CUMMULATIVE TOXICITIES ON LIPID PROFILE AND GLUCOSE FOLLOWING ADMINISTRATION OF ANTI-EPILEPTIC, ANTI-HYPERTENSIVE, ANTI-DIABETIC AND ANTI-ARRHYTHMIC DRUGS

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ABSTRACT
Reporting of undesirable drug reactions is a problem in all countries, even those with sophisticated drug regulatory bodies. However we can expect a horrible picture in developing countries like Pakistan where drug regulatory control is very poor, hence present study has been exclusively designed to explore the outcome of individual administration of antiepileptic, antihypertensive, antidiabetic, antiarrhythmic drugs and their combinations on lipid profile and glucose. The study was conducted on healthy rabbits of either sex. Biochemical tests were performed at the completion of dosing i.e. on 61st day and again after drug-free interval of 15 days.
Present study provides detailed evaluation of adverse effects on lipid profile and glucose, results of the study suggests that animals received amiodarone-glibenclamide-verapamil-oxcarbazepine combination did not revealed any significant changes but animals received amiodarone-glibenclamide-losartan potassium-oxcarbazepine and amiodarone-glibenclamide-captopril-oxcarbazepine combinations revealed significant changes. However more studies on large number of animals and human beings are required to justify the use of multiple drug administration, since trial in man is the only way of establishing drug interactions.

Keywords: Cumulative toxicities, lipid profile, glucose.

INTRODUCTION

"Adverse Drug Reaction" (ADR) is a response to a drug which is deleterious and unintentional, occurs at doses generally used in man for prophylaxis, diagnosis, or treatment of disease, or the amendment of physiological function (Lazarou *et al.*, 1998; Rabinovitz *et al.*, 2001).

Adverse drug reactions including interaction are universal problems of foremost concern (Oshikoya and Awobusuyi, 2009) occur often in current medical practice (Routledge *et al.*, 2004), reporting 3.2-7% of acute hospital admissions (Cullen *et al.*, 2006). They influence both children and adults with varying extent, causing both morbidity and fatality. In appendage to the costs, adverse drug reactions have a key influence on public health by imposing a substantial economic burden on the society and health-care systems (Oshikoya and Awobusuyi, 2009). Patients with cardiovascular disease are predominantly exposed to adverse drug reactions due to impact of heart disease on drug metabolism, concomitant drug administration and old age. The potential of adverse drug reactions for a particular cardiovascular drug varies from individual to individual, the disease treated, and the magnitude of exposure to other drugs (Faulx and Francis, 2008).

Adverse drug reactions are still considered the major problems of drug therapy (Schlienger, 2000). Regardless of the efforts to reduce the frequency of adverse drug events; morbidity and fatality from drug induced disease remain elevated. Numerous types of interactions occur with drugs such as drug-drug, drug-disease, drug-food, drug-alcohol, drug-herbal products, and drug-nutritional status (Mallet *et al.*, 2007). Drugs may interact with other simultaneous administration of drugs or any dietary supplement which may be pharmacodynamic or pharmacokinetic in nature. The induction or inhibition of drug metabolizing enzymes is a predominantly significant cause of clinically major interactions. However, although some interactions make the life hostile, many are only imaginary or clinically insignificant; however it is always judicious to check interactions in a suitable reference work (Pleuvry, 2005).

A patient is often presented with several other pathological states along with epilepsy; such as hypertension, arrhythmias, and diabetes. It is therefore essential to recognize the safe combination of the drugs to be used in particular condition, hence present study was specifically designed to achieve multiple objectives such as to assess the toxicities of multiple drug administration and to explore relatively safe combination for individuals with multiple disorders, not to predict but rather to warn the users and prescribers, of the possible dangers, to discourage the use of combination which have high cumulative toxicities in animals and to suggest more useful combination in developing countries where drug regulatory control is very poor.

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MATERIALS AND METHODS

Choice of Animals

In present study rabbits were selected as experimental animals because of several reasons such as biochemical and histopathological changes produced in rabbits are relatively similar as observed in humans, large quantity of blood samples can be obtained with ease, general physiology of rabbits is similar to humans, rabbits are easily available, easy to handle and economical (Feroz *et al.*, 2010).

The study was conducted on ninety healthy white rabbits of both sexes weighing from 1500-1800 gram. Animal were accommodated in separate cages, under controlled conditions of temperature (22 ± 2°C) and humidity (50-60%). All animals were given green leafy diet and water regularly.

