POTENTIATION OF ANTI-DIABETIC EFFECT OF METFORMIN AND SITAGLIPTIN WITH OFLOXACIN IN ALLOXAN INDUCED DIABETIC RABBITS

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Summary

The study was conducted to investigate the interactions that may be observed between Ofloxacin and anti-diabetic drugs (Metformin and Sitagliptin) in alloxan induced diabetic rabbits. Male albino rabbit were randomized into five groups. Diabetes induced by Alloxan monohydrate 200mg /kg body weight to all animals intravenously into the marginal ear vein. The first, control group received vehicle, The second group received Metformin 30 mg/kg, The third group received Sitagliptin10 mg/kg, The fourth group received Metformin and Ofloxacin 30 mg/kg +10mg/kg, The fifth group received Sitagliptin and Ofloxacin 10 mg/kg + 10mg/kg. blood glucose level was monitored at the regular intervals of time and it was determined that the group II(treated with Metformin 30mg) animals shows 20.2% decrease at 6^{th} hour, group IV (treated with Metformin 30mg and Ofloxacin 10 mg) animals shows 34.2 % decrease at 8^{th} hour, group III (treated with Sitagliptin 10 mg) animals shows 11.5 % decrease at 4^{th} hour and group V (treated with Sitagliptin 10 mg and Ofloxacin 10 mg) animals shows 25.3% decrease at 8^{th} hour in blood glucose level, in conclusion the observed interactions between anti-diabetic drugs (Metformin and Sitagliptin) and Ofloxacin is established study report suggests careful monitoring of blood glucose levels in the case of patients on co-administration of both drugs.

Key words: Ofloxacin, Metformin, Sitagliptin, Alloxan monohydrate, Blood glucose.

Introduction

India with the largest number of people with diabetes has secured the dubious distinction of being the 'diabetic capital' of the world. It has been projected that by 2025, India will have more than 60million people with diabetes.[1] Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, altered metabolism of lipids, carbohydrates, proteins and an increased risk of complications from vascular diseases.[2] Diabetes occurs due either to decreased synthesis of insulin or to defective secretion of insulin from beta cells of islets of Langerhans. Literature study reveals that diabetic patients develop multiple pathology such as fungal infection, cardiovascular disorders, nephropathy, retinopathy, neuropathy, sexual impotence, hyperacidity and respiratory tract infections.[3] Patients with multiple medical problems taking multiple medications are at increased risk of drug–drug interactions as new medications are added to their drug regimen.[4] both antibiotics and oral hypoglycemic drugs are being increasingly used in many therapeutic areas. The former agents are important for controlling bacterial infection and diabetic nephropathy respectively, and are being administered increasingly at earlier stages in these conditions. The latter drugs are widely used for effecting the lowering of blood glucose.

Ofloxacin is a fluorinated carboxyquinolone. The fluoroquinolones are a series of synthetic antibacterial agents which are used for the treatment of a variety of bacterial infections. It is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase.[5] Metformin and sitagliptin are extensively used in worldwide to treat patients with T2DM. Both drugs are considered to be insulin "sensitizers". However, the detailed mechanism of action of these two drugs is still unraveled and further investigations are needed to compare their clinical efficacy in different models of insulin resistance.

Metformin, the only available biguanide in the market, inhibits glucose production potentially through effects on adenosine monophosphate activated protein kinase (AMPK).[6] The treatment of prediabetic patients with metformin decreased new diagnosis of T2DM by 31% with more pronounced reduction in young under 45 years by 44% and in the obese with body mass index (BMI) >35 by 53% in the Diabetes Prevention Program.[7]

Sitagliptin represent a new class of oral antihyperglycemic agent to treat patients with type 2 diabetes. DPP-4 inhibitors improve fasting and postprandial glycemic control without hypoglycemia or weight gain. Sitagliptin was approved by the U.S. Food and Drug Administration (FDA) on October 17, 2006.[8] Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal.[9]

Despite recent advances in the management of both diabetes and infectious diseases, diabetic patients remain at increased risk of infection. Although intensive blood glucose control significantly reduces vascular complications in both type 1 and type 2 diabetes the relationship between glycaemia and infections is less well established. This study determines the interaction of Ofloxacin in Alloxan induced diabetic rabbits simultaneously treated with oral hypoglycemic drugs (Metformin and Sitagliptin).

