

Research Article



Pharmaceutical and Biochemical Studies on Fast Dissolving Tablets of Metformin Hydrochloride for the Purpose of Emergency Cases in Patients with Type II Diabetes Mellitus

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ABSTRACT

Although metformin hydrochloride (MH) has a high aqueous solubility but depicts poor bioavailability, however, formulation of lyophilized tablet (LT) is eventually very important especially in emergency cases in Type 2 diabetes mellitus to obtain a rapid onset of action by the virtue of high bioavailability, moreover avoidance to large extent the hepatic effect. This was achieved by developing a metformin hydrochloride (MH) lyophilized tablet (LT) which dissolves-rapidly in the mouth, therefore, needing not be swallowed. The LT was prepared by dissolving the drug in an aqueous solution of highly water-soluble carrier materials consisting of gelatin, glycine, and sorbitol. The mixture was dosed into the pockets of blister packs and then was subjected to freezing and lyophilization. Biochemical test was carried out using 20 volunteers to compare glucose reducing capacity of both LT and commercial conventional formula (CCF). The dissolution characteristics of MH from the LT were investigated and compared to the plain drug, the physical mixture (PM) and commercial conventional formula (CCF). Results obtained from dissolution studies showed that LT significantly improved the dissolution rate of the drug compared to the plain drug, PM and CCF. More than 98% of MH in LT was dissolved within 2 min compared to only 95%, 90% and 30% for PM, plain drug and CCF dissolved during 10 min. Initial dissolution rate of MH in LT was almost three fold higher than MH powder alone during 2 min. Crystalline state evaluation of MH in LT was conducted through infrared spectroscopy (FTIR), x-ray powder diffraction (XRPD), and scanning electron microscope (SEM) to denote eventual solid state during the process. There was a significant reduction in blood glucose in diabetic volunteers in the first four hours postprandial. These promising results obtained with metformin hydrochloride LT may be a guide for controlling blood glucose level (BGL) especially in emergency cases in small doses.

Keywords: Metformin hydrochloride, lyophilized tablets, dissolution rate, scanning electron microscope, glucose blood level.

INTRODUCTION

Type 2 Diabetes mellitus (T2DM) is characterized by an increase in blood glucose concentration due to resistance of insulin action. High blood glucose (hyperglycemia) is the main pathogenic factor for the development of diabetic complications including coronary heart disease, retinopathy, nephropathy, and neuropathy^{1,2}. In addition, chronic hyperglycemia leads to progressive impairment of insulin secretion and to insulin resistance of peripheral tissues which is known as glucose toxicity³⁻⁶. Treatment of diabetes has been mainly focused on maintaining normal blood glucose levels by using either insulin or oral hypoglycemic agents (OHAs)⁷. The mechanism of action of the anti-diabetic agents used for the treatment of type 2 diabetes, include increasing insulin release, increasing insulin sensitivity, controlling hepatic glucose release or inhibiting intestinal glucose absorption⁸. Often, therapy with insulin and OHAs become less effective in controlling hyperglycemia, particularly as a result of weight gain, worsening insulin resistance and progressive failure of insulin secretion due to glucose toxicity. Insulin therapy alone or with hypoglycemic agents can produce weight gain due to reducing glucose excretion⁹⁻¹¹. Among commonly used OHAs, thiazolidinediones and sulphonylurea contribute to weight gain, whereas metformin causes weight loss and dipeptidyl peptidase-4 inhibitors are weight neutral¹². Overall, there is a need for novel agents which can effectively control blood sugar level without producing

weight gain or Hypoglycemia. World Health Organization estimate for the number of people with diabetes worldwide, in 2000, is 171 million, which is likely to be at least 366 million by 2030¹³. Non-insulin dependent (Type 2) diabetes mellitus is a heterogeneous disorder characterized by an underlying insufficiency of insulin. This insufficiency results from defective insulin utilization and can be corrected by administration of one or more of the currently available oral hypoglycemic agents¹⁴. Combination therapy have various advantages over monotherapy such as problem of dose-dependent side effects is minimized, a low dose combination of two different agents reduces the dose-related risk, the addition of one agent may counteract some deleterious effects of the other, using low dosage of two different agents minimize the clinical and metabolic effects that occur with maximal dosage of individual component of the combined tablet¹⁵. Metformin is an oral biguanidine first-line choice of drug. Metformin has an oral bioavailability of 50–60% under fasting conditions, and is absorbed slowly, having short biological half life of 1.5-4.5 h¹⁶. However, frequent dosing schedule and risk of gastrointestinal symptoms makes its dose optimization complicated. Peak plasma concentrations (Cmax) are reached within 4 to 8 hours with extended-release formulations¹⁷. Type-2 diabetes mellitus is a chronic progressive disorder characterized by defective insulin secretion and increased insulin resistance. It is widely accepted that it required intense and tight glycaemic