Animals were uniformly divided into ten groups. Each group consists of 9 animals, one group treated as a control while the other remaining group received the drug singly and in combination. Before administration of drug, visible health of these animals was observed during the conditioning period under the laboratory environments for a week specifically noticing loss of hair, diarrhea, edema, ulceration and lack of activity.

Dosing

Animals were treated on daily dosing basis for a period of 60 days. Drugs were administered through oral route in normal therapeutic doses after forming suspension in DMSO as under:

- Amiodarone: 4.285 mg/kg
- Glibenclamide: 0.125 mg/kg
- Losartan potassium: 0.892 mg/ kg
- Oxcarbazepine: 18.5 mg/kg
- Verapamil: 1.714 mg/kg
- Captopril: 0.512 mg/Kg

Groups received drugs in following pattern.

- Group A: Control group received saline only
- Group B: Amiodarone
- Group C: Glibenclamide
- Group D: Losartan potassium
- Group E: Oxcarbazepine
- Group F: Verapamil
- Group G: Captopril
- Group H: Amiodarone-Glibenclamide-Losartan-potassium-Oxcarbazepine (AGLO)
- Group I: Amiodarone-Glibenclamide-Verapamil-Oxcarbazepine (AGVO)
- Group J: Amiodarone-Glibenclamide-Captopril-Oxcarbazepine (AGCO)

Blood samples of about 7 ml were collected in gel tubes through cardiac puncture technique (Feroz *et al.*, 2010), after completion of dosing period i.e. 61st day and again after a drug free interval of 15 days. Serum was separated by centrifugation at 3000 rpm for 15 minutes in 14K Humax centrifuge. Lipid profile and glucose were analyzed on Humalyzer 3000 (Semi-automatic chemistry analyzer, Model # 16700) (Human Germany) using standard kits supplied by Human.

Assessment of lipid profile

Total cholesterol (TC) was estimated by CHOD-PAP method; triglyceride (TG) by GPO-PAP methods (Trinder, 1969) and high density lipoprotein-cholesterol (HDL-C) was estimated by the method of Gordon *et al.*, 1977 and Friedewald *et al.*, 1972. The low density lipoprotein-cholesterol (LDL-C) was calculated as follows:

LDL-C = TC- [HDL-C-(TG/5)] mg/dl (Friedewald *et al.*, 1972).

Assessment of glucose

Glucose was estimated by GOD-PAP method. It is an enzymatic colorimetric test method without deproteinisation (Barham and Trinder, 1972).

STATISTICAL ANALYSIS

All values were compared with control by taking mean and standard error to the mean using one-way analysis of variance (ANOVA) followed by post hoc. Data was reported as mean ± standard error to the mean with 95% confidence interval and p-values were observed. Values of $P<0.05$ were considered as significant and $P<0.005$ as highly significant.

Lipid profile

Table 1 reveals the comparison of cholesterol; triglyceride; HDL-C and LDL-C levels in animals of control group, animals received drugs individually and in combinations for a period of 60 days in normal therapeutic doses. While a similar comparison of parameters between the same groups of animals after a drug-free interval of 15 days is presented in table 2.

Animals kept on amiodarone, glibenclamide, and verapamil individually and AGVO in combination did not reveal any significant alteration in cholesterol, triglyceride, HDL-C and LDL-C levels at the end of dosing as well as following drug-free interval.

Animals kept on losartan potassium and captopril individually revealed significant decrease in triglyceride level i.e. 91.94±2.86 mg/dl and 93.40±4.47 with respect to control i.e. 102.65±2.45 mg/dl, while following drug-free interval the level of triglyceride was almost

Table 1: Comparison of lipid profile following 60 days administration of individual drugs and their combinations

Groups/Parameters	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	91.84±2.65	102.65±2.45	3.10±0.13	28.15±1.90
Amiodarone	88.80±0.62	100.51±2.97	3.07±0.04	27.04±1.13
Glibenclamide	93.37±3.07	106.77±4.71	3.28±0.06	23.25±2.08
Los. Pot	92.77±0.57	91.94±2.86*	3.15±0.07	34.87±1.70
Oxcarbazepine	95.08±1.26	97.08±1.45	3.37±0.05*	29.38±2.03
Verapamil	96.90±1.13	108.53±3.32	3.31±0.08	25.24±1.95
Captopril	96.06±2.89	93.40±4.47*	3.32±0.07	34.18±2.26
AGLO	105.15±3.94**	100.20±2.90	2.93±0.05	46.35±3.50**
AGVO	95.93±1.21	104.46±1.31	3.27±0.04	27.41±1.80
AGCO	173.53±4.22**	95.35±3.17	3.40±0.08**	108.83±6.13**

n=9; Mean ± S.E.M.; *p < 0.05 significant with respect to control. **p <0.005 highly significant with respect to control

