Research Article [Sudhakar, 5(9): Sep., 2014:3825-3831]
CODEN (USA): IJPLCP ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Determination of maximum tolerated dose of combination of Lamotrigine and 5-fluorouracil by oral route in swiss albino mice for further *In Vivo* Anticancer activity

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Abstract

The cancer disease has created a great demand for the discovery and development of new drug molecules. The major problem with the cancer chemotherapeutics is the toxicity by the current existing anti cancer drugs and anti tumor drug resistance by the cancerous cells to the anticancer agents. These two major problems can be overcome by the discovery and usage of new novel cancer chemotherapeutic agents and going with the new drug combinational therapeutics. 5-Fluorouracil is an antimetabolite whose usage has reduced due to severe toxicity and drug resistance when used individually. Lamotrigine is an anti epileptic drug which has diversified mechanisms of action, and which is believed to be act as an ideal anticancer agent. In the present study the safe and maximum tolerable dose of the combination of both Lamotrigine and 5-Fluorouracil are determined for further in vivo anticancer activity studies in swiss albino mices.

Key-Words: Toxicology, Drug resistance, Neuro degenerative, Sarcolyis

Introduction

Cancer is a current growing health problem around the world, particularly with the steady rise in life expectancy. According to a recent report (2011) by the World Health Organization, there are now more than 10 million cases of cancer per year worldwide [(1)]. Various therapies have been effectively used and recommended in treating the cancer disease, amongst this chemotherapy is mostly considered in treating different types of cancers [(2)]. Various cancer chemotherapeutic agents have been used individually and in combination with a distinct mechanism of actions [(3)]. The main complication with the cancer chemotherapy is the multiple organ toxicities [(4)] and drug resistance by the cancer cells [(5)]. Now it is very important to discover the drugs to meet all the demands of cancer chemotherapy in treating cancer and achieving patient safety. The drug toxicities can be overcome by reducing the dose or going with the alternate drug or avoiding it. And the drug resistance can be overcome by multiple combination chemotherapy [(6)]. 5-Fluorouracil and Methotrexate are the best and potent antimetabolites used effectively in the treatment of various cancers.

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But the usage of these drugs has been reduced due to the severe multiple organ toxicities [(7, 8)]. The more emphasis has to be paid to the drugs having myriad mechanism of actions and the capability to cure more than one disease. Many existing drugs have been proven to be having the ability to treat cancer productively for instance ASPRIN which is a non steroidal anti inflammatory drug have been proved that it can cure colon cancer [(9)]. The other exemplar is LAMOTRIGINE, which is an anti epileptic drug has been stated that it is having the ability to inhibit an enzyme dihydrofolate reductase (weak inhibitor) [(10)] and can inhibit histone deacylase [(11)]. In the present study the acute oral toxicity study and maximum tolerable dose of the both the drugs in combine (in combination) were done and determined in swiss albino mices for further in vivo anticancer activity. The other perfect rationale for going with this combination is that, lamotrigine has a side effect of suicide behavior, ideation in some bipolar disordered patients [(12)], while 5-Fluorouracil is a suicide inhibitor [(13)].

Material and Methods

In the present study the acute oral toxicity studies in mice for determination of maximum tolerated dose of combination of Lamotrigine and 5-Fluorouracil was done based upon the guidelines of OECD 420.(OECD

GUIDELINE FOR TESTING OF CHEMICALS: Acute Oral Toxicity – Fixed Dose Procedure.)

Animals used

The mouse (*Mus musculus*) of weight 20-25 grams are selected as test system, they are procured from Sugen life sciences, tirupati. Mouse has been historically shown to be a suitable model for dose range finding studies and acute toxicity assessment and is recommended by the OECD and other regulatory authorities. The results of the study are expected to be of value in predicting the acute toxicity of the test item in human beings. The mice were maintained at the standard temperature and conditions and fed as per the prescribed guidelines.

Drugs used

Lamotrigine and 5-Fluorouracil

Dose Administration

A dose volume of 10 ml/kg body weight was administered for each mouse by oral route. Mice were fasted 4 hours prior to dosing and further four hours post-dosing.