Materials and Methods

Chemicals

The entire drugs required in the project work were obtained as a gift samples, Sitagliptin from Merck Pharmaceuticals Pvt. Ltd., Metformin from Cipla Pharmaceuticals Pvt. Ltd. (Indore, M.P.), Ofloxacin from Mecleods Pharmaceuticals Pvt. Ltd (Baddi, H.P.)

Animals

Male albino rabbit weighing between1.2-2 kg were procured from veterinary college of Mhow, Dist Indore M.P. and were kept in mesh bottom iron cages to avoid caprophagy. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44-56% and light and dark cycles of 12 hours. The experimental protocol has been approved by the IAEC and by the regulatory body of the government (Animal Ethical Committee number 1196/a/08/CPCSEA). All animals were fed with cauliflower, cabbage, carrot and tap water for 10 days before the experiment. Food and water were withdrawn 14 hours prior to the experiment.

Experimental design

Rabbits were randomly divided into five groups. The first, control group received Vehicle, The second group received Metformin 30 mg/kg, The third group received Sitagliptin10 mg/kg, The fourth group received Metformin 30 mg/kg after half an hour drug Ofloxacin 10mg/kg was given to same group, The fifth group received Sitagliptin 10 mg/kg after half an hour drug Ofloxacin 10mg/kg was given to same group. Blood samples were collected at 1, 2, 3,4,6,8 and 24 h intervals by puncturing marginal ear vein in all experiments. Blood glucose levels were determined by commercially available diagnostic kits (Accu-check).

Statistical analysis

All statistical analyses were performed using Graph pad. All values were presented as means ± S.E. (standard error). Comparisons among groups were made by application of one-way analysis of variance ANOVA followed by Bonferroni test. Differences were considered statistically significant if $p< 0.05$.

Results

After the treatments of all groups with their respective drugs their blood glucose level was monitored at the regular interval of time and it was determined that group I animals treated with vehicle show elevation of blood glucose level up to 394 mg/dl approx which was considered as 100 % elevation and comparing with it by one way ANOVA (Bonferroni test) the group II(treated with Metformin 30mg) animals shows 20.2% decrease at 6th hour, group IV (treated with Metformin 30mg and Ofloxacin 10 mg) animals shows 34.2 % decrease at 8th hour, group III (treated with Sitagliptin 10 mg) animals shows 11.5 % decrease at 4th hour and group V (treated with Sitagliptin 10 mg and Ofloxacin 10 mg) animals shows 25.3% decrease at 8th hour in blood glucose level (Table-1&2). All the data were statically significant as $p< 0.05$.

Table 1 Interaction effect on blood glucose level

Group No.	I	II	III	IV	V
Drug	Negative control (vehicle)	Metformin	Metformin + Ofloxacin	Sitagliptin	Sitagliptin + Ofloxacin
Dose	1ml/100gm	30mg	30 +10mg	10 mg	10 + 10 mg
OBS / Time	**Blood glucose level mg/dl**				
0 hr	392.2±1.02	394.4±1.80	393.4±1.44	394.2±1.43	392±0.55
1 hr	393.8±0.80	383.1±1.1 ^{a***}	381.3±0.7 ^{a***} ^{b}	390.2±0.8 ^{c}	388.3±0.7 ^{c**} ^{d}
2 hr	395.2±0.41	377.8±0.59 ^{a***}	374.5±0.29^{a***} ^{b**}	386.9±0.54^{c***}	385.3±0.33 ^{c***} ^{d}
3 hr	391.5±0.56	372.1±0.54 ^{a***}	369.5±0.42^{a***} ^{b*}	379.6±0.42^{c***}	377.6±0.42 ^{c***} ^{d}
4 hr	394.1±0.47	358.8±0.70 ^{a***}	351.1±0.60^{a***} ^{b***}	365±0.57 ^{c***}	354.5±0.71 ^{c***} ^{d***}

