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In vitro and *In vivo* Studies of Drug-Drug Interaction between Metformin and Cefepime

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Abstract

A diabetic individual gradually becomes more prone to infections and can be prescribed Metformin concurrently with a broad spectrum antibiotic like Cefepime. Our research examines the possibility of drug-drug interaction between Metformin and Cefepime in a number of *in vitro* and *in vivo* parameters. The *in vitro* tests including Differential Scanning Calorimeter, Scanning Electron Microscope and Fourier Transform Infra Red analysis were found to reveal visible changes in melting points, morphological structures and rearrangement of functional groups due to the interaction. The disc diffusion method was used to compare the antimicrobial properties of all the test samples and the antimicrobial potential of Cefepime was found to be suppressed moderately after in vitro interaction with Metformin. The alteration of the in vivo antidiabetic activity of Metformin was evaluated in streptozotocin-induced Long-Evans Rats, and the anti-hyperglycemic effect of Metformin was detected to decrease significantly in the resulting product of this interaction.

Keywords: Drug-drug interaction; Metformin; Cefepime; Type II diabetes; Physicochemical properties of drugs; Therapeutic activity suppression

Introduction

Administration of more than one drug at the same time might be intended for treating single or multiple pathological conditions. Every therapeutic agent triggers a particular or a number of specific pharmacologic responses to its recipients. If a second drug is given concurrently or prior to or immediately after the first one, the clinical consequences of the first drug can be altered. This phenomenon is termed as drug-drug interaction (DDI) [1]. The effects of drug-drug interactions can lead to serious adverse drug reactions (ADR). Two millions of adverse drug-drug interactions have been reported in USA previously, among which 26% were avoidable [2,3]. In many cases, combined actions of several drugs might be beneficial also. Combination therapies are also used to alleviate each other's adverse effects [4]. At present time, numerous researches concerning drug-drug interaction and successful application of novel drug delivery methods have already been conducted to overcome the unwanted results. Use of biodegradable polymers and interpenetrating polymeric networks are increasing day by day to formulate controlled released and sustained released forms of a variety of therapeutic agents to avoid drug related adverse events [5-7].

Concurrent use of multiple drugs has been found to be more frequent in treating chronic non-curable illness including diabetes mellitus, hypertension and organ transplantation [8]. More than 300 million people are suspected to develop diabetes within 2025, and around 90-95% of the affected people may be recognized with type II diabetes [9]. In third world developing countries, diabetes has already been found to cause severe health complications at an epidemic level. The situation is at no contrast for the developed countries as well [10]. The more terrifying news is that, alarming percentages of children and adolescents have been identified with type II diabetes [11]. Evaluation of pharmacological activities and drug delivery methods of conventional medications in long term diabetes management is still under consideration. For example, administration of Insulin via oral route along with the efficacy and challenges of this method have been studied worldwide to increase patient compliance [12]. Simultaneously, search for novel bioactive molecules against type II diabetes are also ongoing in both synthetic and natural product chemistry.

Type II diabetes is an Insulin-Independent Diabetes Mellitus (IDDM) and is a non-curable endocrine disorder where the diabetic individual fails to produce sufficient insulin to maintain optimum glycemic control [13]. Metformin is the most clinically recognized first-line treatment option to manage this critical condition. In addition to glucose lowering activity, it augments in weight reduction, lipid profile lowering and endothelial function enhancement. It is also prescribed as a blood glucose lowering agent even prior to developing persistent hyperglycemia. Besides, Metformin is also widely prescribed because of its antidiabetic, antioxidant, anti-tumorigenic and anticancer potentials [14-17]. Thus, a patient taking Metformin can be prescribed concurrently with a number of other therapeutic agents for considering different pathological conditions.

Diabetic patients gradually become immuno-suppressed and suffer from a number of complications leading by diabetes. Diabetic patients are more susceptible to microbial infections, especially to skin infection, urinary tract infections (UTIs) and foot infections [18-20]. Those infections are usually treated with various antibiotics. Antimicrobial resistance is the natural consequence of repetitive and overuse of antibiotics. Fourth generation Cephalosporin (Cefepime) is becoming popular because of this increasing resistance rate.

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Cefepime showed greater potency and improved spectrum of activity against many Gram-positive, multi-resistant Gram-negative and beta-lactamase producing organisms [21,22]. Therefore, it might be inferred that co-administration of Cefepime and Metformin is a probable event, and their effects of interaction should be evaluated to avoid undesired outcomes.

