

Combinative therapeutic approach for better blood sugar level control in alloxan diabetic mice

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Abstract

In the present study the combined therapeutic approach of using an isolated compound from a herb and an oral hypoglycemic drug in alloxan-induced diabetic mice is investigated. Herbal drugs can have beneficial effects in terms of reducing the dosage, side effects and duration of action of synthetic drugs. The results show that combined therapy with 4-hydroxyisoleucine and pioglitazone is more beneficial than pioglitazone alone and also more beneficial than the combination of 4 hydroxyisoleucine and glyburide in the treatment of diabetes. (Int J Diabetes Metab 14: 104-105, 2006)

Key words: 4 hydroxyisoleucine, fenugreek, glyburide, pioglitazone

Introduction

Diabetes mellitus is a metabolic disorder treated by oral hypoglycemic agents such as sulphonylureas, biguanides, thiazolidinediones, meglitinide derivatives, and alpha glucosidase inhibitors.¹ Moreover, herbal powders are very popular among diabetic subjects. It is observed that many patients simultaneously use medicinal plant powders or extracts along with sulphonylureas. However, the standardization of powders and extracts cannot be carried out by the patient himself/herself and therefore fluctuations in the blood glucose level (BGL) are commonly observed. *Trigonella foenumgraecum* Linn. (fenugreek; family: Leguminosae) is known to possess hypoglycemic actions. Seeds of fenugreek are known to exhibit hypoglycemic activity when taken orally.² The general practice followed by Indians is to soak the seeds in water overnight and drink the water extract next morning. There is a paucity of data on the interaction of synthetic antidiabetics with isolated herbal ingredients. The objective was to study the interaction between 4 hydroxyisoleucine (4HI), glyburide (Gly) and pioglitazone (Pgz).

Materials and Methods

Animals

Swiss Albino mice (25 to 30g) were obtained from National Toxicological Centre, Pune. The animals were housed under standard condition of temperature (25 ± 2°C), 12h/12h light dark cycles and fed with standard pelleted diet (Chakan Oil Mills, Sangli). Water was given *ad libitum*. Animal handling was performed as per Good Laboratory Practice. The research proposal was approved by "The Institutional Animal Ethics Committee (IAEC)" of Poona College of Pharmacy.

Drugs

The oral antidiabetic drug Glyburide (Gly) was obtained as

gift from Ranbaxy Pvt. Ltd., New Delhi. Pioglitazone (Pgz) was obtained from Alembic Specia, India in the form of tablets of Piolem -15. Appropriate dilutions were made for preparation of a suitable dose (0.1 ml/10g of body weight) that was administered orally. Alloxan monohydrate was purchased from Spectrochem, India. 4HI was isolated from fenugreek seeds by column chromatography.

Induction of diabetes and estimation of Blood glucose

Diabetes was induced by intravenous administration of 70 mg/kg alloxan monohydrate solution through the tail vein. Blood was withdrawn by the retroorbital plexus technique (ROP) and blood glucose was estimated by the glucose oxidase peroxidase (GOD/POD) method using a kit purchased from Accurex Biomedicals Pvt. Ltd., (Mumbai, India).

Experimental design

Healthy mice were made diabetic by giving alloxan monohydrate (70 mg/kg) solution intravenously as described by Dunn and Letchie³. After 48 hours blood was withdrawn and blood glucose level was estimated. Animals with blood glucose of more than 200 mg/dl were called 'diabetic' and were selected for the study. Alloxan-induced diabetic mice were divided into 6 groups (n=6 in each group). Group I (Glucose, 2.5%), Group II (4HI, 40 mg/kg), Group III (Gly, 10 mg/kg), Group IV (4HI 40 mg/kg + Gly 10 mg/kg), Group V (Pgz 10 mg/kg), Group VI (4HI 40mg/kg + Pgz 10 mg/kg). An intraperitoneal glucose tolerance test (IPGTT) was carried out in overnight fasted diabetic animals to determine the glucose tolerance⁴. Blood was withdrawn at the start of the experiment. Half an hour after drug administration, glucose (2.5%) was administered to the animals by the intraperitoneal route. BGL was estimated at 0 min, 30, 60 and 120 min after glucose administration.