Study design

Since both the drugs Lamotrigine and 5-Flurouracil are already marketed and clinically used, a large data is available on their acute toxicological studies (i.e. the information about the LD50 values of the both the drugs). It has been determined that the oral LD50 value of Lamotrigine in mouse is 269 mg/kg [(14)]. The oral LD50 of 5-Fluorouracil in mouse is 115 mg/kg [(15)]. Now, in the present study the doses are administered at a starting dose of their LD50 values, if the mice doesn't show any signs of toxicity then for the next group of mice the doses are administered with an increase of $1/4^{th}$ of their LD50 values, in case if the mice shows any signs of toxicity then the doses are administered to the next group of animals in such a way that $1/4^{th}$ of the dose has been reduced from the LD50 dose values. Similarly if the mice shows any signs of toxicity, for the next group of mice the doses are reduced to half of the LD50 dose values of the both the drugs and are administered to the mice. Subsequently the doses are reduced until the animals don't show any signs of toxicity. And finally a single cross over dose administration is done between the safe doses and prior toxic doses i.e., the last safe Lamotrigine dose and prior toxic 5-Fluorouracil dose for one group and the last safe 5-Fluorouracil dose and prior toxic Lamotrigine dose for another group of mice, for determination of safe and maximum tolerable dose. . The Mice were observed individually after dosing for signs of toxicity and mortality at least once during the first 30 minutes, 1, 2, 3 and 4 hours. Subsequently, the Mice were observed twice a day for morbidity and mortality for a period of 14 days. Individual body weight was recorded for all animals on the day of commencement of treatment, weekly intervals and on the day of sacrifice.

Toxicological evaluations

For the determination of safe and maximum tolerable dose, after administration of each dose all the groups of mice are subjected for examination for signs of toxicity for 14 days. The signs of toxicity are observed by and evaluated by physical signs of toxicity, weight variation studies, histopathological, haematological and biochemical variation. By considering all the toxicological data of the study a safe and maximum tolerable dose is determined and concluded.

Results and Discussion

In the present study the animals are administered by the starting doses of the LD50 values of the both the drugs to the group-1 mice and they had shown the severe signs of the toxicity, the group-4 mice at the dose of $1/4^{th}$ of the LD50 values of the both the drugs had shown no signs of toxicity, finally a single cross over design were done between the safe doses and prior toxic doses. The groups division of mice and dose administration were done as explained in table-1.

After administration of the corresponding doses to different group of mice mentioned in table-1, the mice are examined for physical signs of toxicity, the observed physical signs of toxicity are as follows:

Physical signs of toxicity

Group-1: The group-1 mice which received Lmotrigine (269 mg/kg) and 5-fluorouracil (115 mg/kg) orally had shown the signs of toxicity like Somatomotor incordination, Tachypnea, Tachycardia, loss of Rightening reflex the mice had reached the moribund condition within 16-18 hours after drug administration and the mice were humanely sacrificed and were observed for the weight variation, hematological, biochemical and histopathological observations.

Group-2: Due to the above signs of toxicity the group-2 mice were administered with Lmotrigine(201.75 mg/kg) and 5-fluorouracil(86.25 mg/kg) orally and the mice had shown the signs of toxicity like Somatomotor incordination, Tachypnea, Tachycardia, loss of Rightening reflex the mouse had reached the moribund condition within 24-26 hours after the drug administration and the mice were humanely sacrificed and were observed for the weight variation, hematological, biochemical and histopathological observations.

Group-3: Due to the above signs of toxicity the group-3 mice were administered with Lmotrigine(134.5 mg/kg) and 5-fluorouracil(57.5 mg/kg) orally and the

mice had shown the signs of toxicity like Somatomotor incordination, Tachypnea, Tachycardia, loss of Rightening reflex but, the mice had shown the signs of recovery after 6 hours of drugs administration and then completely recovered and the mice were observed for 14 days for the signs of toxicity and weight variation and after the 14 days time period of observation the mouse were observed for the weight variation, hematological, biochemical and histopathological observations.

