[Chem. Pharm. Bull. 26(7)2238—2241(1978)]

UDC 547.963.05.08 : 612.11-08

Blood Chemistry in Alloxan Diabetic Rabbits[1]

Toshiaki Nishihata, Noboru Yata, and Akira Kamada

Faculty of Pharmaceutical Sciences, Osaka University[2]

(Received December 5, 1977)

As a preliminary study of pharmacokinetic behaviors of drugs in the living body under alloxan diabetes, endogenous physiological parameters such as levels of glucose and acetone bodies in blood and total protein, albumin and insulin levels in serum were determined employing rabbits under normal and alloxan diabetic conditions.

Levels of glucose, acetone bodies, total protein and albumin were increased under alloxan diabetic conditions. The increases were reduced to the normal levels by insulin treatment.

From changes of levels of blood glucose, levels of insulin, total protein and albumin in serum and body weight after ceasing insulin treatment, the stages of hyperglycemia as well as hyperproteinemia and hyperalbuminemia were considered to be suitable for the study of pharmacokinetic behaviors of drugs in alloxan diabetic rabbits.

Keywords——diseased state; alloxan diabetic rabbits; endogenous parameters; blood glucose; acetone bodies; serum protein; serum insulin

An experimental diabetes in rabbits induced by intravenous administration of alloxan has been used to clarify pathological changes in diabetes and symptoms of the disease in human.

Changes in endogenous parameters such as GOT value have been studied in animals under alloxan diabetes.[3] Many workers have studied on the physiological changes of such endogenous parameters in reference to the nosogenesis of diabetes.[4]

In case of studying elimination and disposition of a drug in the living body under the condition of a disease, picture of the disease should be checked and, if possible, it is required to sustain the condition of diseases at constant symptoms.

Presently, as a preliminary experiment for the study of pharmacokinetic behavior of drugs in the living body under alloxan diabetes, endogenous parameters in terms of levels of glucose and acetone bodies in blood, and protein and insulin in serum were measured employing rabbits under alloxan and insulin treatments.

Experimental

Materials——Alloxan on the market was used without further purification. Insulin zinc suspension (J.P. IX) on the market was used in the insulin treatment. All other reagents were of analytical grade.

Preparation of Alloxan Diabetic Rabbits——Alloxan diabetic rabbits were prepared after Mita *et al.*[5] Twenty per cent aqueous solution of alloxan freshly prepared with redistilled water was intravenously injected in the ear vein of rabbits weighing 1.7—3.0 kg at a dose of 0.5 ml/kg. Then, the concentration of blood glucose was determined once a day for several days. Rabbits sustained over 150 mg% of glucose level from seventh through tenth day after the alloxan administration were used for the following experiments. In the following experiments, the concentration of blood glucose was checked once a week to confirm the diabetic conditions.

1) This report forms Part I of "Pharmacokinetic Behaviors of Drugs in the Diseased State."
2) Location: *133-1, Yamada-Kami, Suita, Osaka.*
3) R.A. Levinson and E. Englert, Jr., *Diabetes*, **19**, 683 (1970); J.A. Kofood, C.E. Bozzini, and R.A. Alippi, *ibid.*, **19**, 732 (1970); M. Rodrignez-Lopez and C. Lopez-Auijada, *Life Sci.*, **10**, 57 (1971).
4) C.C. Rerup, *Pharmacol. Rev.*, **22**, 485 (1970).
5) S. Mita, T. Nakazima, M. Toshioka, and G. Chiba, *Yakugaku Zasshi*, **88**, 166 (1968).

