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EFFECT OF STREPTOZOTOCIN INDUCED DIABETES AND ADMINISTRATION OF INSULIN ON SOME BLOOD CLOTTING PARAMETERS AND PLATELET COUNT IN MALE ALBINO RATS

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SUMMARY

The prothrombin time, activated partial thromboplastin time, fibrinogen concentration and platelet count were determined in diabetic and insulin treated diabetic mature male albino rats. The values of prothrombin and activated partial thromboplastin time showed significant decrease in the diabetic and semi-diabetic rats at 10 and 20 days following treatment, fibrinogen concentration increased in diabetic rats, 10 and 20 days post-insulin administration and in the semi-diabetic rats after 20 days. Platetet count increased significantly in the diabetic rats treated with 4 U insulin daily after 20 days, although the clotting parameters were not affected. The results presented are indicative of coagulation activation in diabetic rats and the periodic administration of insulin will reverse the adverse effects of diabetes.

INTROUCTION

Diabetes mellitus is a naturally occuring disease which affects humans (Cahill and McDevitt, 1981) and recorded in many animal species such as cattle (Mostaghni and Ivoghli, 1977), sheep (Phillips et al., 1970), dog (Williams et al., 1981) and cat (Moise and Reimers, 1983). In the horse, diabetes in all reports has been associated with pancreatic destruction secondary to strongyle migration (Jeffrey 1968). Moreover, increased plasma concentration of any of the diabetogenic hormones (i.e. glucocorticoids, epinephrine, glucagon or growth hormone) due to excessive secretion, impaired degradation or exogenous administration will result in insulin antagonism in peripheral tissues and the development of diabetes mellitus. For experimental purposes, diabetes can be produced by either surgical removal of the pancreas or by injection of streptozotocin, a chemical which selectively poisons the β cells (Peterson et al., 1981).

Diabetic animals may be regarded as models of the disease in man and have been used to study the etiologies, complications, treatment and prevention of the disease. Diabetes mellitus have been found to be associated with an increased risk of cardiovascular disease, especially myocardial infarction, cerebrovascular and peripheral vascular diseases. These complications account for 80% of the deaths in people with non-insulin dependent diabetes (Abott et al., 1987).

Kwaan (1992) found that the increased risk of thromboembolism in diabetes mellitus is due to changes in the hemostatic mechanism including abnormal platelet function leading to platelet activation, increase in several clotting factors, decrease in natural anticoagulants and impaired fibrinolytic activity.

The aim of the present study is to investigate the hemostatic mechanism including prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen concentration and platelet count and glucose level in diabetic and insulin-treated diabetic male albino rats.

MATERIALS AND METHODS

Forty male albino rats (160-200 g body wt.) were

maintained on standard rat laboratory diet and water ad libitum throughout the study. Rats were housed in stainless steel cages and exposed to a 10 h on -14 h off lighting cycle and studied after initial adaption one week to experimental conditions.

Diabetes mellitus was induced in thirty rats by a single intraperitonial injection (0.1 ml) of 65 mg/kg b.w. streptozotocin (STZ) (Upjohn, Kalamazoo, MI, U.S.A.), dissolved in sodium citrate buffer (o.1 M citric acid, pH 4.5) and used within 10 minutes after dissolving. Other ten rats received an equal volume of the vehicle and were kept as control (Group A). 3 days after STZ injection, the thirty rats were classified into three groups of 10 rats each:

Group B: STZ treated, diabetic rats.
Group C: Was maintained by daily S.C. injection of insulin (U-40 NPH lletin-lihy) 1 U/day at 9.00 a.m. for twenty days: Uncontrolled diabetic rats.
Group D: Was maintained by daily S.C. injection of insulin 4 U/day at 9.00 a.m. for twenty days: Controlled diabetic rats.

Control (Group A) and untreated diabetic rats (Group B) recevied daily S.C. injection of o.1 ml NPH vehicle only for twenty days.

Blood samples were collected by orbital sinus puncture using citrated capillary tubes under light ether anaesthesia from all rats, on days 10 and 20 after the injection of insulin or the vehicle. Citrated blood samples were collected into 3.8% sodium citrate solution and centrifuged at 3000 r.p.m. for 10 minutes and citrated plasma were obtained and used for the determination of PT, APTT and fibrinogen concentration. EDTA blood samples were collected and used for platelet count, according to the method of Rees and Ecker (1923). Blood was also collected into plain tubes and allowed to clot at room temperature for one hour. Serum was removed follwing centrifugation and used for determination of glucose level. PT and APTT were determined according to the method of Dacie and Lewis (1984) and fibrinogen conentration according to the method of Von-Clauss (1957), using commercial reagents from "Orient-Hoeckst" Company and the "bio-Mericux" fibrometer. Glucose was determined using glucose oxidase reagent (Boehringer) according to the method of Schmidt and Pfeiffer (1971).