Group-4: Due to the above signs of toxicity the group-4 mice were administered with Lmotrigine (67.25 mg/kg) and 5-fluorouracil (28.75 mg/kg) orally and the mice had shown the signs like Somnolence, Lethargic the animal was observed for 14 days for the signs of toxicity and weight variation and after the 14 days time period of observation the mice were observed for the weight variation, hematological, biochemical and histopathological observations.

Now a single cross over study was done between the last safe and its prior toxic dose.

Group-5: Group-5 mice were treated with Lmotrigine(134.5 mg/kg) and 5-fluorouracil(28.75 mg/kg) the mice had shown the signs of toxicity like Somatomotor incordination, Tachypnea, Tachycardia, loss of Rightening reflex, circling movements the mice had shown the signs of recovery with in a time period of 10-12 hours and the mouse had got recovered from the toxic signs. The mice were kept in observation for a time period of 14 days. After the completion of 14 days of time period the mice were observed for the weight variation, hematological, biochemical and histopathological observations.

Group-6: The mouse which received Lmotrigine (67.25 mg/kg) and 5-fluorouracil (57.5 mg/kg) had shown no signs of toxicity and the animal were kept in observation for about 14 days for signs of toxicity and weight variation. After the 14 days time period of observation the mice were observed for the weight variation, hematological, biochemical and histopathological observations.

Hematological observations

Hematological analysis was performed for all drug treated groups of mice by collecting blood samples from the mice retro orbitally. Hematological parameters like hemoglobin concentration, hematocrit, erythrocyte count, total leukocyte, platelet count, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration was analyzed. The results of hematological observations are shown in table-2.

Biochemival observations

The biochemical parameters like SGOT, SGPT of various dose treated groups of mice were analyzed and are explained in table-3.

Weight variation observations

The weight variations of different dose treated groups of mice were observed periodically and are shown in table-4.

Histopathological observations

Histopathological examination of vital organs like brain, liver, kidney, lungs, colon, heart and spleen were performed. The changes observed are as follows:

Control group:

All organs were observed and noticed as normal state of organs.

Group-1:

The following histopathological observations have been noticed for the organs of group-1 mice which were treated with Lamotrigine (269 mg/kg) and 5-Fluorouracil (115 mg/kg) orally.

Brain: Mild demyelinating changes.

Lungs: Emphysematous changes.

Heart: Hemorrhage between myocardial cells, mild-moderate congestion.

Liver: Severe congestion, swollen hepatic cells, mild sinusoidal hemorrhages, proliferation of bile ducts and endothelial cells, binucleated hepatic cells, cloudy swelling.

Spleen: Spleenic hemorrhages.

Colon: Colon congestion.

Kidney: Severe congestion, intertubular hemorrhages, glomerular hemorrhage and severe Congestion, massive inter tubular hemorrhage.

Group-2:

The following histopathological observations have been noticed for the organs of group-2 mice which were treated with Lamotrigine (201.75 mg/kg) and 5-Fluorouracil (86.25 mg/kg) orally.

Brain: Meningial hemorrhages, proliferation of blood vessels, congestion of choroid plexus.

Lungs: Emphysematous changes, bronchectasis, alveolar macrophages, infiltration of mononuclear cells in between alveoli.

Heart: Sarcolysis, mild hemorrhages, cloudy swelling changes.

Liver: More congestion, swollen hepatic cells, mild sinusoidal hemorrhages, proliferation of Bile ducts and endothelial cells, binucleated hepatic cells, cloudy swelling, thrombus formation and hepatic lesions.

Spleen: Spleenic hemorrhages.

Colon: Normal.

Kidney: Congestion, intertubular hemorrhages.

Group-3:
The following histopathological observations have been noticed for the organs of group-3 mice which were treated with Lamotrigine (134.5 mg/kg) and 5-Fluorouracil (57.5 mg/kg) orally.
Brain: Grouping of cells in molecular layer.
Lungs: Mild areas of congestion, perivascular and peribronchial lymphoid aggregates, emphysematous changes, alveolar hemorrhages.
Heart: Sarcolysis.
Liver: More congestion, swollen hepatic cells, mild sinusoidal hemorrhages, proliferation of bile ducts and endothelial cells, binucleated hepatic cells, cloudy swelling, thrombus formation and hepatic lesions.
Spleen: Spleenic hemorrhages.
Colon: Normal.
Kidney: Mild congestion, proliferation of mesengial cells.