control to prevent several cardiovascular complications. Metformin hydrochloride is an orally administered biguanide, widely used in the management of type-2 diabetes, a common disease that combines defects of both insulin secretion and insulin action¹⁸.

The present study was aimed to formulate flash tablets of Metformin HCl using glycine and sorbitol to obtain a rapidly dissolved LT and absorbed from mouth aiming to reduce the high BGL in a short onset.

MATERIALS AND METHODS

Materials

Metformin HCl, gelatine, glycine, and sorbitol, were purchased from Sigma Chemical Co., St. Louis. All water used was distilled and de-ionized. All other chemicals were of reagent grade and used as received.

Preparation of fast dissolving tablets by lyophilization technique (LT)

A 2% w/v solution of gelatin in water was prepared by first soaking the gelatin in water until complete hydration. The hydrated gelatin was stirred using a magnetic stirrer until a clear solution was obtained. Different weights of glycine and sorbitol were added to the gelatin solution while stirring until completely dissolved. Glycine (used to prevent shrinkage of the tablet during manufacturing) and sorbitol (used to impart crystallinity, hardness, and elegance to the tablet) are well-known and acceptable materials used in preparing freeze-dried tablets. The percentage excipient used was optimized during the formulation process to result in a strong and elegant tablet that could be handled with ease. An accurately weighed amount of MH powder (10 % w/v) was then dissolved in the aqueous solution of gelatin, glycine, and sorbitol. One milliliter of the resulting suspension was poured into each of the pockets of a tablet blister pack to result in MH dose of 100 mg in each tablet. The tablet blister packs, each containing 10 tablets, were then transferred to a freezer at -22°C and kept in the freezer for 24 h. The frozen tablets were placed in a lyophilizer for 24 h using a Novalyphe-NL 500 Freeze Dryer (Savant Instruments, Holbrook, NY) with a condenser temperature of -52°C and a pressure of 7×10^{-2} mbar. The LTs were kept in desiccators over calcium chloride (0% relative humidity) at room temperature until further used. Four blister packs containing a total of 40 tablets were produced in each run. Eight randomly selected tablets (two from each pack) were assayed for drug content uniformity. The mean % drug content was found to be $97.88\% \pm 1.40$. The quantitative amounts of ingredients used in the preparation of LT are tabulated in table 1.

Preparation of physical mixture

MH was uniformly mixed with gelatin, glycine and sorbitol in the same percentage used in the lyophilized tablet (LT) using a mortar and pestle. The prepared mixtures were kept in a desiccator until used.

Table 1: Qualitative amounts of the ingredients used in the preparation of LT

Ingredients	%w/v
Metformin HCL	10
Gelatin	2.5
Sorbitol	1, 1.5, 2
Glycine	1,1,1

Infrared analysis (FTIR)

The x-ray diffraction patterns of MH and LT were performed in infrared spectrophotometer (Gensis II, Mattson, (England). Radiation was provided by a copper target (Cu anode 2000W:1.5418 high intensity x-ray tube operated at 40 KV and 35MA). The monochromator was a curved single crystal (one PW1752/00). Divergence slit and receiving slit were 1 and 0.1 respectively. The scanning speed of geniometry (PW/050/81) USED WAS 0.02.20/5 and the instrument were combined with a Philips PM8210 printing recorder with both analogue.

X-ray powder diffraction analysis (XRPD)

X-ray diffraction experiments were performed in a Scintag x-ray diffractometer (USA) using Cu K α radiation with a nickel filter, a voltage of 45 kV, and a current of 40 mA. Diffraction patterns for MH and lyophilized tablet (LT) were obtained.

