

Study of antimicrobial property of some hypoglycemic drugs

Abstract

In the present work, a comparative antimicrobial study of different hypoglycemic drugs (Metformin, Phenformin, and Rosiglitazone) was carried out. The main objective was to ascertain the antimicrobial activity by using "non-antibiotics" as the test substances. The antimicrobial activity was carried out against different bacteria and fungi namely *Bacillus liceniformis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Bacillus subtilis*, *Staphylococcus aureus* subsp., and *Staphylococcus epidermidis* by using disc diffusion method and agar dilution method. Ciprofloxacin was taken as the standard antibiotic. The entire procedure was carried out in an aseptic area under the laminar flow by inoculating the bacterial strain to the agar media in which the drug solution was added. Different concentrations (300 and 400 µg/ml) of the standard antibiotic and selected drugs were subjected for minimum inhibitory concentration, and zone of inhibition tests and the antimicrobial activity of the selected drugs were determined.

Key words:

Disc diffusion method, hypoglycemic drugs, antimicrobial activity

Introduction

Antibiotics are one of our most important weapons in fighting bacterial infections and have benefited the health-related quality of human life. But over the past few decades, these health benefits are under threat as the commonly used antibiotics have become less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug-resistant bacteria. Thus, it has become essential to investigate newer drugs with lesser resistance. Drugs belonging to different pharmacological classes such as antihistamines (diphenhydramine, bromodiphenhydramine,^[1] and promethazine^[2]), psychotropics (promazine,^[3] chlorpromazine,^[4] fluphenazine, and trifluoperazine), antihypertensive (Methyl-DOPA,^[5]), local anaesthetics (procaine), and hypoglycemic possess powerful antibacterial activity. Such chemotherapeutic agents have been grouped together and are now entitled as "non-antibiotics."^[6,7] This article describes the detailed antimicrobial activity of some hypoglycemic drugs (metformin, phenformin, and rosiglitazone) whose antimicrobial activity has never been studied before.

Materials and Methods

Bacteria

A total of eight different varieties of both gram-positive and -negative bacteria (listed in [Table 1]) were obtained from M.T.C.C Institute of Microbial Technology, Chandigarh. These were preserved in freeze-dried stage. These were

Table 1: Variety of bacteria used in test

Strains as coded	Name of bacteria	Source
A	<i>Bacillus liceniformis</i> * 429	M.T.C.C Institute of Microbial Technology, Chandigarh 160036, India
B	<i>Escheriaceae coli</i> 40	
C	<i>Proteus vulgaris</i> 426	
D	<i>Pseudomonas aeruginosa</i> 424	
E	<i>Shigella flexneri</i> 1457	
F	<i>Bacillus subtilis</i> 441	
G	<i>Staphylococcus aureus</i> subsp. <i>au</i> 87	
H	<i>Staphylococcus epidermidis</i> 2639	

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of human origin, identified as described by Barrow and Feltham.^[8]

Media

Liquid media were prepared consisting of Beef extract (1%), yeast (2%), peptone (5%), sodium chloride (5%), agar (15%), and distilled water (q.s. 1l). The pH was adjusted to 7.4±0.2 with sodium hydroxide solution. This media provided the best environment for the growth of the microbes.

Preparation and standardization of test inoculums

Stock culture was obtained by aseptically transferring a loop full (2 mm) of the organism into 100 ml of sterile nutrient broth. These were incubated for 24 hours at 37°C±1°C. From this, 1 ml of stock culture was aseptically transferred to 9 ml of sterile water containing 0.05% Tween. This was cyclonized and 1 ml was transferred to 9 ml of sterile water containing Tween 80. This was continued to get a 10¹⁰ dilution factor. From each of the dilution, 0.2 ml of the water was spread on sterile agar plates. These plates were incubated for 18 hours at 37°C±10°C. After incubation, the number of colonies from the plate which was cultured from the maximum diluted tube is taken. Then the number of microorganisms in the stock was calculated and expressed as colony-forming unit/milliliter (cfu/ml).

Preparation of various concentration of drugs

Accurately weighed 50 mg of metformin, phenformin, and rosiglitazone were dissolved in 0.7 ml of dimethyl sulphoxide (DMSO) and 50 ml of sterile water to get conc. of 50 µg/ml (stock solution). From the above stock solution, 0.05, 0.1, 0.2, 0.3, and 0.4 ml solution were pipetted and were diluted and the volume was made up to 10 ml with the nutrient agar which give solution of conc. 50, 100, 200, 300, and 400 µg/ml, respectively.

Assessment of the antibacterial activity by using minimum inhibitory concentration

Measured volumes of stock solution of the drug were individually added aseptically to molten nutrient agar in concentration of 50, 100, 200, 300, and 400 µg/ml. The agar plates containing drugs were refrigerated overnight and subsequently dried for 2 hours at 37°C before use. Small squares were demarcated at the back of the agar-containing portion of the plate with a marker to specify the actual location for each test organism. The inoculums for determination of sensitivity pattern consisted of one loop full of an overnight growth culture of the test organism. The average size of the inoculums was about 10⁵ cells contained in a 2-mm diameter standard loop. When the nutrient agar plates containing the drug and also the control plates having equal containing the drug and also the control plates having equal volume of solvent were made ready, the overnight growth culture of each test organism was spot inoculated by checker-board technique, on the marked area of the plate, and these were then incubated for 72 hours at 37°C.^[9]

Determination of zone of inhibition by disc diffusion method

Pure ciprofloxacin was taken as standard antibiotic for comparison of the result. Two sets of two dilutions (100 and 200 µg/ml) each of drugs and ciprofloxacin in DMSO were prepared in sterile Mc-Cartney bottles. Sterile nutrient agar plates were prepared and incubated at 37°C for 24 hours to check for any sort of contamination. Two sterile filter paper discs (Whatman no. 1) of 4 mm diameter were soaked in different dilution of the drugs and placed in an appropriate position of the surface of flooded plate, marked as quadrants at the back of the petridish. The petridishes were incubated at 37°C for 14 hours and the diameter of zones of inhibition in mm was recorded. Similar procedure was adopted for the pure ciprofloxacin, and the cores providing zone diameter were compared accordingly. The experiment was repeated in triplicate, and the average results were noted down.