Results

In the group treated with 4HI + Gly, a significant (P<0.001) lowering of BGL at 30 min was seen when compared to the 4HI + Pgz group. At 60 min only 4HI showed a significant (P<0.05) decrease in BGL when compared to the glucose

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Table 1: Effect of 4HI and Pgz on blood glucose concentrations in alloxan induced diabetic mice after IPGTT

Groups	Treatment	Before glucose	After glucose administration			
			0 min	30 min	60 min	120 min
I.	Glucose (2.5%)	314.07 ± 28.98	446.64 ± 42.05	467.58 ± 10.39	415.28 ± 21.25	445.45 ± 9.4
II.	4HI	267.52 ± 9.11	331.93 ± 20.32	461.28 ± 9.53	321.36 ± 16.05 ^a	271.67 ± 34.27 ^b
III.	Gly (10 mg/kg)	310.04 ± 18.88	379.23 ± 19.02 ^a	400.60 ± 7.77 ^c	372.25 ± 16.31	283.00 ± 35.64 ^b
IV.	4HI(40 mg/kg) + Gly (10 mg/kg)	303.06 ± 29.85	531.19 ± 31.19	416.71 ± 12.08 ^{b,d}	412.42 ± 18.04	383.80 ± 37.96
V.	Pgz (10 mg/kg)	369.66 ± 23.71	421.92 ± 21.48	530.16 ± 3.27	507.25 ± 44.18	435.16 ± 24.67
VI.	4HI(40 mg/kg) + Pgz (10 mg/kg)	340.16 ± 22.36	419.51 ± 22.08	521.93 ± 16.36	455.79 ± 8.15	318.02 ± 39.1 ^{a,c}

Values are mean ± S.E.M, n=6 in each group; Statistical analysis by one – way ANOVA followed by Student – Newman – Keuls test; P values < 0.05^a, P < 0.01^b, P < 0.001^c compared to Group I and P < 0.001^d as compared to group VI, P < 0.05^e, when compared to group V. Glucose was administered intraperitoneally and other drugs were given orally. IPGTT = Intraperitoneal glucose tolerance Test

treated group but at 120 min, the 4HI + Pgz group showed a significant (P<0.05) decrease in BGL when compared to glucose and Pgz only groups. The change in BGL in the 4HI + Gly group was insignificant when compared to glyburide only group at 120 min.

Discussion

4HI is considered to be an insulin secretagogue.⁵ Glyburide, which is a second generation sulphonylurea, acts by stimulating the release of insulin from pancreatic beta cells⁶ and so development of tolerance and exhaustion of pancreas is commonly experienced by patients. Pioglitazone is an insulin sensitizer acting primarily on peroxisome proliferator activated receptor gamma (PPAR-γ).⁷ Unlike oral sulphonylureas, pioglitazone enhances tissue sensitivity to insulin rather than stimulating insulin secretion. The combination of 4HI and Pgz may result in increasing the sensitivity of insulin. Thus, the present work provides conclusive support for the combined treatment of 4HI with selective oral hypoglycaemics. The present investigation also showed that a synergistic effect of 4HI + Pgz is more effective than 4HI + Gly treatment. Pretreatment of the animals with 4HI + Gly and 4HI + Pgz for half an hour improved the glucose tolerance, decreasing the BGL in alloxan diabetic mice. The beneficial effect of 4HI + Gly at 30 min may be due to the release of insulin whereas delayed effect shown by 4HI + Pgz may be due to the increased sensitization of the receptor. It is thus apparent that combinative therapy with 4HI and pioglitazone would be more beneficial than pioglitazone alone.

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