Short Communication

In vivo Oxidation of Cholesterol in Streptozotocin Induced Diabetes Rat

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Introduction

Cholesterol is present in tissues and in plasma lipoproteins either as free cholesterol or combined with a long chain fatty acid, as cholesterol or combined with a long chain fatty acid, as cholesterol ester. Cholesterol is the precursor of all other steroids in the body such as corticosteroids, sex hormones, bile acids. and vitamin D. A little more than half the cholesterol of the body arises by *in vivo* synthesis (about 700 mg d) and the remainder is provided by the average diet. [1]

In diabetes the plasma cholesterol level is usually elevated and this may play a role in the accelerated development of the arteriosclerotic vascular disease which is a major long-term complication of diabetes in man. In severe diabetes, cholesterol synthesis is decreased, part of the rise in plasma cholesterol level is due to an increase in the cholesterol containing very low-density and low-density lipoproteins secondary to the great increase in circulating trigycerides. Another factor may be a decline in hepatic degradation of cholesterol which when it exceeds the decline in cholesterol synthesis, contributes to the rise[2]. Because cholesterol is very important metabolic precursor which plays a central role in our biological system so that we were interested to investigate the metabolic pattern in diabetic conditions of this precursor.

In this paper we have seen the effect of diabetes on the metabolism of ^{14}C-cholesterol. It was investigated by Ip Intraperiotoneal (IP) injection of unio-formly labelled ^{14}C- cholesterol. Streptozotocin induced diabetic rats were used separately and compared with sham control rats. This investigations include the areas in volved in the studies of the in vivo oxidation of (U-^{14}C) labelled cholesteol from which we

can get some metabolic pattern in terms of oxidation of the respected compound in diabetes [3].

Materials and Methods

(a) Animals and Management

Animal : Long-evans rats of black and white strain were used this study. Adult animals aged 75 to 120 days and weighing between 200 to 250 gms were selected. Total number of rats was ten. Five was used as Sham Control and five for induction of diabetes. The experimental animals were obtained from the animal resources branch, diabetic association, Shabagh, Bangladesh.

Maintenance : Each of the selected rats was housed in a screen bottomed cages (GEO. H. Wahmann Manufacturing Co. Baltimore MD) and maintained in a constant temperature environment with 12 hrs of artificial light per 24 hours. The temperature of the room in which the rats kept was 26±5 °C. The rats were fed on a good quality basal diet[4] and water *ad libitum*. The diet supplied for 7 days to each rat was approximately isocaloric (Approximately, 20 gm of diet per rat per day).

(b) Induction of diabetes

Diabetes was induced in the experimental rats by injection of streptozotocin. The drug (1 mg steptozotocin containing 22 mg cirtric acid in 1 ml saline solution 200 g B.W.) was administered intraperitoneally after 24 hrs of fasting. The Sham Control rats were given equal amount of normal saline. Ten rats were used in total, five for induction of diabetes and five for sham control. The degree of occurrence of diabetes was monitored after 7 days by estimating blood glucose level using glucose strip in a reflectometer (Reflemat, labora Mannheim; GMbH fur Labortechnic) and by urine analysis for detecting increased concentration of glucose of Benedict reagent. One drop of blood was collected from a stab incision at the ventral position of the base of the tail for the examination of blood glucose level by reflectometer. Then blood glucose was estimated by modified Somogyi-Nelson method [5].

(c) Radiotracer specification

The compound ^{14}C cholesterol, 55.7 μci/mmol (Code : CF A229, Batch 64) was supplied by ICN Isotope and Nuclear Division. Campus Drive, Irvine, U.S.A. Its purity was 99% as checked by Paper chromatography.

(d) Administration of Radiotracer

A tracer dose 4 μci holesterol (U-^{14}C) 0.2 ml saline solution rat were administer IP in diabetic rats following an overnight fasting. The Sham control rat were also given equal amount of radioactie cholesterol.

(e) Collection of $^{14}CO_2$

Immediately after injection of radiotracer the rat was placed in a metabolic chamber attached with a $^{14}CO_2$ trapping system. The expired $^{14}CO_2$ was aspired with a water pump through 3 ml 10% KOH in a counting vial to trap $^{14}CO_2$. The flow was kept slow enough to allow complete trapping. Fresh trapping solutions in counting vials were provided after every 10 minutes for a period of 4 hours. The expired ^{14}C-CO_2 evolved as a result of *in vivo* oxidation of the radiotracer was collected in twenty-four (6X 4=24) vials. To prevent leakage of $^{14}CO_2$ in the laboratory a safety trap containing 250 ml 10% KOH solution was connected in series with the metabolic chamber.

(f) Measurement of raidoactivity

The trapped $^{14}CO_2$ samples were counted in a Packard tricard liquid scintillation spectra-meter (Model No: 3255) in BIRDEM. Ten millilitre instagel scintillation cocktail (Packard) was used in each counting vial. The samples were counted thrice for 1 min using the green channel, with automatic background subtraction. Results are the mean of three counts, in CPM (count per minutes). Counting efficiency (CE) was determined using an internal standard (CE varied between 86-88%) and a standard curve for varying degree of quenching was constructed. Tmax in minutes was claculated from the graph afte plotting CMP against time using the formaula.