Determination of Concentration of Glucose, Protein, Albumin and Acetone Bodies in Blood or Serum——Blood glucose level was determined with an *o*-toluidine–boric acid method with some modifications employing a test kit of Glucose Test Wako® (Wako Pure Chemical Ind. Co.). Total serum protein and serum albumin were determined with a test kit of A/G-Test Wako® (Wako Pure Chemical Ind. Co.). Acetone bodies in whole blood were determined according to Procos[6] with some modifications. An unsealed ampoule (1 ml) containing 0.5 ml of heparinized fresh blood was placed in a glass-stoppered test tube which contained 4.0 ml of a vanillin reagent. The vanillin reagent was freshly prepared by dissolving vanillin in 4 N KOH solution to make 2.0%. After closing the test tube tightly, it was kept for 60 min at 55° in a water bath. The color developed in the vanillin solution was spectrophotometrically determined at 430 nm. A control sample of distilled water instead of blood was used as a reference following the procedure described for blood sample.

Determination of Urinary pH[7]——Urine was collected through a ureteral catheter into a reservoir which was cooled with ice.

Determination of Serum Insulin——Serum insulin levels of rabbits under normal and alloxan diabetic conditions were determined by a radioimmunoassay method with Phadebas Insulin Test® (Pharmacia Co., Ltd.) which was an assay kit with a Sephadexbound antibody.

Oral Glucose Tolerance Test——An oral glucose tolerance test was made in normal and alloxan diabetic conditions. Rabbits were kept fast for 24 hr prior to the test. Fifteen per cent aqueous solution of glucose was administered to rabbits by intubation at a dose of 7 ml/kg. Thirty minutes after the first administration, the same dose of glucose was orally administered. Concentration of blood glucose was determined at 15, 30, 60, 75, and 150 min after the first administration of glucose.

Administration of Insulin——Administration of insulin to alloxan diabetic rabbits was performed by two dosage schedules: One method was to administer insulin zinc suspension to femoralis muscle once a day for 4 days at a dose of 1 unit/body and the other was to administer the same dose of insulin on alternate days for four weeks.

Results and Discussion

Fig. 1 shows a typical change of concentrations of glucose, acetone bodies, albumin and insulin in a rabbit under conditions of normal, alloxan diabetes and insulin treatment.

Levels of glucose and acetone bodies in whole blood increased about two weeks after the administration of alloxan.

Levels of serum albumin also increased with an increase of blood glucose level. Changes of serum total protein were similar to those of albumin.

Levels of serum insulin decreased with an increase of blood glucose level.

At the hyperglycemic state, urinary pH lowered with an increase of acetone bodies level. The lowered pH is an feature of diabetic acidosis.

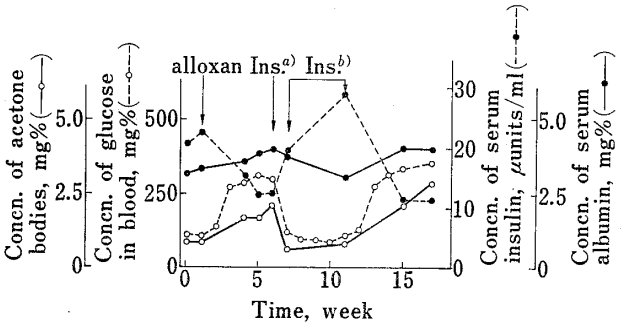

Fig. 1. Changes of the Concentration of Glucose, Acetone Bodies, Albumin and Insulin in Blood or Serum in Alloxan Diabetic Rabbit

Ins.a) and Ins.b) mean the administration of insulin zinc suspension for 4 days and 4 weeks, respectively.

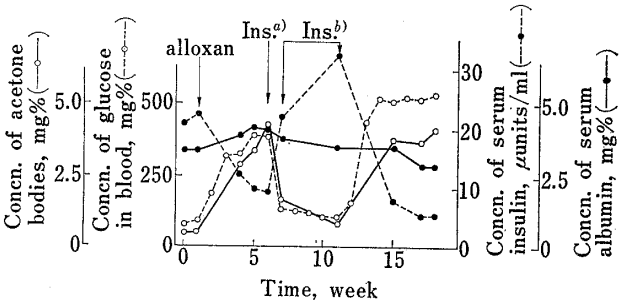

Fig. 2. Changes of the Concentration of Glucose, Acetone Bodies, Albumin and Insulin in Blood or Serum in Alloxan Diabetic Rabbit which was considered to be in an advanced Diabetic State

Ins.a) and Ins.b) mean the administration of insulin zinc suspension for 4 days and 4 weeks, respectively.