Results

Minimum inhibitory concentration (MIC) and zone of inhibition were taken as parameters for the assessment of the antimicrobial property of the selected drugs. The MICs of metformin, phenformin, and rosiglitazone are given in [Tables 2-4], respectively. Similarly, the zone of inhibition of these drugs is summarized in [Table 5].

Discussion

Both zone of inhibition and MIC are taken as parameters for ascertaining the antimicrobial activity. Maximum literature suggests that antimicrobial activity is carried out using the antibiotics which involves the use of antibodies. It is an obvious expectation an antibiotic will always have some antibiotic activity. Since last decade, there has been a tremendous increase in the spread of glycaemia. The motive of choosing the “non-antibiotics” class was to know whether the above stated drugs have any antimicrobial activity or not and at what dose they were found to contain such antimicrobial activity. If a hypoglycemic drug contains some antimicrobial activity, that it would be an added advantage for the patient. It could produce more immunity

Table 2: MIC of metformin

Name of the bacteria	Concentration of drug in µg/ml					
	50	100	200	300	400	500
<i>Bacillus liceniformis</i>	+	+	+	-	-	-
<i>Escheriaceae coli</i>	+	+	+	+	-	-
<i>Proteus vulgaris</i>	+	+	+	+	-	-
<i>Pseudomonas aeruginosa</i>	+	+	+	-	-	-
<i>Shigella flexmeri</i>	+	+	+	-	-	-
<i>Bacillus subtilis</i>	+	+	+	-	-	-
<i>Staphylococcus aureus</i>	+	+	+	+	+	-
<i>subsp. au</i>						
<i>Staphylococcus epidermidis</i>	+	+	+	+	+	+

Table 3: MIC of phenformin

Name of the bacteria	Concentration of drug in $\mu\text{g/ml}$					
	50	100	200	300	400	500
<i>Bacillus liceniformis</i>	+	+	+	-	-	-
<i>Escheriaceae coli</i>	+	+	+	+	-	-
<i>Proteus vulgaris</i>	+	+	+	+	-	-
<i>Pseudomonas aeruginosa</i>	+	+	+	-	-	-
<i>Shigella flexmeri</i>	+	+	+	-	-	-
<i>Bacillus subtilis</i>	+	+	+	-	-	-
<i>Staphylococcus aureus subsp. au</i>	+	+	+	+	+	-
<i>Staphylococcus epidermidis</i>	+	+	+	+	+	+

Table 4: MIC of rosiglitazone

Name of the bacteria	Concentration of drug in $\mu\text{g/ml}$					
	50	100	200	300	400	500
<i>Bacillus liceniformis</i>	+	+	+	-	-	-
<i>Escheriaceae coli</i>	+	+	+	+	-	-
<i>Proteus vulgaris</i>	+	+	+	+	-	-
<i>Pseudomonas aeruginosa</i>	+	+	+	-	-	-
<i>Shigella flexmeri</i>	+	+	+	-	-	-
<i>Bacillus subtilis</i>	+	+	+	-	-	-
<i>Staphylococcus aureus subsp. au</i>	+	+	+	+	+	-
<i>Staphylococcus epidermidis</i>	+	+	+	+	+	+

"0" = control, "+" = growth, "-" = no growth

Table 5: Zone of inhibition of different drug by disc diffusion method

Name of bacteria	Zone of inhibition in cm							
	Metformin		Phenformin		Rosiglitazone		Ciprofloxacin	
	300 $\mu\text{g/ml}$	400 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$	400 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$	400 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$	400 $\mu\text{g/ml}$
<i>Bacillus liceniformis</i>	8	12	9	12	9	13	20	22
<i>Escheriaceae coli</i>	5	11	5	10	6	11	19	20
<i>Proteus vulgaris</i>	5	10	7	11	7	12	18	21
<i>P. aeruginosa</i>	9	12	11	14	10	13	21	23
<i>Shigella flexmeri</i>	10	13	10	12	11	14	21	24
<i>Bacillus subtilis</i>	11	14	9	13	12	15	19	21
<i>S. aureus</i>	6	7	6	8	6	8	18	22
<i>S. epidermidis</i>	5	7	5	7	6	9	17	22

in the body. On comparing the result of MIC, among the selected drugs, metformin and rosiglitazone were found to have more antimicrobial activity when compared with phenformin. Phenformin was found to have antimicrobial properties at a concentration of 400 $\mu\text{g/ml}$ when compared with 300 $\mu\text{g/ml}$ of metformin and rosiglitazone. The results for the zone of inhibition revealed that the standard ciprofloxacin was having around twice microbial activity when compared with the drugs under investigation at a concentration of 200 and 300 $\mu\text{g/ml}$. Moreover the drugs were found to be more effective against *Shigella flexmeri* and *Pseudomonas aeruginosa* when compared with the other bacterial strains. Thus, these hypoglycemic drugs namely metformin, phenformin, and rosiglitazone can also be used as antimicrobials at a concentration of 400–500 $\mu\text{g/ml}$.

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