If Metformin and Cefepime interact and alter each other's efficacy or concentration at the target sight, the patient can have serious clinical manifestations. To illuminate the results of interaction between Metformin and Cefepime, the *in vitro* examinations were performed. The *in vitro* tests were followed by the *in vivo* evaluation for assuming probable consequences of interaction.

Materials and Methods

Materials

Metformin HCl (99.25%) was obtained from Amico Laboratories Ltd. and Cefepime HCl (99.05%) was obtained from Incepta Pharmaceuticals Ltd., Bangladesh. HPLC grade methanol was purchased from Active Fine Chemicals Ltd., Dhaka, Bangladesh; Chloroform and Potassium bromide (KBr) were purchased from Sigma Aldrich, USA. The microorganism suspension, filter papers and saline water were collected from the Biomedical Research Laboratory, University of Dhaka. The standard Kanamycin disc (30 µgm/disc) and nutrient agar media used for disc diffusion method were of Oxoid Ltd., Basingstoke, Hampshire, England and Merck, India, respectively.

Instrumentation

Differential Scanning Calorimeter (DSC) (DSC-60 WS, Shimadzu, Japan) was used for performing DSC analysis. Scanning Electron Microscope (SEM) (JEOL, JSM-6490LA, USA) and Fourier Transform Infra Red (FT-IR) Spectrophotometer (FT-IR 8400S, Shimadzu, Japan) were used to investigate surface topography changes and functional groups alterations, respectively. Electronic analytical balance (AND GH-200, Japan) was used for completing both DSC and FT-IR experiments. An autoclave (model RAU-530, Rexmed Industries Co. Ltd., Taiwan) and incubator (BD 53, Binder, Germany) were used for the disc diffusion method. The blood glucose levels of Rats were determined with the help of Insulin ELISA kit (Linco research, Inc. USA) and centrifugation machine (Z 36 HK, Hermle Labortechnik Gmbh, Germany).

Preparation of mixture MC-1

Equivalent weights to 50 mg each of Metformin and Cefepime were taken and mixed properly in a 10 ml beaker. Few drops of methanol were added to the mixture as a liquid vehicle for interaction and the mixture was allowed to evaporate overnight at room temperature. After two days, methanol was evaporated completely, and the solid residue was scratched with spatula and collected as a solid homogeneous 1:1 mixture (MC-1) of Metformin and Cefepime. The procedure was repeated for preparing mixture MC-1, as per the research requirements.

DSC analysis

The required weights (within a range of 2-5 mg) of Metformin, Cefepime and MC-1 were taken in sealed aluminum pans. The temperature range of DSC runs for this test was 0°C to 350°C with the rate of increasing temperature at 10°C/minute. The total experiment was performed under the flow of nitrogen gas at 20 ml/minute.

Scanning Electron Microscope (SEM) analysis

For SEM analysis, the most common mode of detection (by the emission of secondary electrons) was used. All the samples (Metformin, Cefepime and MC-1) were coated with thin platinum layers for performing the test.

Fourier Transform Infra Red (FT-IR) analysis

An appropriate quantity of KBr was mixed separately with Metformin, Cefepime and MC-1 at a ratio of 100:1, and ground in an agate mortar to prepare pellets (~100 mg) for the IR investigation. The range for recording the spectra was fixed at 4000-400 cm^{-1}, and the resolution was 2 cm^{-1}.

Determination of antimicrobial activity of MC-1

The conventional disc diffusion method was used for evaluating antimicrobial activity. All the experiments were carried out under clean bench. Inoculum suspension in nutrient agar media was prepared with reference microorganisms by proper incubation. The inoculum suspension was transferred in petridishes and allowed to solidify. Solutions of desired concentrations of Metformin, Cefepime, MC-1 and blank were prepared by dissolving in chloroform. Filter paper discs were impregnated with test samples solutions and allowed sometimes to evaporate the solvent. Impregnated discs along with the standard (Kanamycin 30 µg/disc) were placed in close contact of the petridishes and incubated for 24 hours at 37°C. Zone of inhibition were measured in millimeter unit [23].

