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EFFECT OF PRIMIDONE ON THERAPEUTIC EFFICACY OF MIFEPRISTONE

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ABSTRACT

A drug interaction can be defined as an interaction between a drug and another substance that prevents the drug from performing an expected action. Primidone is used to treat complex partials, simple partials, generalized tonic-clonic seizures, myoclonic, a kinetic seizures and it has been a valuable alternative to propranolol in the treatment of essential tremor. Mifepristone acts as a competitive receptor antagonist at the progesterone receptor. The present study shows the Abortifacient activity, Serum and Uterus Progesterone, Protein, Alkaline phosphatase, Cholesterol, SGPT, SGOT levels by using wister albino rat (150-200) gm and also uterus histopathological studies was performed. Vaginal smears from each rat were monitored daily. Only rats with normal estrous cycles were selected for this experiment. In the present study, the effect of Primidone (CYP3A4 inducer) on the abortifacient activity of Mifepristone in pregnant rats is being investigated. Primidone may cause failure of abortifacient effect of Mifepristone as a result of drug-drug interaction.

KEY WORD

Abortifacient activity, Alkaline Phosphatase and Serum progesterone.

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INTRODUCTION[1-9]

Whenever two or more drugs are being taken, there is a chance that there will be an interaction among the drugs. A drug interaction can be defined as an interaction between a drug and another substance that prevents the drug from performing as expected. This definition applies to interactions of drugs with other drugs (drug-drug interactions), as well as drugs with food (drug-food interactions) and other substances.

Many drugs may increase or decrease the activity of various cytochrome P450 isozymes either by inducing the biosynthesis of an isozyme (enzyme induction) or by directly inhibiting the activity of the cytochrome p450 (enzyme inhibition). This is a major source of adverse drug interactions, since changes in cytochrome p450 enzyme activity may affect the metabolism and clearance of various drugs. For example, if one drug inhibits the cytochrome p450-mediated metabolism of another drug, the second drug may accumulate within the body to toxic levels. Hence, these drug interactions may necessitate dosage adjustments or choosing drugs that do not interact with the cytochrome p450 system. Such drug interactions are especially important to take into account when using drugs of vital importance to the patient, drugs with important side-effects and drugs with small therapeutic windows, but any drug may be subject to an altered plasma concentration due to altered drug metabolism.

A classical example includes anti-epileptic drugs. Phenytoin, induces CYP1A2, CYP2C9, CYP2C19, and CYP3A4. Substrates for the latter may be drugs with critical dosage, like amiodarone or carbamazepine, whose blood plasma concentration may either increase because of enzyme inhibition in the former, or decrease because of enzyme induction in the latter.

Cytochrome P450 3A4 (abbreviated CYP3A4), a member of the cytochrome P450 mixed-function oxidase system, is one of the most important enzymes involved in the metabolism of xenobiotics in the human body. CYP3A4 is involved in the oxidation of the largest range of substrates of all the cytochrome P450s. As a result, CYP3A4 is present in the largest quantity of all the cytochrome P450s in the liver.

The cytochrome P450 proteins are monooxygenases that catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids, and other lipids. This protein localizes to the endoplasmic reticulum, and its expression is induced by glucocorticoids and some pharmacological agents. This enzyme is involved in the metabolism of approximately half the drugs that are used today, including acetaminophen, codeine, ciclosporin (cyclosporin), diazepam, and erythromycin. The enzyme also metabolizes some steroids and carcinogens. Most drugs undergo deactivation by CYP3A4, either directly or by facilitated excretion from the body.

Primidone

Primidone is an anticonvulsant of the pyrimidinedione class whose active metabolites, phenobarbital (major) and phenylethylmalonamide (PEMA) (minor). It is used to treat complex partial, simple partials, generalized tonic-clonic seizures, myoclonic, a kinetic seizures and it has been a valuable alternative to propranolol in the treatment of essential tremor. It is the drug that inducing the metabolic enzyme cytochromeP450s.

