be explained by its affinity for the β-cell of the pancreas. In the long run, streptozotocin may be of great importance not only in understanding the mechanisms of diabetogenesis and oncogenesis but also as an antitumour agent for which the Food and Drug Administration has just approved the patent for production by the Upjohn Co. for therapy of apudomas.

Full proceedings of the meeting are to be published by Elsevier/North-Holland, Amsterdam, New York.