Total percent of dose converted at

$$T_{max} = \frac{\mu ci\ ^{14}CO_2}{\mu ci \text{ injected}} \times 100$$

Result

Estimation of glucose level in streptozotocin injected rats after 7 days clearly indicate the establishment of diabetes in experimental group of rats (data not shown). *In vivo* conversion of cholesterol (U-^{14}C) to $^{14}CO_2$ by stretozotocin induced diabetic (SZID) and sham control (SC) rats is presented in Figure 1. Time after injection for the maximum rate of conversion to $^{14}CO_2$ (T_{max} in minutes) was found to be higher in SC rat when compared to that in SZID rat (T_{max}=110 min and T_{max}=50 min, respectively). The reverse situation occured in case of acetate metabolism in diabetes[6]. Figure 1 also shows that the percent of dose converted to $^{14}CO_2$ at T_{max} is higher in SC rat than in SZID rat. (0.080% and 0.040% 0. respectively). Mimicing the same fate as seen in case of acetate metabolism. This may indicate why in diabetes hypercholesterolemia occurs anomalously.

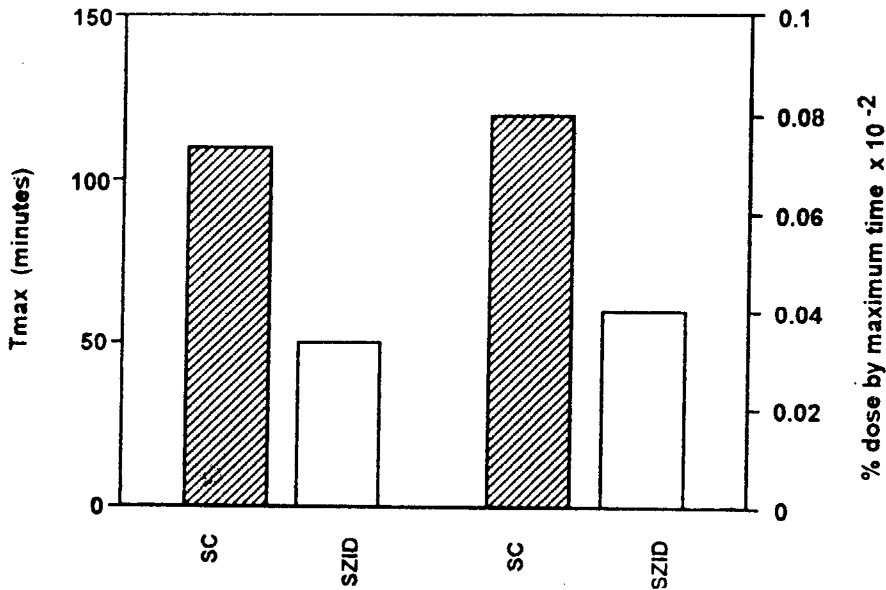

Fig. 1. *In vivo* oxidation of intrapertioneally injected cholesterol (U-^{14}C) to $^{14}CO_2$ by streptozotocin induced diabeteis (SZID) and sham contol (SC) rats expressed in terms of T_{max} and percent of dose converted to $^{14}CO_2$ at T_{max}.

Discussion

Little and Block[7] reported that the rat can oxidize a large amount of ^{26}C of ^{14}C cholesterol (i.e. 26 position labelled) to radioactive $^{14}CO_2$ Nineteen percent of ^{14}C dose was recovered in the form of respiratory $^{14}CO_2$ in the first 24 hrs and 40% by the end of 84 hours. But in our experiments only 0.08 X $10^{2\%}$ in SC rat and only 0.04 X $10^{-2\%}$ in SZID rat of total dose were found to be utilized for production of $^{14}CO_2$ from ^{14}C-cholesterol by respiration. In our case the labelled U-^{14}C was uniform and this may account for the low results. The results thus indicate that the oxidation of free leading to long term complication of arteriosclerotic vascular diseases. In severe diabetes cholesterol synthesis is decreased and rise of plasma choleterol level is due to an increase in the cholesterol containing very low density lipoprotein (VLDL) and low density lipoprotein (LDL) with a decline in hepatic degradation of cholesterol. Diabetes also inhibit cholesterol synthesis to a lesser degree than fatty acid synthesis[8]. This may indicate why in diabetes hypercholesterolemia occurs anomalously. Therefore, the decreasd metabolic oxidation of free cholesterol during diabetes might be either due to its decreased

conversion into acetate for which $^{14}CO_2$ production was so low or this may be perhaps due to the increased gluconeogenesis by diabetic liver before appreciable quantities penetrate into muscle cells for oxidation or due to both.

Summary

In-vivo oxidation of uniformly (U-^{14}C) labelled cholesterol in streptozotocin induced diabetic (SZID) rat models vis-a-vis sham control (SC) was studied. Total number of rats was ten. Five was used as Sham Control and five for induction of diabetes. Adult animals aged 75 to 120 days and weighing between 200 to 250 mg were selected. The rats were fed on a good quality basal diet and water *and libitum* for 7 days. A tracer dose of 4 μci cholesterol (U-^{14}C)/0.2 ml saline solution were administered IP to the rats following an over night fasting. The rate of oxidation of cholesterol (U-^{14}C) was not only significantly appeared 50 minutes earlier in SZID (Tmax=50) than SC rats (Tmax=110), the percent of dose converted to $^{14}CO_2$ was also about 50% lower in the diabetic animals.

Reference

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