6) J. Procos, *Clin. Chem.*, **7**, 97 (1961).
7) M. Gibaldi, B. Grundhoter, and G. Levy, *Clin. Pharmacol. Therap.*, **16**, 520 (1975).

The abnormal levels of blood glucose, acetone bodies, serum albumin and serum insulin after the administration of alloxan recovered to the normal levels by insulin treatments for 4 weeks.

Diabetic acidosis also disappeared by the insulin treatment.

However, after the withdrawal of insulin treatments, levels of glucose, acetone bodies and albumin increased again up to the levels before insulin was administered.

These findings suggest that these levels under alloxan diabetic state can be reversibly recovered to normal ones by insulin treatments and that changes of pharmacokinetic parameters of a drug under normal and diabetic conditions may be repeatedly studied in each animal by intermittent treatment with insulin.

The findings shown in Fig. 1 were observed in three of four rabbits used in the present experiments. But, the rest one rabbit showed a different pattern in levels of blood glucose, serum albumin, serum total protein and serum insulin, especially after the withdrawal of insulin treatment (Fig. 2). In this rabbit, blood glucose increased up to 350 mg% after alloxan was administered. And after the withdrawal of insulin treatment, blood glucose increased to higher levels than those before the insulin treatment began. Remarkable decreases of serum insulin and albumin were observed after withdrawal of insulin treatment.

This rabbit lost its body weight even during the insulin treatment and showed a symptom of an advanced stage of diabetes.

In glucose tolerance test in man, it is generally accepted that the value at 120 min has a significant meaning in criteria of diabetes.

Time course of the concentration of glucose in glucose tolerance test in one of the three rabbits is shown in Fig. 3. The higher glucose level under alloxan diabetes at 120 min after the first administration of glucose approached to the normal level by insulin treatments for 4 weeks. After withdrawal of the insulin treatment, the level increased up to that under diabetes.

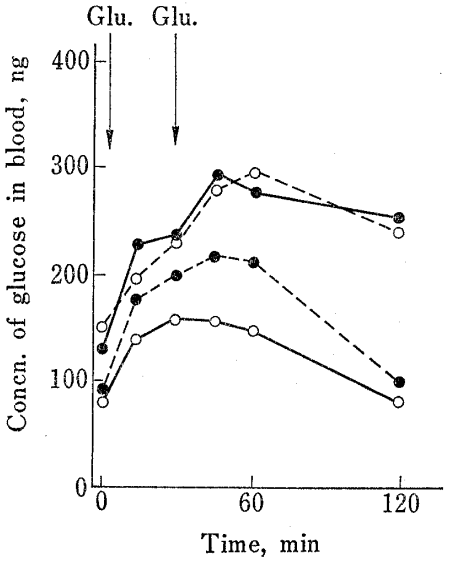

Fig. 3. Changes of the Concentration of Glucose in Blood in Oral Glucose Tolerance Test in Alloxan Diabetic Rabbit

---●---, normal; ---○---, diabetic; —○—, next day after the final administration of insulin in 4 weeks-insulin treatment; —●—, 3 weeks afetr ceasing insulin treatment.