Mifepristone

Mifepristone is a synthetic steroid compound. In the presence of progesterone, Mifepristone acts as a competitive receptor antagonist at the progesterone receptor (in the absence of progesterone, Mifepristone acts as a partial agonist) used as oral contraceptives and Abortifacient agent. It is the drug metabolized by the enzyme Cytochrome P450s.

MATERIALS AND METHODS

Experimental procedures (Abortifacient activity) [10]

Colony bred adult female albino rats of Wistar strain (150–200 g) were maintained under controlled standard animal house conditions with access to food and water ad libitum. Vaginal smears from each rat were monitored daily. Only rats with normal estrous cycles were selected for the experiment antifertility activity was determined as described by Khanna and Chowdhary. The female rats were caged with male rats of known fertility in the ratio of 2:1 in the evening of proestrous and examined the following morning for the evidence of copulation. Rat exhibiting the copulation plug or thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day 1 of pregnancy and those rats were divided into groups 4 different groups (n=6 per group). The Female Sprague-

Dawley strain rats were randomized into 4 different groups (n=6 per group).

Group I: Vehicle control. The animals received distilled water only.

Group II: The animals received Primidone 22 mg/kg p.o from 8-12 days of pregnancy.

Group III: The animals received Mifepristone in sunflower seed oil 10 mg/kg s.c from 8-12 days of pregnancy.

Group IV: The animals received combined treatment of Mifepristone in sunflower seed oil 10 mg/kg s.c and Primidone 22mg/kg p.o from 8-12 days of pregnancy.

Percentage of Abortifacient Activity[8]

The percentage of abortifacient activity was determined by following formula (Williamson).

Abortifacient = No. of litters delivered in C - No. of litters delivered in T x 100/ No. of litters delivered in C

Where,

C - Control, T- Test.

Histopathological Studies of Uterus[10]

After the study period, the rats were anaesthetized with pentobarbitone sodium (30mg/kg, i.p). Uterus were dissected out perfused with chilled saline to remove blood and blood clots and fixed in 10%w/v formalin saline 24h, dehydrated in isopropyl alcohol and then embedded in paraffin. The paraffin blocks are sectioned at 5 m intervals and stained with haematoxylin-eosin and mounted with Canada balsam. Histological measurement and photographs are taken with a Carl Ziss Jena ampulla type photomicroscope (magnification 100X). The diameter of uterus, thickness of myometrium and thickness of endometrium are measured using compound microscope in µm. The eyepiece micrometer is calibrated with stage micrometer.

Statistical Analysis

All data will be expressed as mean ± SEM. The statistical significance between groups will be compared using one way ANOVA, followed by Dunnet's t-test (multiple comparisons). $P < 0.05$ will be considered as significant.

SUMMARY AND DISCUSSION

In our present study mature female rats mated in 2:1 ratio, Data comparision was made between Group 1 vs Group 4 and b. Group 3 vs Group 2 and 4. Statistical significance was done by ANOVA, followed by dunnett's multiple comparison tests. Significant elevation in progesterone level in combination treated groups. Total cholesterol was shown in Table No.1 and 2, total protein, alkaline phosphatase was significantly increased in combination treated groups. Serum SGOT, SGPT was significantly increased in combination treated groups. Liver enzyme-cytochrome p450 was significantly increased in primidone treated groups. Uterus weight was significantly reduced in combination treated groups was show in Table No.3. Histopathological findings of the combination treated group showed reduction in the thickness of endometrium, myometrium and diameter of uterus. Abortifacient activity of mifepristone was abolished by the combinational treatment of mifepristone + primidone was shown in Figure No.1.