Scanning electron microscopic analysis (SEM)

Surface morphology of MH and lyophilized tablet LT, was examined by SEM (Jeol JSM-6400, Tokyo, Japan). Photograph was taken at magnification of 1200.

Dissolution study (DS)

The dissolution of physical mixture (PM), LT and commercial conventional formula (CCF) compared to the plain drug, were determined using dissolution tester (VK 7000 Dissolution Testing Station, Vankel Industries, Inc., NJ) following the USP paddle method. All tests were conducted in 900 mL of distilled water maintained at $37 \pm 0.5^{\circ}\text{C}$ with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 100 mg. After specified time intervals, samples of dissolution medium were withdrawn, filtered, and assayed for drug content Spectrophotometrically at 232 nm after appropriate dilution with water. The experiment was carried out in triplicate.

Study subjects

The study was conducted according to the Helsinki Declaration and Resolution 8430 of 1993 by the Ministry of Social Protection. It was also approved by the Ethics Committee of the School of Medicine, at Universidad de Antioquia. 20 adult male were recruited for this study; ages 25.2 ± 1.6 years; weight 68.5 ± 5.2 kg; height 1.65 ± 0.06 m. Volunteers were divided into four groups; healthy group and three type 2 diabetes mellitus groups after having been medically examined and clinically tested:

complete blood count, urinalysis, blood biochemistry were normal (with the exception of diabetic volunteers), and HIV, hepatitis B free. All volunteers were briefed on the study details and they all agreed and signed a written informed consent. All volunteers were free to leave the study at any time.

Study design

The study was carried out to compare the capacity of LT and CCF to lower the high BGL following administration of single doses of 100 mg (LT) and 500 mg (CCF). All volunteers were prohibited from taking medicines and smoking for 3 days before the beginning of the studies to the end of the test. All subjects were fasted for at least 9 hrs before the study day. The obtained data of first group (healthy group) were used as a reference. The other three groups treated or non treated with LT and CCF in a cross over technique. All volunteers took a breakfast (a loaf of bread of high carbohydrate content, two cucumbers, cheese of high fat content and one orange). Postprandial, LT were administered to be absorbed from mouth and CCF were ingested with 200 ml of water. The volunteers were kept without any effort to the end of the test. No food was allowed for eight hours after dosing. Blood specks (one drop) were taken from the thumb using injection pen at the following time points: 0 (pre-dose), 1h, 2h, 3h, 4h, 5h, 6h, 7h, 8h after administration. The blood specks were analyzed using ACCU-CHEK Active device (fig 1).

Test working

The blood specks withdrawn from thumb were applied to the test area on the test strip. This area is containing sensitive chemicals. When blood is applied to the test area, a chemical reaction takes place (glucose dye oxidoreductase mediator reaction, synonym: PQQ-dependent glucose dehydrogenase mediator reaction), causing the color of the test area to change. The meter registers this color change and converts the signal obtained into a blood glucose result in mg/dl. The test was carried out at ambient temperature.

Statistical analysis

In order to assess the effects of treatment, period and sequence of administration, analysis of variance (ANOVA) for the cross design (Statistica 6.0, Statsoft Inc, 2001) was carried out. A p value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

In table 1, the formulation containing glycine/sorbitol in a ratio of (1:1) depicted higher drug content compared to the ratio of (1:1.5 & 1:2), good formed tablets and the best elegance, therefore ratio (1:1) was selected to proceed the study.

Infrared analysis

Figure (2) depicted IR spectra of MH(a), PM(b) and LT(c). The IR spectra revealed no change in both functional

group and fingerprint regions of drug, PM and LT. This indicated that there is no change in both chemical and physical properties of the drug after formulation with the tested excipients.