Test animal selection and categorization

Streptozotocin (STZ)-induced diabetic Long-Evans Rats were collected from the animal house of North South University (NSU), Bangladesh. The selected rats were induced diabetes by administering subcutaneous injection of STZ at the dose 100mg/kg body weight after their postnatal day to induce mild type II diabetes [24]. The blood glucose levels were monitored after the intervals of 0 day, 7 days, 14 days, 20 days and 60 days to ensure the induction of type II diabetes. All the rats used in present experiment were male, weighing 150-200 g and two months old. In the previous researches, STZ-induced diabetic models have been found to resemble various stages of type II diabetes mellitus like impaired glucose tolerance, persistent hyperglycemia and insulin resistance. So, STZ-induced diabetic models can be considered to have advantages over other animal models for studying type II diabetes [25].

Experiment

Collected rats were divided into three groups as A, B and C, each group containing six rats. All the three groups were treated with respective test samples. The blood glucose levels of those rats were determined with the help of insulin-ELISA kit [26], both after 0 minute and 60 minutes of the test samples administration.

Group A:	This group was considered as the control group. Only saline water (0.9% sodium chloride) was given orally with the help of a feeding needle.
Group B:	This group was considered as the standard group. An oral solution of Metformin (50 mg/kg body weight) dissolved in saline water (0.9% sodium chloride) was given with the help of a feeding needle.
Group C:	This group was provided an oral solution of MC-1 (100 mg/kg body weight) dissolved in saline water (0.9% sodium chloride) with the help of a feeding needle.

Collection of plasma from test animals

About two milliliter blood was drawn from tail vein of the rats and centrifuged for 10 minutes at 4000 rpm. After the centrifugation, the supernatant plasma was collected and preserved at -20˚C until analysis.

Statistical analysis

The blood glucose levels were expressed as Mean ± Standard Deviation (SD). For the calculating mean, SD and 't-test' SPSS software (IBM corporation, USA) was used. The 'p value' obtained from 't-test' was used to indicate the degree of difference in glucose lowering capacity among various groups. The 'p value' was considered significant for $P<0.05$.

Results and Discussion

Evaluation of alteration in physicochemical properties through DSC

DSC is a thermoanalytical technique which gives definite thermograms. The thermogram is considered as a representative of melting point and primary physical attribute of the molecule [27]. Thermograms of Metformin and Cefepime showed sharp melting endotherm at 229°C and 182.92°C, respectively but the thermogram of their mixture MC-1 showed no melting endothermic revealing peak up to 350°C, i.e. no melting endotherm neither at 182.92°C nor 229°C. It was an obvious indication to the formation of a new complex. The complex was evaluated both immediately after preparation (blue curve, Figure 1) and after a particular time period (black curve, Figure 1).

Surface topography analysis by Scanning Electron Microscope (SEM)

SEM study has been used successfully for analyzing their surface topography in various molecules or microspores and the subsequent changes in surface topography due to the interaction [28]. In SEM showed distinct morphological structures of Metformin and Cefepime. Both of the compounds did not have any crystals of definite structure. Metformin molecules are comparatively oval and have smooth edges. They can be shaped in different sizes probably due to lamp formation because of the hygroscopic nature of Metformin. The Cefepime crystals are also of irregular shapes. The mixture MC-1 molecules were irregular crystals having sharp edges. Lamp formation was also observed in the case of MC-1. Subtle portions were seen that may be the separate particles of MC-1. These changes in surface topography might have been the results of the interaction.

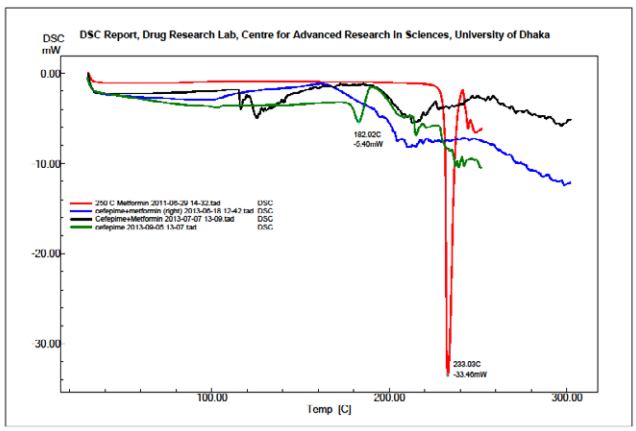

Figure 1: Thermograms of Metformin, Cefepime and MC-1.

