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Effect of temperature on the potency & pharmacological action of insulin

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Background & objectives: **Since proper storage of insulin is necessary for its action, the present study was undertaken to determine the extent to which improper temperature storage conditions could have contributed to the potency of the three insulin formulations tested.**

Methods: **Two human insulin formulations (regular and biphasic) from three different manufacturers were stored at 5 different temperatures. *In vitro* potency of insulin was determined by high performance liquid chromatography on every seventh day for a period of 28 days. For the *in vivo* study, insulin tolerance test was done by injecting human regular insulin intraperitoneally to rabbits on the 25th day of storage. Blood glucose was determined at 0, 15, 30 and 60 min after insulin injection using glucometer.**

Results: **Storage at 32 and 37ºC showed 14-18 per cent decrease in potency of insulin in both the formulations on 28th day for all the three brands. Also the rabbits receiving insulin stored in 32 and 37ºC did not show a significant decrease in blood sugar level when compared to those receiving insulin stored at 5ºC.**

Interpretation & conclusions: **Improper storage of insulin decreases the potency and hence the pharmacological action of insulin. Patients should be educated on the proper methods of storage, and free supplies of insulin for more than two weeks use should not be dispensed.**

Key words Insulin - pharmacological action - potency

It is an established fact that labile drugs and vaccines show decrease in potency if not stored under controlled temperatures[1,2]. Insulin is one such labile drug, sensitive to extreme temperatures and sunlight and hence needs to be stored under refrigeration between 2-8°C[3]. Previous studies have shown that during storage and use, insulin is degraded by hydrolytic reactions or transformed to higher molecular weight components[4,5]. Hence it is recommended that insulin vials should be stored under refrigeration between 2-8°C when the vials are unopened and be protected from light[3,6].

However in a pilot study conducted at the Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, a 1050 bedded tertiary care teaching hospital, blood sugar level in 131 type 1 diabetic patients (73 males and 58 females with average age of 42.2 yr), showed that 59 per cent (77 of 131) were hyperglycaemic indicating that the glycaemic control was poor in these patients. Also 56 per cent (49 of 77) of these hyperglycaemic patients stored their insulin vials at room temperature. Though adequate cold storage was maintained in the hospital, the question

was, whether improper storage of the insulin by patients attending the diabetic clinic be one of the main factors contributing to poor glycaemic control in these patients? Also discrepancies exist in the storage recommendation and shelf-life for opened insulin vials, manufactured and marketed by the different drug companies in India. Novo Nordisk, Bangalore, in its package insert for human insulin recommends storage at room temperature upto 25°C for 4 wk but should not be kept in a refrigerator. Eli Lily, Gurgaon, recommends that the vials can be refrigerated or stored below 30°C for up to 4 wk, and according to Biocon, Bangalore, opened vials can be stored at room temperature up to 25°C for 6 wk.

In India, the temperature remains well above 25°C in many parts of the country, including Tamil Nadu and Puducherry for most of the year. Moreover, most of the insulin stability studies have been conducted in western countries where the ambient room temperature is usually within 25°C[7-9]. The extent to which these data can be extrapolated to the Indian environment is questionable. Hence we undertook this study to compare the potency of human insulin from three different manufacturers after storing at different temperatures and durations.

Material & Methods

The study was conducted in the Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India from February 2007 to November 2007. Two human insulin formulations namely regular and biphasic insulin (30/70) were purchased from the open market from three different manufacturers namely Novo Nordisk, Eli Lily and Biocon. Pure insulin powder which was used as reference standard was procured from Sigma-Aldrich Chemicals, USA. Sodium dihydrogen phosphate (RM 256 Extrapure) was obtained from Hi-media, Mumbai, India. Orthophosphoric acid (A.R Grade) and hydrochloric acid (HCL) (A.R.Grade) were obtained from S.D. Fine-chem Ltd., Poicha, India. Acetonitrile (HPLC grade) was obtained from Merk Limited, Mumbai, India. Acrodisc syringe filters of 0.45 µm and 25 mm diameter were obtained from Pall Life Sciences, USA.

The animal study was approved by the Institute Animal Ethics committee. New Zealand white rabbits of either sex weighing approximately 2 kg were used. The rabbits were maintained in a 12 h light-dark cycle and fed on normal diet and had free access to water.

Human insulin formulations (regular and biphasic) were stored at 5 different temperatures based on manufacturers recommendations and the way patients were storing insulin in their homes, *i.e.*, 5°C (refrigerator), 25°C (air conditioned room), 26°C (mud pot), 32°C (room temperature) and 37°C (room temperature in summer). Samples were withdrawn from these vials every 7 days for a period of 28 days and analyzed by HPLC. The duration of the study was fixed as 28 days, since this was the shelf-life of insulin once the vial is opened. Assuming that there will not be any significant difference in insulin potency (within batch variation) two vials from each batch were analyzed.