Table 2: Comparison of lipid profile following drug-free interval of 15 days of individual drugs and their combinations

Groups/Parameters	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	91.61±2.52	102.12±2.46	2.98±0.12	30.56±1.68
Amiodarone	88.18±0.75	100.26±2.93	2.96±0.04	28.81±1.25
Glibenclamide	93.80±3.10	106.7±4.59	3.16±0.05	26.38±2.06
Los. Pot	92.66±0.65	95.72±1.94	3.04±0.06	34.43±1.22
Oxcarbazepine	94.82±1.33	96.91±1.52	3.26±0.05**	31.38±1.96
Verapamil	96.01±1.27	104.27±1.81	3.18±0.07	29.60±1.90
Captopril	95.37±2.87	95.64±3.69	3.21±0.07	36.05±1.65
AGLO	104.31±3.91**	97.97±2.48	2.83±0.05	48.71±3.31**
AGVO	94.98±1.33	102.40±2.11	3.13±0.04	30.85±1.62
AGCO	99.73±1.18*	94.72±3.13	3.26±0.05**	37.88±2.70**

n=9; Mean ± S.E.M.; *p < 0.05 significant with respect to control. **p <0.005 highly significant with respect to control.

comparable to control. On the other hand there was no significant change in cholesterol, HDL-C and LDL-C levels at the end of dosing as well as following drug-free interval. Conversely animals kept on oxcarbazepine individually revealed significant increase in HDL-C level i.e. 3.37±0.05 mg/dl with respect to control i.e. 3.10±0.13 mg/dl, which was remained significant even following drug-free interval. Conversely there was no significant change in cholesterol, triglyceride and LDL-C levels at the completion of dosing as well as following drug-free interval.

Animals kept on AGLO combination revealed highly significant elevation in cholesterol and LDL-C levels i.e. 105.15±3.94 mg/dl and 46.35±3.50 mg/dl with respect to control values i.e. 91.84±2.65 mg/dl and 28.15±1.90 mg/dl respectively, which was remained significant even following drug-free interval. Conversely there was no significant change in triglyceride and HDL-C levels at the completion of dosing as well as following drug-free interval. On the other hand animals kept on AGCO combination showed highly significant elevation in cholesterol, HDL-C and LDL-C levels i.e. 173.53±4.22 mg/dl, 3.40±0.08 mg/dl and 108.83±6.13 mg/dl with respect to control values i.e. 91.84±2.65 mg/dl, 3.10±0.13 mg/dl and 28.15±1.90 mg/dl respectively, which were remained significant even following drug-free interval. Conversely there was no significant change in triglyceride level at the end of dosing and following drug-free interval.

Glucose

Table 3 reveals the comparison of glucose level in animals of control group; animals received drugs individually and in combinations for a period of 60 days in normal therapeutic doses. While a similar comparison of parameter between the same groups of animals after a drug-free interval of 15 days is presented in table 4.

Animals kept on amiodarone, losartan potassium and verapamil alone and AGLO, AGVO in combination did not reveal any significant alteration in the level of glucose at the end of dosing as well as following drug-free interval.

Table 3: Comparison of glucose following 60 days administration of individual drugs and their combinations

Groups/Parameter	Glucose (mg/dl)
Control	122.20±7.60
Amiodarone	111.84±3.30
Glibenclamide	75.20±5.79**
Los. Pot	119.67±4.0
Oxcarbazepine	142.17±4.54**
Verapamil	123.33±2.31
Captopril	146.06±4.72**
AGLO	102.50±6.12
AGVO	111.88±3.11
AGCO	145.44±2.93**

Table 4: Comparison of glucose following drug-free interval of 15 days of individual drugs and their combinations

Groups/Parameter	Glucose (mg/dl)
Control	123.40±7.40
Amiodarone	113.76±3.10
Glibenclamide	104.50±5.40
Los. Pot	122.30±4.30
Oxcarbazepine	141.50±4.70
Verapamil	124.39±2.30
Captopril	144.30±4.30*
AGLO	102.60±6.10
AGVO	112.11±3.10
AGCO	137.90±3.60*

n=9; Mean ± S.E.M.; *p < 0.05 significant with respect to control. **p <0.005 highly significant with respect to control

Animals kept on glibenclamide individually revealed highly significant decrease in the level of glucose i.e. 75.20±5.79 mg/dl with respect to control i.e. 122.20±7.60 mg/dl at the end of dosing. This highly significant decrease was found to be insignificant following drug-free interval. Conversely animals kept on oxcarbazepine and captopril individually revealed highly significant increase in the level of glucose i.e. 142.17±4.54 mg/dl and 146.06±4.72 mg/dl with respect to control i.e. 122.20±7.60 mg/dl at the end of dosing. On the other hand following drug-free interval oxcarbazepine was found to be insignificant but captopril was still remained significantly high i.e. 144.30±4.30 mg/dl with respect to control i.e. 123.40±7.40 mg/dl.