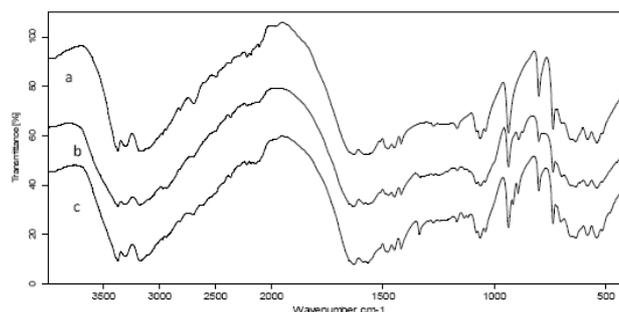


Figure 1: FTIR of plain MH(a), PM(b) and LT(c)

X-ray powder diffraction analysis (XRPD)

Figure (2) depicted the x-ray diffraction pattern of LT. The diffraction pattern of the LT showed absence, broadening, and reduction of major MH diffraction peaks indicating that mostly an amorphous form (disordered state) existed in LT. These results could explain the observed enhancement of rapid dissolution of MH in LT.

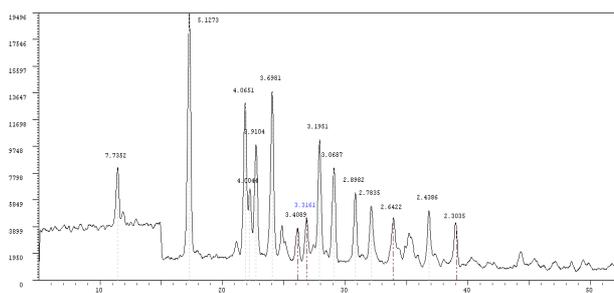


Figure 2a: XRPD of plain drug

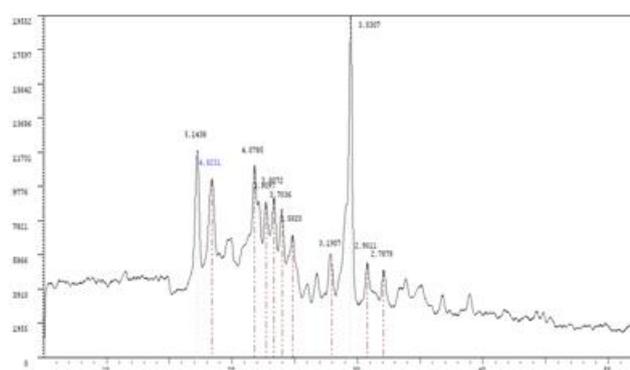


Figure 2b: XRPD of LT

Scanning Electron Microscopy (SEM)

Figure (3) depicted SEM micrographs of LT. The results showed that the drug has crystals of different shapes (feather, rod, finger). Whereas, the micrographs of LT revealed a lattice shape matrix. This could therefore indicate that MH particle size has been reduced as result of disappearance of characteristic crystal shapes of the drug. This will accelerate dissolution.

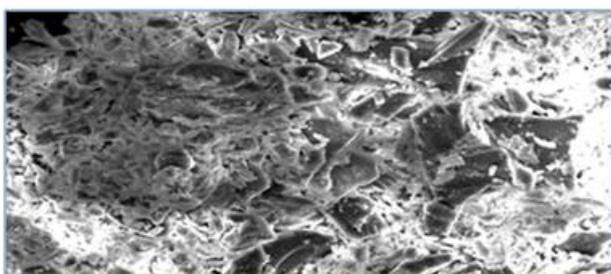


Figure 3a: SEM of plain drug

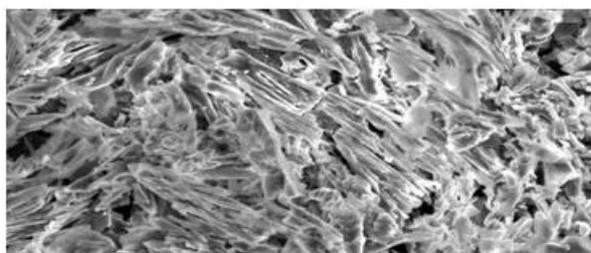


Figure 3b: SEM of LT

Dissolution Study (DS)

The dissolution profiles of PM, LT, and MH powder alone in distilled water at 37°C are shown in Figure(4) MH in the LT was immediately dispersed and almost completely dissolved in 3 min (99.88%). Initial dissolution rate of MH in the LT increased markedly (about two fold) compared to MH powder alone whereas the dissolution rate of MH in, PM (60.45%) was (about 1.5 fold) the dissolution rate of MH powder. The dissolution rate of LT were about 9.5 times the dissolution rate of CCF.