Detection of alterations in functional groups through FT-IR

In FT-IR Spectra, several peaks are obtained in specific frequency ranges and every frequency range stands for different functional groups or various vibrational bands [29].

The present experiment was concerned with the interaction of Metformin hydrochloride and Cefepime hydrochloride. The FT-IR spectra of Metformin, Cefepime and MC-1 showed the mode of interaction. Metformin HCl molecule was observed moderate to strong FT-IR peaks for N-H stretching at 3392 cm^{-1}, $(CH_3)_2N$ absorption at 2814 cm^{-1}, N-H deformation at 1477 cm^{-1}, C-N stretching at 1167 $cm^{-1}$and C-N-C deformation at 580 cm^{-1}. The Cefepime HCl defining strong vibrational bands were found for O-H stretching at 2936 cm^{-1}, β-lactam C=O stretching at 1774 cm^{-1}, amide C=O stretching at 1656 $cm^{-1}$and carboxylic C=O stretching at 1425 cm^{-1}. For the resulting complex MC-1, the peaks for methylene vibration or $(CH_3)_2N$ absorption at 2814 cm^{-1}, O-H stretching at 2936 cm^{-1} and amide C=O stretching at 1656 cm^{-1} were abolished. In addition, no other peak was obtained between 2960-2850 cm^{-1} and 1680-1630 cm^{-1} which were the frequency ranges for methylene vibration of Metformin and amide C=O stretching of Cefepime molecule, respectively.

After FT-IR analysis, it was evident that Metformin and Cefepime interacted with each other by displacing the methyl, hydroxyl and amide groups present in their molecules. Therefore, the formation of intermolecular hydrogen bonding (either stable or intermediate) might be predicted as one of the probable reason for interaction. Though the nuclei of these two therapeutic agents seemed to be retained outside, the pharmacological activity as well as the interaction results can be altered considerably inside the body.

Evaluation of antimicrobial activity

Cefepime is a potent broad spectrum antibiotic.As a result of drug-drug interaction, Cefepime can completely or partially lose its antimicrobial activity. In disc diffusion method, the following zones of inhibition were obtained. Table 1

Kanamycin and Cefepime both are potent broad spectrum antimicrobials. Metformin is an oral hypoglycemic agent, with no antimicrobial property. From the *in vitro* antimicrobial testing, Cefepime was found to show an antimicrobial activity almost resembling the standard in a concentration of 100 µg/disc. The test sample (MC-1) was tested in three different concentrations of 100 µg/disc, 200 µg/disc and 300 µg/disc. In the case of MC-1 at the dose of 100 µg/disc, the zones of inhibition were significantly lesser. As MC-1 was prepared by mixing both of the drugs at a 1:1 ratio of their weights, 100 µg/disc MC-1 may contain lesser amount of Cefepime which ultimately showed smaller zone of inhibition. When the concentration of MC-1 disc was raised three times the concentration of Cefepime, the zones of inhibition for many of the microorganisms became moderate or little lesser than the zones of inhibition for Cefepime. In these cases, amount of Cefepime were supposed to be equal or greater than the discs containing Cefepime alone. It can be inferred from the results that, the antimicrobial susceptibility to MC-1 was seen because of the existing β-lactam rings and the moderate declining in antimicrobial activity of MC-1 were observed due to interaction with Metformin.

Determination of blood glucose levels after test samples administration

The glucose lowering activity of Metformin in type II diabetic

Test microorganisms	Diameter of zone of inhibition (mm)					
	Kanamycin 30 µg/disc	Metformin 100 µg/disc	Cefepime 100 µg/disc	MC-1 100 µg/disc	MC-1 200 µg/disc	MC-1 300 µg/disc
Gram positive bacteria						
Bacillus cereus	35	-	22	14	14	20
Bacillus megaterium	35	-	30	15	17	18
Bacillus subtilis	37	-	32	15	21	26
Staphylococcus aureus	32	-	23	14	17	16
Sarcinalutea	34	-	28	18	22	24
Gram negative bacteria						
Escherichia coli	34	-	33	17	20	23
Pseudomonas aeruginosa	37	-	27	11	14	17
Shigellaboydii	38	-	27	18	22	27
Vibrio mimicus	37	-	26	13	15	19
Vibrio parahemolyticus	36	-	23	08	09	08
Fungi						
Aspergillus niger	35	-	28	18	19	23
Sacharomyces cerevacae	35	-	27	16	21	22

Table 1: Alterations in antimicrobial activity of Cefepime due to complexation with Metformin.