Table No.1: Lipid Profile

S.No	Parameters		Control I	Primidone II	Mifepristone III	Mifep + primi IV
1	Serum Total Cholesterol mg/dl	Day 12	200.7 ± 2.98	196.6 ± 5.09 ***	89 ± 4.03 ***	171.8 ± 11.11 ***
		Day 19	121.7 ± 2.76	110.3 ± 2.53 ***	94.59 ± 1.50 **	110.9 ± 3.55 **
2	Uterus Total Cholesterol mg/dl	Day 12	130.1 ± 2.20	130.3 ± 2.56 ***	80 ± 2.34 ***	111.5 ± 2.61 ***
		Day 19	181.6 ± 4.42	179.6 ± 3.21 ***	122.4 ± 2.93 ***	172.8 ± 2.52 ***

Table No.2: Abortifacient activity

S.No	Parameters		Control I	Primidone II	Mifepristone III	Mifep + primi IV
1	Number of litters		8 ± 0.25	7.5 ± 0.22 ***	---	1.3 ± .33 **
2	Effect of uterus weight (gms)		2.66± 0.421	2.31± 0.421***	7.16 ± 0.749 ***	4.33 ± 0.3 **
3	Effect of serum Progesterone (ng/ml)	Day 12	154.3 ± 1.44	151.6 ± 3.32 ***	76.52 ± 1.04 ***	145.1 ±4.39 ***
		Day 19	145.4 ± 1.89	144.5 ± 1.75 ***	129.6 ± 1.86 ***	136 ± 0.53*
4	Effect of uterus Progesterone (ng/ml)	Day 12	28.94 ± 0.74	25.76 ± 1.06 ***	7.70 ± 0.32 ***	30.79 ±2.29 ***
		Day 19	21.79 ± 0.94	19.18± 0.39 ***	13.66 ± 0.45 ***	21.19 ± 0.61***

Table No.3: Protein Profile and Uterus diameter

S.No	Parameters	Control I	Primidone II	Mifepristone III	Mifep + primi IV
1	Total Protein mg/dl	70.82 ± 2.97	69.85± 3.32 ***	41.07 ± 3.64 ***	61.86 ± 1.85 ***
2	Alkaline Phosphatase ka/dl	237.3 ± 2.23	244.3 ± 8.71 ***	164.7 ± 5.08 ***	199.8 ± 4.29 ***
3	Serum SGOT IU/dl	35.46 ± 1.44	32.96 ± 0.73 ***	21.55 ± 0.87 ***	25.83 ± 0.81 *
4	Serum SGPT IU/dl	34.11 ± 0.34	34.61 ± 1.08 ***	18.65 ± 1.29 ***	30.89 ± 1.70 ***
Effect on uterus diameter					
5	Uterus Diameter mm/mg t.w	3.41 ± 0.14	3.02 ± 0.25 ***	5.84 ± 0.32 ***	4.04 ± 0.36 **
Estimation of hepatic enzyme cytochrome p450					
6	Liver CYP3A4 Level in nmol/mg	0.5697±0.001	0.709 ± 0.002 ***	0.6114 ± 0.002 ***	0.6609 ± 0.002 ***
Histopathology Study of uterus					
7	Thickness of endometrium µm/mg, t.w	96.54 ± 3.65	97.8 ± 4.5 ***	126.54 ± 5.32 ***	105.64 ± 3.42 **
8	Thickness of myometrium µm/mg t.w	49.83 ± 1.98	48.54 ± 1.43 ***	76.87 ± 2.01 ***	54.98 ± 1.76 **

SIGNIFICANT: *P<0.05, **P<0.01, ***P<0.001, ^{NS} non significant.