The HPLC method was performed as described in Indian Pharmacopoeia 1996[10]. The analysis was done using a Shimadzu HPLC system equipped with LC-10AD*vp* solvent delivery module, SPD-10A*vp* UV-VIS detector, a 200 µl injection loop, a SIL-HT*c* auto sampler and CTO-10AS*vp* column oven (Shimadzu Corporation, Japan). The column used was of stainless steel measuring 250 mm in length and 4.6 mm diameter packed with a stationary phase of octadecyl silane (ODS) of 5 µm particle size maintained at 45°C (Thermo Hypersil - Keystone, Thermo Fisher Scientific, USA). The detection wavelength was about 214 nm.

Regular insulin was linear over the range 1-10 units/ml (40-400 µg/ml), and got eluted at 10.8 min. The mean recovery of compounds was consistent, being 101 per cent for regular insulin and 82 per cent for regular insulin in biphasic insulin. The limit of quantification of insulin was 20 µg/ml. The intra- and inter-day coefficients of variation were less than 3.8 and 8.7 per cent respectively.

Pure insulin reference standard (0.4 mg) was dissolved in 5 ml of 0.025 M HCl to make up to 20 units/ml. From this serial dilution of 1, 5 and 10 units/ml was prepared and used as the calibration range. The working concentration was 5 units/ml. Standards were prepared freshly every 7th day.

For regular insulin formulation 0.2 ml of sample was added to 0.8 ml of 0.03 M HCl to make upto 5 units/ml, mixed well, allowed to stand for 1 h and passed through syringe filters and subjected to HPLC analysis. For regular insulin in biphasic insulin formulation, 1 ml of sample was added to 4 µl of 5M HCl, mixed well, allowed to stand for 1 h. From this, 0.2 ml of supernatant was mixed with 0.8 ml of 0.03 M HCl to make up to 5 units/ml and filtered using syringe filters and subjected to HPLC analysis. Potency of biphasic insulin was reported in terms of regular insulin.

Insulin tolerance test in rabbits: The rabbits were divided into 5 groups with 6-7 animals in each group. Each group received regular human insulin of three different brands stored at 5 different temperatures for 25 days. The vial was opened on the 25th day. Rabbits were deprived of food but not of water for 2 h before the start of the experiment. Insulin was injected at the dose of 0.75 units/kg body weight intraperitoneally[11]. The blood sugar was determined at 0, 15, 30 and 60 min from the ear vein using a glucometer[12] (Optium Xceed, Abbott Diabetes Care Inc., USA).

Statistical analysis: The concentration of insulin by the HPLC method was reported as mean. Indian pharmacoepia recommends a deviation of up to ± 10 per cent of the stated label claim of insulin and this was followed. Data were expressed as mean ± standard error of mean (SEM). Comparisons between groups was done using one way ANOVA, followed by the Dunnett's multiple comparisons test. $P<0.05$ was considered as significant. Statistical analysis was done using the GraphPad InStat version 3.00 statistical software (GraphPad Software Inc., San Diego, California, USA).

Results

All the three brands of insulin for both the formulations did not differ considerably in their insulin content during various sampling time and temperatures (pharmaceutically equivalent). There was no difference in concentration of insulin in vials stored at 25 and 26°C compared to those stored at 5°C on all five sampling days. There was a 14 and 18 per cent decrease in the concentration of regular insulin in vials stored at 32 and 37°C on day 28 for all the three brands. There was a 11 and 14 per cent decrease in the concentration of regular insulin in biphasic insulin formulation in vials stored at 32 and 37°C on day 28.

Insulin injection showed a considerable decrease in blood glucose levels with respect to basal glucose at 15, 30 and 60 min (Table). Animals injected with insulin from vials stored at 32°C and 37°C did not show a significant decrease in blood glucose when compared with those receiving insulin stored at 5°C for all three brands.

There was a significant increase in the area under the curve (AUC) when blood sugar was plotted against time, of rabbits treated with insulin from vials stored at 32°C and 37°C (Fig.) when compared with those receiving insulin that was stored at 5°C.