Group-4:
The following histopathological observations have been noticed for the organs of group-4 mice which were treated with Lamotrigine (67.25 mg/kg) and 5-Fluorouracil (28.75 mg/kg) orally.
Brain: Congestion of choroid plexus, proliferation of blood vessels, demyelinating changes in cerebrum.
Lungs: Mild alveolar hemorrhages, peribronchial lymphoid aggregates, bronchodilation.
Heart: Mild cloudy swelling, diffuse areas of congestion.
Liver: Mild congestion, karyomegaly.
Spleen: Spleenic hemorrhages.
Colon: Normal.
Kidnay: Diffusive areas of congestion and hemorrhages.

Group-5:
The following histopathological observations have been noticed for the organs of group-5 mice which were treated with Lamotrigine (134.5 mg/kg) and 5-Fluorouracil (28.75 mg/kg) orally.
Brain: Proliferation of blood vessels, gliosis, atrophy of neurons.
Lungs: Moderate emphysematous changes, mild areas of congestion.
Heart: Mild sarcolysis.
Liver: Infiltration of inflammatory cells in between hepatic cells i.e., extensive infiltration of mononuclear cells and neutrophils in between hepatic cells, mild congestion, karyomegaly.
Spleen: Spleenic hemorrhages, gaint cells, R.e cell hyperplasia.
Colon: Normal.
Kidney: Mild congestion.

Group-6:
The following histopathological observations have been noticed for the organs of group-6 mice which were treated with Lamotrigine (67.25 mg/kg) and 5-Fluorouracil (57.5 mg/kg) orally.
Brain: Mild proliferation of blood vessels.
Lungs: Diffusive alveolar hemorrhages, peribronchial lymphoid aggregates.
Heart: Diffused areas of congestion, mild hemorrhages.
Liver: Normal.
Spleen: Mild spleenic hemorrhage.
Colon: Desquamation of lining of epithelium.
Kidney: Moderate.
The most considerable histopathological toxicities of the present study are shown in the figure1, 2, 3, 4, 5 and 6.
By considering the results obtained from the present studies, through all the toxicological data it is very explicit that the combination is very toxic at all different doses, but the group-5 mice for which the drugs administered at Lamotrigine (67.25 mg/kg) and 5-Fluorouracil (57.5 mg/kg) is relatively safer than the other administered doses. Thus it is concluded that the combination of Lamotrigine at adose of 67.25mg/kg orally and 5-Fluorouracil at a dose of 57.5 mg/kg orally is the maximum tolerable dose for further in vivo anticancer activity in swiss albino mices.

Acknowledgement

I, K. Sudhakar thankfully acknowledge SUGEN LIFE SCIENCES, tirupati India, for providing all necessary facilities and also grateful to C. Shanmuga reddy for his valuable guidance and assistance in pursuing the research. I am also greatly thankful to Dr. Chi, Srilatha, Department of Veterinary Pathology, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh for her valuable guidance. It is great privilege to me to mention my professor Dr. D. Shivaraman for his valuable guidance from Vagdevi College of Pharmacy and Research Centre, Nellore and I would like to thank all the individuals who have contributed for the successful completion of the work for their encouragement and support.

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Table1: Experimental Design

GROUP	DOSE mg/kg		DOSE VOLUME	NO.OF ANIMALS
	Lamotrigine	5-Flurouracil		
Control	Normal saline	Normal saline	10ml/kg	5
Group 1	269	115		5
Group 2	201.75	86.25		5
Group 3	134.5	57.5		5
Group 4	67.25	28.75		5
Group 5	134.5	28.75		5
Group 6	67.25	57.5		5