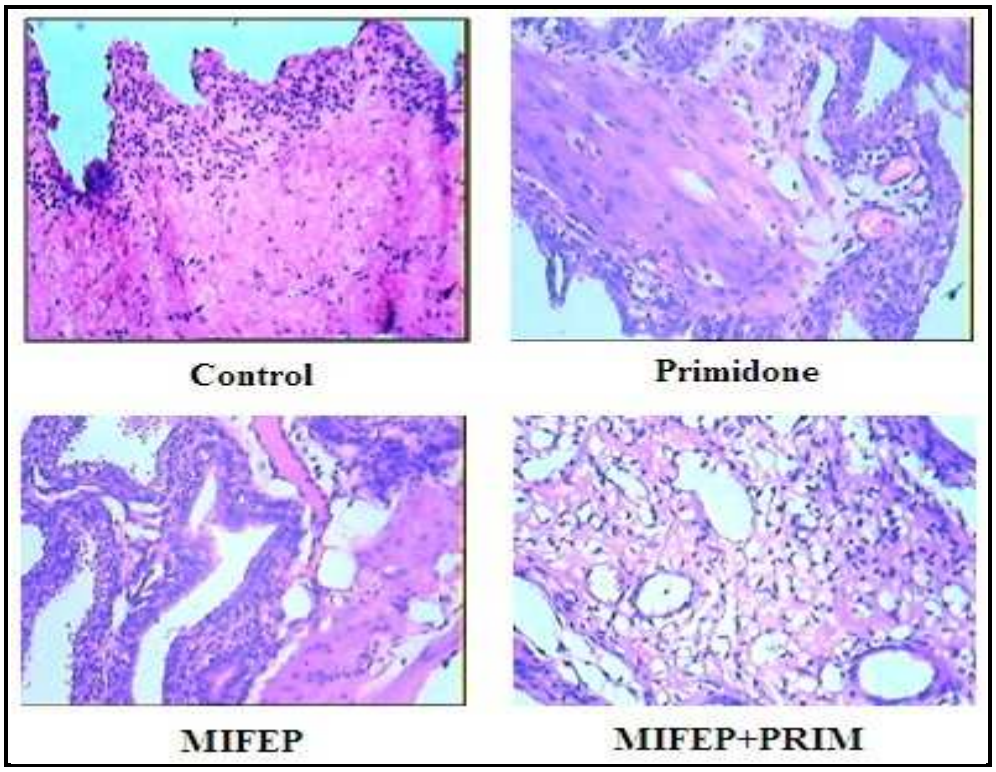

Figure No.1: Representative microscopic examination (100x) under polarized light of uterine sections

CONCLUSION

In the present study, investigated that the effect of primidone (CYP3A4 inducer) on the abortifacient activity of mifepristone in pregnant rats. The cause of failure of abortifacient effect of mifepristone may be due to drug-drug interaction. The mechanism has point towards inhibiting therapeutic efficacy of mifepristone by primidone.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Piaggio G et al. Meta-analysis of randomized trials comparing different doses of Mifepristone in emergency contraception, *Contraception*, 68(6), 2003, 447.
2. Rafi N, Bachorick Paul S and Albers J. Lipids, lipoproteins and apolipoprotein, Tietze text book of clinical chemistry, *W B Saunders Company, Philadelphia*, 5th edition.
3. Nicholas V. Carroll, Robert W. Longley, Joseph H. Roe. The determination of glycogen in Liver and muscle by use of anthrone reagent, *The journal of biological chemistry*, 220(2), 1956, 583-593.
4. Naito H K. Role of apolipoproteins in vascular diseases, *J. Clin. Immuno assay*, 9, 1986, 155.
5. Reitman S, Franker S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, *Am J Clin Path*, 28, 1957, 56-63.
6. Herbert K. Lipids, In clinical chemistry; Theory, Analysis and Co-relation, 1984, 1182-1230.
7. Lin H L, Zhang H, Hollenberg P F. Metabolic activation of mifepristone [RU486; 17beta-hydroxy-11beta-(4-dimethylaminophenyl)-

17alpha-(1-propynyl)-estra-4,9-dien-3-one] by mammalian cytochromes P450 and the mechanism-based inactivation of human CYP2B6, *J Pharmacol Exp Ther*, 329, 2009, 26-37.

8. Williamson E M, Okpako D T and Evan F G. Selection, preparation and pharmacological evaluation of plant material, *Pharmacological Methods in Phytother. Res*, 1, 1996, 191-12.
9. Xiaoming Cui, Ann Thomas, Valerie Gerlach, Ronald E. White, Richard A. Morrison, Cheng K C. Application and interpretation of hPXR screening data: Validation of reporter signal requirements for prediction of clinically event CYP3A4 inducers, *Biochemical pharmacology*, 76, 2008, 680-689.
10. Khanna U, Garg S K, Vohra S B, Walia H B, Chaudhury R R. Antifertility screening of plants.II. Effects of six indigenous plants on early pregnancy in albino rats, *Indian J Med Res*, 57, 1969, 237-44.

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