Discussion

Our results showed that storage at higher temperatures (32 and 37°C) decreased the potency of insulin in both formulations for all the three brands by 14 to 18 per cent. This value is higher than the cut-off recommended by the Indian Pharmacopoeia which is 10 per cent[10]. This decrease in the *in vitro* potency was further supported by the data from the *in vivo* study wherein there was a 9.5 to 14 per cent mean decrease in blood glucose levels in rabbits, injected with regular insulin of all the three brands, stored at temperatures higher than

Table. Mean blood sugar levels in rabbits after intraperitoneal administration of insulin stored at different temperatures for 24 days

Time (min)	Temperature (°C)				
	5	25	26	32	37
	Blood glucose (mg/dl)				
Novo Nordisk					
0	104.6 ± 1.5	105.3 ± 1.3	105.1 ± 1.1	107.6 ± 1.2	105.8 ± 1.4
15	77.6 ± 1.1	77.5 ± 1.0	77.6 ± 1.6	79.6 ± 1.8	80.8 ± 1.8
30	54.6 ± 1.2	54.6 ± 1.3	54.8 ± 1.1	56.8 ± 1.5	59.3 ± 1.1
60	53.5 ± 1.2	53.5 ± 1.2	53.6 ± 1.4	58.5 ± 0.8*	60.8 ± 1.3†
Eli Lily					
0	102.6 ± 1.5	102.6 ± 2.3	103.3 ± 1.5	102.5 ± 1.9	102.1 ± 1.9
15	78.6 ± 1.1	78.5 ± 1.2	78.6 ± 1.8	79.3 ± 1.1	80.3 ± 1.1
30	54.6 ± 1.6	54.8 ± 1.6	54.8 ± 1.5	58.1 ± 1.4	59.8 ± 1.3
60	55.1 ± 1.5	55.1 ± 1.3	55.0 ± 0.9	60.3 ± 1.2*	62.8 ± 1.2†
Biocon					
0	102.8 ± 1.0	103.3 ± 1.0	102.6 ± 1.9	104.1 ± 1.3	102.1 ± 1.4
15	78.6 ± 1.2	78.8 ± 1.0	78.8 ± 1.3	80.5 ± 1.6	81.3 ± 1.9
30	55.5 ± 1.5	55.5 ± 1.6	55.6 ± 1.2	57.8 ± 1.1	59.5 ± 1.1
60	54.0 ± 1.4	54.0 ± 1.2	54.6 ± 1.4	60.3 ± 1.1*	63.0 ± 1.1†

Values are means ± SEM (n = 6 to 7 rabbits per group); *$P<0.05$ and †$P<0.01$ when compared to rabbits injected with insulin stored at 5°C, using one way ANOVA, followed by Dunnett's multiple comparison test

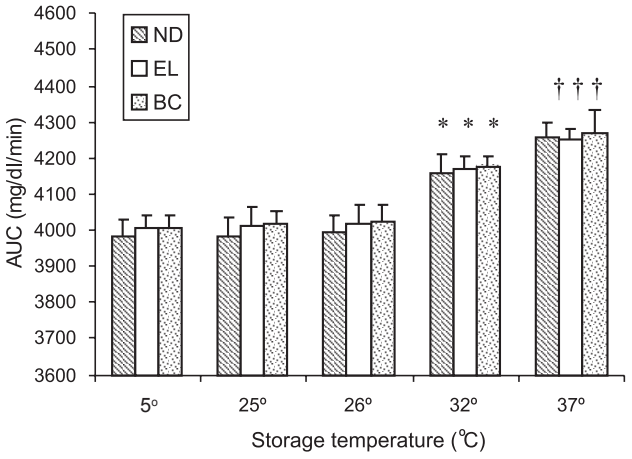

Fig. Area under curve (AUC) of blood sugar levels in rabbits after intraperitoneal administration of Novo Nordisk (ND) Eli Lily (EL) and Biocon insulin (BC) stored at different temperatures for 24 days. Values are mean ± SEM (n = 6-7 rabbits/group) **P*<0.05 and †*P*<0.01 when compared to AUC values of rabbits receiving insulin from vials placed at refrigerator (5ºC).

recommended as shown earlier[13,14]. The greater marginal fall in the *in vitro* potency compared to *in vivo* could be due to the pharmacologically active insulin degradation products, namely desamido insulin which itself has half the biological activity as insulin thus accounting for the greater *in vivo* biological activity[4].

Our results showed that the loss of potency at 32 and 37ºC (room) temperatures started after three weeks. Thus when storage cannot be assured at cool temperatures, insulin vials may be used only for 2 wk.

Both *in vitro* and *in vivo* studies showed no significant loss of potency of insulin in vials stored at 25 and 26˚C by the end of four weeks. Thus the insulin in vials stored in mudpot (26˚C) retained the potency or biological activity supporting the previous studies[15,16].

To conclude, our study has shown that insulin stored at high temperatures lose its potency and biological activity. Thus, storage in refrigerator is the ideal method of storage of insulin vials. However, when adequate storage cannot be assured at cool temperatures, insulin vials may be used within two weeks of opening. Diabetic patients need to be educated properly about the temperature and duration of storage of insulin vials[17] to maintain adequate glycaemic control.

Conflict of interest: None.

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