RESULTS

Table (1): Effect of streptozotocin induced diabetes and insulin administration on serum glucose level, some blood clotting parameters and platelet count in male albino rats

study group	Days after injection	Glucose (mg/dl)	PT (Seconds)	APTT (seconds)	Fibrinogen (mg/dl)	Platelet count ($x10^3$/c.mm)
Group A	10	111±3.0	15.26±0.13	37.0±0.42	284±2.7	260±3.9
	20	114±3.3	15.32±0.21	38.7±0.72	293±3.6	267±3.2
Group B	10	316±3.2*	11.50±0.34*	33.7±0.45*	313±3.1*	263±2.3
	20	326±4.1*	10.64±0.31*	27.4±0.71*	332±2.7*	262±3.1
Group C	10	152±4.6*	13.23±0.22*	34.4±0.38*	291±1.8	269±2.0
	20	161±3.6*	12.40±0.34*	30.5±0.59*	310±2.1*	272±3.1
Group D	10	110±2.3	15.27±0.29	38.0±0.35	275±2.7	261±2.4
	20	120±4.7	15.07±0.22	38.1±0.54	292±3.6	294±3.1*

± S.E.
* Significantly different from control values at ($P < 0.01$).

Data presented in table (1) showed the effect of induced diabetes mellitus and administration of insulin on serum glucose level, PT, APTT, fibrino gen concentration and platelet count in male abino rats.

Serum glucose level of animals treated with STZ (group B) was elevated by 284% after 10 days and 286% after 20 days of insulin administration in comparison with their respective control, while the animals which received insulin I U/day (group C) had elevated level of glucose 137% after 10 days and 142% after 20 days post-insulin administration than that of the control gorup. However, animals injected with insulin 4 U/day (Group D) showed no significant differences in serum glucose level in comparison with control gorup.

Both the prothrombin time and activated partial thromboplastin time showed a significant decrease in their values in the uncontrolled diabetic (Group C) and diabtic gorup (Group B) of rats after 10 and 20 days post-insulin administration, as compared with their control (group A). No significant differences were observed between controlled diabetic (Group D) and control gorup.

Significant increase have been observed in the fibrinogen concentration in the diabetic group of rats (Group B) after 10 and 20 days and in the uncontrolled diabetic gorup (group C) after 20 days post-insulin administration in comparison with their respective control group. No significant variation between the controlled diabetic group of rats (group D) and control gorup.

Significant increase in the platelet count was noticed in the controlled diabetic group of animals (group D) after 20 days post insulin administration in comparison with control group. However, the uncontrolled diabetic (group C) and diabetic (group B) groups of rats showed no significant differences with their control gorup

DISCUSSION

The decrease in PT and APTT in diabetic (group B) and uncontrolled diabetic rats (group C) might be due to an increase of clotting factors involved in the extrinsic and intrinsic pathways of blood coagulation respectively. The results of APTT are in agreement with those of Van-Wersch et al. (1990), who observed a decreased APTT values in diabetic patients.

There have been scanty studies on coagulation factor concentrations in the plasma of diabetic subjects. Factor VII (Proconvertin) studies have been reported from the extrinsic pathway. Fuller et al. (1979) and Carmassi et al. (1992) reported that mean factor VII concentrations were higher in diabetic subjects.

Intrinsic pathway coagulation factor abnormalities were studied by Egeberg (1963) who reported increased concentrations of factors XII (Hageman factor) and IX (Christmas factor) in diabetic subjects. The same investigator reported normal concentrations of factor II (Prothrombin) but a 23% increase in factor V (Proaccelerin) activity in diabetic subjects. In addition, Fuller et al. (1979) reported normal concentration of factor II and increased concentrations of factor V and factor X (Stuart factor) in insulin-dependent diabetic patients.

Mayne et al. (1970) and Koert et al. (1991) studied factor VIII (Antihaemophilic factor) concentrations and found significant increases in diabetic subjects who, as a gorup, had a high incidence of vascular disease.

The data of plasma fibrinogen obtained in this study agree with those of Carmassi et al. (1992) who found that fibrinogen concentrations in diabetic patients increased significantly above those in normal controls.

Moreover, Kwaan (1992) reported that hyperfibrinogenemai in diabetics is a significant risk factor for ischemic heart disease and there is unfavorable effect conferred by the increased plasma fibrinogen level. Fibrinogen is one of the important plasma proteins that contribute to the blood viscosity. Increased blood viscosity is associated with an increased risk of atherosclerosis.

Another report showed that increased fibrinogen concentrations in diabetics may result in part from

cells with release of cytokines such as interleukin which increases hepatic synthesis of fibrinogen and other acute-phase proteins (Andrew et al., 1992).

Concerning the blood platelet, the present work revealed a significant increase in the platelet count in the controlled diabetic rats (administrated with insulin 4 U/day) after 20 days of treatment, compared with the normal controls. These data concide with those obtained by Negrev (1990) who found that adminstration of rats with insulin stimulates thrombocytopoiesis and rises the biosynthesis of its specific humoral regulator thrombocytopoietin which stimulates the bone marrow megakaryocytes and increases the thrombocyte number. In addition, Muhlard et al. (1979) reported early increases in platelet adhesiveness and aggregation in rats treated with streptozotocin.

Thus, it can be concluded from these data that impairment of the hemostatic balance in diabetic subjects; that is a possible hypercoagulable state, represents an important factor in the pathogenesis of atherosclerotic complications. Nevertheless, the observation that many of the abnormalities described are reversible when hyperglycemia was corrected in the controlled insulin-dependent diabetic rats, has given impetus to the development of improved systems of glucose control for diabetic subjects.

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