Table 2: Hematological observations

Group	RBC (10^6/µL)	WBC (10^3/µL)	HGB (g/Dl)	HCT (%)	MCV (Fl)	MCH (Pg)	MCHC (g)	PLT (/µL)
Control	5.77±0.04* 5.77±0.02**	13.41±0.56* 13.41±0.32**	16.1±0.36* 16.1±0.20**	43.74±0.85* 43.74±0.49**	53.8±1.75* 53.8±1.01**	23.19±0.67* 23.19±0.38**	35.03±0.30* 35.03±0.17**	25±2* 25±1.15**
Group 1	4.71±0.23* 4.71±0.13**	12.23±0.35* 12.23±0.20**	13.39±0.41* 13.39±0.24**	36.26±0.43* 36.26±0.24**	49.41±0.31* 49.41±0.18**	22.41±0.58* 22.41±0.33**	31.08±0.26* 31.08±0.15**	27.67±0.57* 27.67±0.33**
Group 2	5.18±0.15* 5.18±0.08**	12.96±0.12* 12.96±0.07**	14.87±0.36* 14.87±0.20**	41.34±0.34* 41.34±0.19**	53.13±0.32* 53.13±0.18**	21.97±0.17* 21.97±0.10**	32.36±0.33* 32.36±0.19**	28.33±0.57* 28.33±0.33**
Group 3	5.48±0.34* 5.48±0.2**	13.35±0.38* 13.35±0.21**	14.74±0.11* 14.74±0.66**	41.92±0.19* 41.92±0.11**	48.4±0.83* 48.4±0.48**	22.81±0.36* 22.81±0.21**	34.31±0.40* 34.31±0.23**	18.67±0.57* 18.67±0.33**
Group 4	5.64±0.12* 5.64±0.06**	12.4±0.34* 12.4±0.19**	15.49±0.32* 15.49±0.18**	43.25±0.81* 43.25±0.46**	53.44±0.35* 53.44±0.20**	23.32±0.28* 23.32±0.16**	35.49±0.30* 35.49±0.17**	21±0* 21±0**
Group 5	5.59±0.15* 5.59±0.09**	13.54±0.07* 13.54±0.04**	15.32±0.38* 15.32±0.04**	42.69±0.38* 42.69±0.21**	52.27±0.57* 52.27±0.33**	22.43±0.43* 22.43±0.25*8	35.04±0.24* 35.04±0.13**	18.33±0.5* 18.33±0.33**
Group 6	5.68±0.23* 5.68±0.13**	13.25±0.15* 13.25±0.08**	15.49±0.49* 15.49±0.28**	42.63±0.49* 42.63±0.28**	51.08±0.37* 51.08±0.21**	22.13±0.33* 22.13±0.19**	35.21±0.33* 35.21±0.19**	23.67±0.57* 23.67±0.33**

Inference: MEAN±S.D*, MEAN±S.E**

Table 3: Biochemical observations

Group	SGOT (U/L)	SGPT (U/L)
Control	34±0.25* 34±0.14**	21.67±0.66* 21.67±0.38**
Group 1	38.3±0.13* 38.3±0.07**	26.1±0.36* 26.1±0.21**
Group 2	37.32±0.35* 37.32±0.20**	25.11±0.23* 25.11±0.13**
Group 3	33.26±0.55* 33.26±0.32**	22.09±027* 22.09±0.15**
Group 4	32.26±1.08* 32.26±0.62**	19.11±0.20* 19.11±011**
Group 5	33.16±0.11* 33.16±0.06**	21.76±0.86* 21.76±0.49**
Group 6	31.75±0.35* 31.75±0.20**	18.45±0.41* 18.45±0.23**

Table 4: Weight variations

Group	Weight in gm		
	0 day	7 days	14days
Control	24.20±1.31* 24.20±0.75**	25.51±1.93* 25.51±1.11**	27.19±2.05* 27.19±1.18**
Group 1	22.766±2.12* 22.766±1.12**	-	-
Group 2	21.76±1.3* 21.76±0.77**	-	-
Group 3	23.75±0.71* 23.75±0.41**	24.45±1.64* 24.45±0.94**	25.67±1.81* 25.67±1.04**
Group 4	22.43±1.23* 22.43±0.71**	23.31±1.03* 23.31±0.59**	24.94±0.69* 24.94±0.40**
Group 5	23.94±0.71* 23.94±0.41**	25.47±0.59* 25.47±0.34**	26.13±1.74* 26.13±1.00**
Group 6	24.03±0.34* 24.03±0.18**	25.28±0.56* 25.28±0.32**	26.29±1.17* 26.29±0.67**

Inference: MEAN±S.D*, MEAN±S.E**

Inference: (-) indicates that the mice had reaches the moribund condition befor time period and were humanely sacrificed.