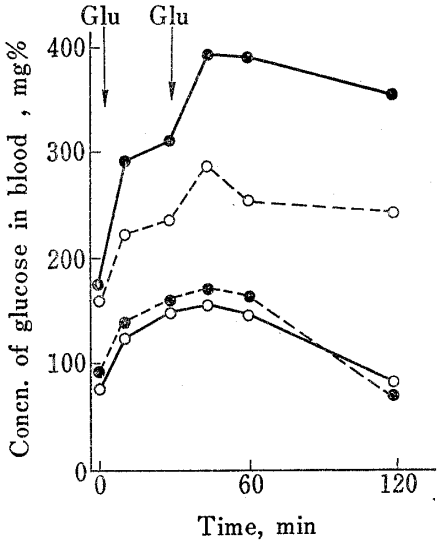

Fig. 4. Changes of the Concentration of Glucose in Blood in Oral Glucose Tolerance Test in Alloxan Diabetic Rabbit which was considered to be in an Advanced Diabetic State

---●---, normal; ---○---, diabetic; —○—, next day after the final administration of insulin in 4 weeks-insulin treatment; —●—, 3 weeks after ceasing insulin treatment.

On the other hand, a rabbit which was considered to be under an advanced diabetes showed different results (Fig. 4). After withdrawal of insulin treatment, glucose level markedly exceeded the level before the insulin treatment began. Thus, in rabbits which showed hypoalbuminemia and loss of body weight with abnormally high levels of blood glucose after withdrawal of insulin treatment, a possible existence of complications with diabetes might be considered.

If there are complications with diabetes, analysis of the experimental results in the pharmacokinetic study of drugs in the diseased state will be more complicated. A constant picture of the disease without complications is desired.

Therefore, the above three rabbits which showed hyperalbuminemia and constant body weight with hyperglycemia even after withdrawal of insulin treatment were considered to be at a constant diseased state suitable for the pharmacokinetic study of drugs in alloxan diabetic rabbits.

Thus, it was concluded that alloxan diabetic rabbits suitable for the study for pharmacokinetic behavior of drugs can be selected by confirmation of hyperglycemia, hyperalbuminemia and constant body weight.

[Chem. Pharm. Bull. 26(7)2241—2246(1978)]

UDC 615.225.2.011.4.074 : 543.426.061

Studies on Ecarazine Hydrochloride (Apiracohl®). II.[1] Spectrofluorometric Determination of Ecarazine Hydrochloride and Its Metabolite in Plasma

Akio Ishii and Takashi Deguchi

Pharmaceuticals Research Laboratory, Kyowa Hakko Kogyo Co., Ltd.[2]

(Received December 12, 1977)

Spectrofluorometric assay for the quantitative determination of ecarazine hydrochloride (I) and its metabolite, 3-methyl-*s*-triazolo(3,4-*a*)phthalazine (III) were described. I and III were extracted from plasma at weakly alkaline pH with chloroform and fluorescence of III was directly measured (Ex 250, Em 410 nm). The chloroform layer containing I was treated with alkali at elevated temperature to give *s*-triazolo(3,4-*a*)phthalazine-2H-3-one (II). The fluorescence of II was measured at an emission wavelength 470 and excitation of 266 nm. There was a linear relationship between concentration of I and fluorometric response up to 10 μg/ml in plasma. This assay was sensitive enough to be useful for the determination of I and III and had a sensitivity limit of 0.2 and 0.07 μg/ml, respectively. Other antihypertensive agents and some metabolites had no detectable effect on the present assay. The method was applied to the determination of plasma levels in rats and dogs after oral or intravenous administration (3 or 10 mg/kg) of I.

Keywords——ecarazine hydrochloride; spectrofluorometry; rat plasma; dog plasma; antihypertensive drugs

Ecarazine hydrochloride (N_1-Carbethoxy-N_2-hydrazinophthalazine hydrochloride, I), a derivative of hydralazine, is well established as a therapeutic agent for the treatment of essential hypertention. In the previous paper,[1] I was found to be metabolized to some extent to hydralazine. In hydralazine therapy, systemic lupus erythematosus (S. L. E.) has been observed as a serious side effect, and a high plasma drug concentration was attributed

1) Part I: A. Ishii, T. Deguchi and H. Takahira, *Yakugaku Zasshi*, 93, 1383 (1973).
2) Location: *1188 Shimotogari, Nagaizumi machi, Sunto gun, Shizuoka, Japan.*