6 hrs	394.8±0.60	344.6±1.14 [a***]	319.8±0.70 [a***] [b***]	369.1±0.65 [c***]	343.6±0.76 [c***] [d***]
8 hr	394.2±0.47	353.3±0.66 [a***]	310±0.47 [a***] [b***]	381.2±0.47 [c***]	331.6±0.66 [c***] [d***]
24 hr	392.5±0.5	393.8±0.30 [a]	394.3±0.34 [a] [b]	392.2±0.77 [c]	393.6±0.61 [c] [d]

OBS-observation, mg/dl- milligram per deca liter, No. of animals in each group = 4
Data expressed as mean ± S.E.M followed by One way ANOVA (Bonferroni test)
[a, c] Group I (negative control), [b] Group II (Metformin treated), [d] Group IV (Sitagliptin treated),
***P<0.001, ** P<0.01, * P<0.05

Table 2 Percentage inhibition in blood glucose level

Group No.	**I**	**II**	**III**	**IV**
Drug / Time	**Metformin**	**Metformin + Ofloxacin**	**Sitagliptin**	**Sitagliptin + Ofloxacin**
0 hr	0%	0%	0%	0%
1 hr	4.0%	4.8%	1.2%	1.9%
2hr	6.3%	7.6%	2.5%	3.2%
3hr	8.6%	9.7%	5.5%	6.3%
4hr	14.0%	17.3%	11.5%	15.8%
6hrs	20.2%	30.1%	9.8%	20.3%
8hr	16.3%	34.2%	4.9%	25.3%
24hr	0%	0%	0%	0%

Discussion

The main objective of our study is to investigate the interactions that may be observed between Ofloxacin and anti-diabetic drugs (Metformin and Sitagliptin) in alloxan induced diabetic rabbits. We are aware of similar study that focuses on flouroquinolones interaction with oral hypoglycemic drugs[10] and fluoroquinolone-associated dysglycemias.[11]

The investigation was carried out on albino rabbits. Alloxan 200mg/kg was administered to animals of each group which is randomly selected and after the time periods of 48 hours their blood glucose level was examined and all of them were found to be diabetic due to the effect of Alloxan which produces diabetes. In clinical practice, Metformin, Sitagliptin and Ofloxacin in therapeutic doses are administered orally. Hence, human therapeutic dose was extrapolated to rabbit based on their body weight for oral dose selection and administered orally. After the treatments of all groups with their respective drugs, Blood samples were collected from the marginal ear vein. Their blood at the regular interval of time i.e. 1, 2, 3, 4, 6, 8, 24 hours respectively blood glucose level was estimation by commercially available diagnostic kits (Accu-check). The results obtained after treatment by oral hypoglycemic (Metformin and Sitagliptin) alone and in combination with Ofloxacin shows a wide range of difference in the decrease of blood glucose level of the diabetic animals, which suggest that there are possibilities of interaction between Metformin + Ofloxacin and Sitagliptin + Ofloxacin because Metformin alone decrease blood glucose level upto 20.2 % but in combination with Ofloxacin it reduces up to 34.2 % while in other combination i.e. of

Sitagliptin and Ofloxacin, Sitagliptin alone decrease blood glucose level up to 11.5 % but in combination with Ofloxacin it reduces upto 25.3 % the reason for this might be the pharmacodynamic and/or pharmacokinetic drug interaction.

In the earlier interaction there can be enhanced insulin release from pancreatic islet cells [12, 13, 14] and while in the later one the Flouroquinolones were reported to inhibit the renal clearance of oral hypoglycemic (Metformin and Sitagliptin) this increases their clearance t½ thereby their activity.

Conclusion

Hence the study report suggests that concurrent administration of ofloxacin with metformin and sitagliptin can result in hypoglycemia. Patients stabilized on either metformin, sitagliptin or both who are started on a fluoroquinolone should have their glucose levels monitored closely.

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