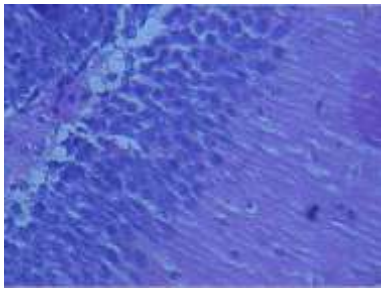
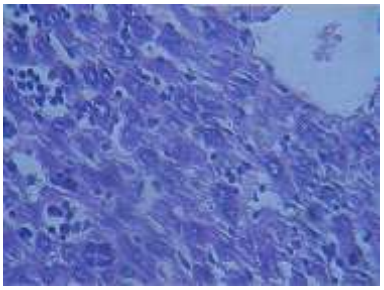


Figure-1



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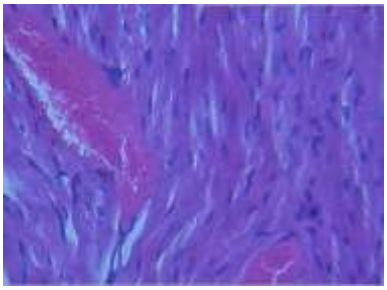

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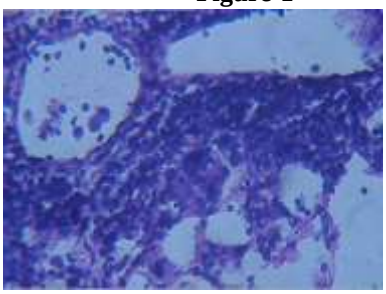

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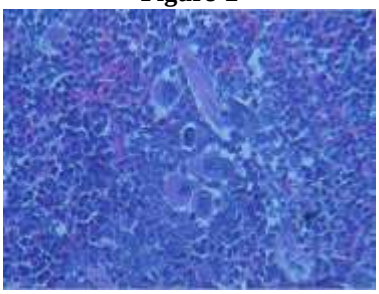

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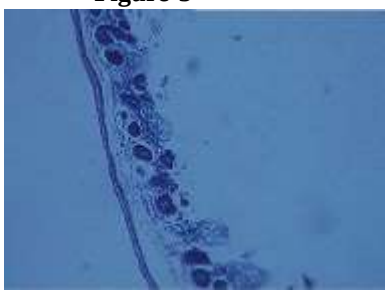

Figure-6

Inference: Figure-1: The brain of group-1 mice showing the Mild demyelinating changes.

Figure-2: The liver of group-2 mice showing more congestion, swollen hepatic cells, mild sinusoidal hemorrhages, proliferation of bile ducts and endothelial cells, binucleated hepatic cells, cloudy swelling, thrombus formation and hepatic lesions.

Figure-3: The heart of group-3 mice showing Sarcolysis.

Figure-4: The lung of group-4 mice showing mild alveolar hemorrhages, peribronchial lymphoid aggregates, bronchodilation.

Figure-5: The spleen of group-5 mice showing spleenic hemorrhages, gaint cells, reticulo endothelial cell hyper plasia.

Figure-6: The colon of group-6 mice showing the desquamation of lining of epithelium.

How to cite this article

Sudhakar K. (2014). Determination of maximum tolerated dose of combination of Lamotrigine and 5-fluorouracil by oral route in swiss albino mice for further *In Vivo* Anticancer activity. *Int. J. Pharm. Life Sci.*, 5(9):3825-3831.

Source of Support: Nil; Conflict of Interest: None declared

Received: 18.08.14; Revised: 26.08.14; Accepted:01.09.14