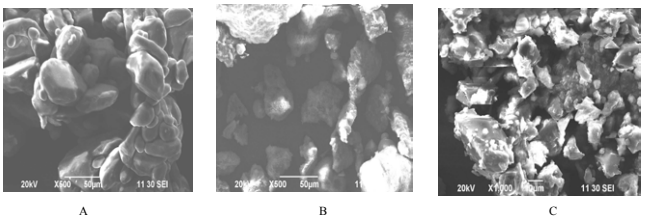

Figure 2: Images in SEM (A=Metformin, B=Cefepime, C=MC-1).

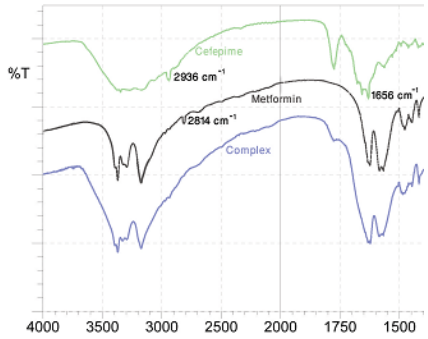

Figure 3: FT-IR Spectra for Metformin, Cefepime and MC-1 (Complex).

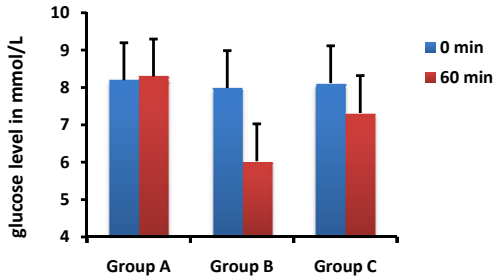

Figure 4: Mean blood glucose level at 0 minute and 60 minutes of administering saline water (group A), Metformin (group B) and MC-1 (group C) in STZ induced diabetic rats. The standard deviations are shown as error bars. The decrease in glucose lowering level in MC-1 group (group C) was statistically significant ($p \leq 0.05$) in comparison to the Metfortmin group (group B) after 60 minutes.

rats, usually reaches a plateau within 60 minutes after single oral administration of Metformin at a dose of 100 mg/kg body weight [30]. Therefore, the glucose levels were detected after 60 minutes for each group.

From the experiment, group A was seen to increase the mean blood glucose level after 60 minutes (from 8.2 ± 0.36 to 8.3 ± 0.45 mmol/L). On contrast, group B was found to reduce the glucose level sharply (from 7.99 ± 0.53 mmol/L to 6.03 ± 0.21 mmol/L). The value of mean blood glucose level was also observed to decrease moderately in case of group C from 8.12 ± 0.19 mmol/L to 7.32 ± 0.27 mmol/L. The mean changes in blood glucose levels (from 0 to 60 minutes) were 0.1 ± 0.0.49 mmol/L for group A, 1.96 ± 0.49 mmol/L for group B and 0.85 ± 0.23 mmol/L for group C. It was evident from the data that the glucose lowering capacity of Metformin was suppressed significantly due to its interaction with Cefepime as the changes in glucose lowering capacity were statistically significant for group C in comparison with group B. This suppression of activity indicates that interaction with Cefepime either reduces or delays the activation of Metformin molecule in diabetic individuals (Figures 2-4).

Conclusion

The *in vitro* investigations clearly indicated that Cefepime and Metformin interacted with each other and formed stable or intermediate complexes. The physicochemical properties, morphology and specific functional groups were also found to be altered in DSC, SEM and FT-IR analyses, respectively. The most crucial fact revealed in this report was the suppression of therapeutic activity of both Metformin and Cefepime. According to the *in vivo* data, the glucose lowering capacity of Metformin was reduced significantly. The prime objective of diabetes management is to control the blood glucose level by properly adjusting the doses of anti-hyperglycemic agents. Failing to prevent hyperglycemia in a diabetic patient is considered as a treatment failure and can also be detrimental to the patient. Besides, as an unwanted consequence of the interaction, the cure for infections will also become uncertain for usual doses of Cefepime. Therefore, concomitant administration of Metformin and Cefepime should be avoided in patients suffering from diabetes and infections.

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