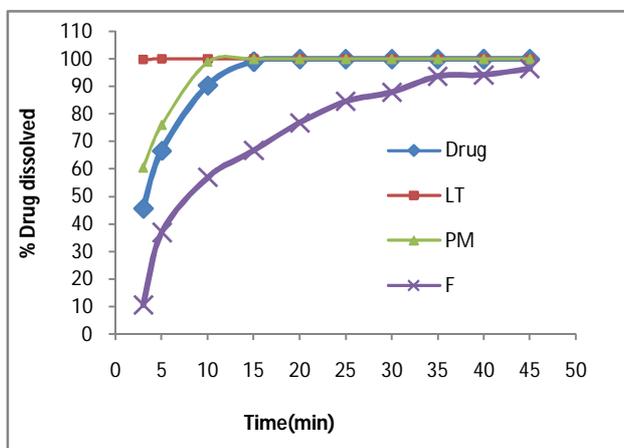


Figure 4: Dissolution pattern of MH in LT, PM, CCF and plain MH in distilled water at 37 ± 0.5 °C.

Biochemical studies

Figure 5 depicted the behavior of BGLs with and without administration of LT and CCF. It was clear that LT reduced BGL to great extent especially at the first four hours compared to CCF and non treated trial, whereas, these results were comparable with those of reference group (healthy group). This may be due to rapid absorption of MH eventually from mouth, therefore depicts a rapid onset of action and hence obvious reduction in BGL even in a smaller dose (100mg) hence normal BGL level was reached after three hours. The unfair lowering effect of

CCF (500mg) on BGL may due to its poor bioavailability (50%) resulting from hepatic effect and other physiological factors present in GIT which retard drug absorption. All these factors delayed its onset of action and brought the blood glucose at its normal level approximately after six hours. Fig 9 showed BGLs chart to give an obvious view that LT tablets gave an excellent results comparable with those of healthy group. This may recommend LT to be used in emergency cases in severe hyperglycemic patients of type 2 diabetes mellitus.

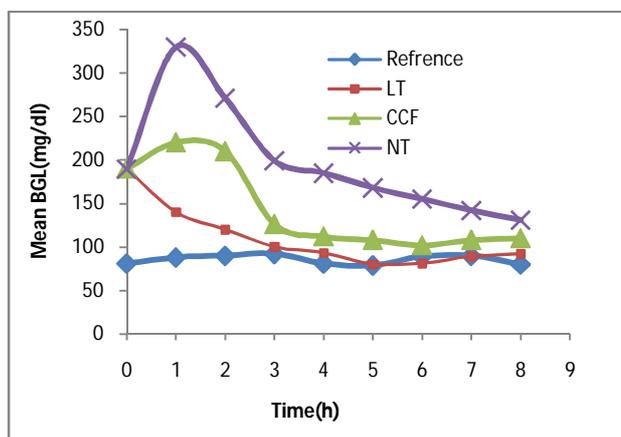


Figure 5: Profile of blood glucose levels before and after administration of LT and CCF formulations at ambient temperature (n=20 with SD); NT: not treated.

Statistical analysis

Analysis of ANOVA depicted that, there is a significant difference (p<0.05) between results of LT and those of commercial conventional formula (CCF) and not treated trial (NT).

Table 2: Analysis of ANOVA (LT vs CCF and NT)

Source	DF	Sum of Squares	Mean Squares	F-Ratio	Prop>F
Column 1	2	34948.963	17474.5	6.6849	0.0049
Error	24	62736.667	2614	-	-
C.Total	26	97685.63	-	-	-

CONCLUSION

We demonstrated that a lyophilized MH tablet made of widely used, safe, water-soluble excipients which are feasible for obtaining a rapid dissolution of MH. We suggest that the prepared MH lyophilized tablet (LT) might have a higher oral bioavailability due to expected flash dissolution in the saliva and avoidance of the hepatic effect than standard dosage forms; therefore, it would be possible to formulate MH in lyophilized tablets having an eventual decreased therapeutic dose resulting in reduced side-effects encountered with MH therapy such as gastrointestinal disturbance. The biochemical study proved the efficiency of LT in lowering the high BGL in tested volunteer.

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