Animals kept on AGCO combination revealed highly significant increase in glucose level i.e. 145.44±2.93 mg/dl with respect to control i.e. 122.20±7.60 mg/dl, which was remained significant even following drug-free interval.

DISCUSSION

The simultaneous use of several medications has greatly increased the risk of undesirable drug reactions and drug-drug interactions particularly in the geriatric population. Administration of multiple drugs may be life threatening and unreasonably expensive, since a wide range of drugs are administered simultaneously which may produce toxicity and affect any organ system. Hence it is extremely important for a prescribing physician to be aware of the toxic profile of the drugs and to be watchful for the occurrence of unexpected adverse reactions (Koh *et al.*, 2005).

Cholesterol and triglycerides are the most important plasma lipids, crucial for formation of cell membrane, synthesis of hormones and offer a source of free fatty acids (Dietschy, 1998). Individuals using diet with increased saturated fat normally have elevated levels of serum cholesterol. The increase in the level of cholesterol occurs because of irregularity in lipoproteins levels responsible for bringing in the blood and that is ultimately because of diet, genetic factors and disorders such as diabetes and an under active thyroid (Durrington, 2003).

Table 1 and 2 shows the outcome of individual and multiple administrations of drugs on lipid profile. Present study shows highly significant decrease in triglyceride level in animals kept on losartan potassium and captopril alone, whereas animals kept on AGLO and AGCO combinations showed highly significant increase in cholesterol level at the end of dosing which remained significant even after drug-free interval. Elevated level of cholesterol also occurs in disorders such as hypothyroidism, obstructive liver disease, nephritic syndrome, uncontrolled diabetes mellitus, post-hepatic cholestasis, primary hyperlipoproteinemia and acute porphyria. Present study also shows highly significant increase in LDL-C level in animals kept on AGLO and AGCO combinations which were remained significant even after drug-free interval. Elevated levels of cholesterol and LDL-C are undoubtedly associated with enhanced threat of coronary heart disease (Brown, 1984) and cerebrovascular morbidity and mortality. There has been a correlation among increased LDL-C and atherosclerosis. Since LDL-C gets deposited in the walls of the blood vessel forming atherosclerotic plaque. There are studies which recommend that lowering LDL-C reduces the risk of coronary heart disease (Aghasadeghi *et al.*, 2008). There was also significant increase in HDL-C in animals kept on oxcarbazepine alone and AGCO in combination at the end of dosing and following drug-free interval; however reason of elevated HDL-C is yet to be explored.

Table 3 and 4 shows the outcome of individual and multiple administrations of drugs on glucose. Present

study shows significant increase in glucose level in animals kept on AGCO combination at the completion of dosing and following drug-free interval. Elevated blood glucose level may be due to elevation in the level of cholesterol and LDL-C, because diabetes mellitus is a group of heterogenous, autoimmune, hormonal and metabolic disorders, frequently occurs along with hypertension, hyperlipidemia and obesity (Mahomed and Ojewole, 2003), which also augmented the possibility of coronary heart disease (Howard *et al.*, 2000) and threat of CVD fatality in diabetic persons may be as high as that in non-diabetic persons with prior myocardial infarction (Haffner *et al.*, 1998). There was also a significant elevation in glucose level in animals kept on captopril and oxcarbazepine alone, however it has to be elucidated. Conversely animal kept on glibenclamide alone revealed highly significant decrease in glucose level because the major mechanism of action of glibenclamide is the stimulation of insulin release and the inhibition of glucagon secretion; conversely it was inverted following drug-free interval.

CONCLUSION

Present study was conducted to evaluate the toxicities of drugs on lipid profile and glucose that are commonly used in patients with multiple disorders. The overall results of the study reveal that animals received amiodarone-glibenclamide-verapamil-oxcarbazepine combination reveals no toxic effects than amiodarone-glibenclamide-losartan potassium-oxcarbazepine and amiodarone-glibenclamide-captopril-oxcarbazepine combinations.

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