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Original Research Article

A Study to Find out Additive Analgesic Effect of Flupirtine Maleate, Tramadol Hydrochloride and Paracetamol in Experimental Pain Management in Mice

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Abstract

Controlled animal experiment was done at Department of Pharmacology, R. G. Kar Medical College and Hospital after getting permission from institutional animal ethics committee to find out a drug with good analgesic & safety profile for long term use in high risk population like hepatic, cardiac, hypertensive, diabetic patients. Flupirtine maleate, Tramadol hydrochloride and Paracetamol these three compounds have known analgesic property of their own and they are relatively safe than NSAIDS and opioids. Conventional Hot plate and Tail flick methods were used to access the analgesic effects of drugs. Total number of 36 screened Swiss albino mice were taken in the experiment. Mices were divided into six groups from Ato F with six mices in each group, where A is the control, B,C,D,E,F are the flupirtine(B), tramadol(C), paracetamol(D), combined half(E) and comboned full doses(F) of these three drugs. Result were noted at 20, 60 and 90 minutes intervals. Analgesic effects between these groups were compaired and p value were extracted. All the drugs individually as well as in combinations with their half and full doses showed an increase reaction time in comparison with control. Combinations in half and full doses also showed more nalgesic property than individual drugs. So, we may conclude that combination of flupirtine, tramadol and paracetamol have supra additive analgesic effects.

Keywords: Flupirtine, Tramadol, Paracetamol, Analgesic, Opioids, Combination.

INTRODUCTION

Pain is a sensory experience of special significance to physicians and basic scientist and it is a common reason for individuals to seek medical care. Pain (algesia) is an intensely subjective experience and is therefore difficult to describe, but it has two features which are nearly universal.

Features are (a)unpleasant & (b)evoked by a external or internal noxious stimuli. Analgesics are drugs that selectively relieves pain by acting in the CNS or on peripheral pain mechanism, without significantly altering consciousness [1]. Analgesics are divided into two groups, Opioids & non-opioids (including anti-inflammatory). Opioids produce analgesia by binding to specific GPCR in brain & spinal cord. It is well established that NSAIDs inhibit the activity of both cyclooxygenase-I & II (COX-I & II) and thereby the synthesis of prostaglandin (PG) & thromboxans. Inhibition of COX-II leads to the anti-inflammatory, analgesic & anti-pyretic effects [2].

Inflammation means pain, redness, swelling and heat that usually developed in response to injury or illness. The management of acute and chronic pain is important for the patient's wellbeing. Though opioids analgesic like morphine, pethedine (Meperidine) is good painkiller but they abuse potential and lots of side effects for prolong use like respiratory depression, tolerance and dependence. Non-steroidal anti-inflammatory drugs (NSAIDS) are also good analgesic but some complication like neuropathy, hypersensitivity, haemolytic anemia, hepatic damage (Reye's syndrome), acid peptic disorder are common adverse effects. The choice of analgesics for acute pain depends on the efficacy, side-effects, complications, pharmacokinetics and its cost-effectiveness.

So, we are in search of drugs with good analgesic property & with good safety profile for long term use in high risk population like hepatic, cardiac, hypertensive, diabetic and cancer patients. Flupirtine maleate, Tramadol hydrochloride and Paracetamol these three compounds have known analgesic property

of their own and they are relatively safe than other NSAIDS and opioids. Co-administration of these three compounds may have additive analgesics effect and we may be able to reduce their dose when these compounds are combined together for management of pain. Also, they can be safely used in severe pain and for long duration. There are only few researches published all over the world in this matter and actual data regarding the efficacy and safety of these three combinations are not available till date.

Flupirtine

Non-steroidal anti-inflammatory and non-opioid. It is triaminopyridine derivatives having a chemical structure 2-amino-3 ethoxy-carboxylamino-6-4 fluro-benzylamine-pyridine. The basic molecule used for synthesis of flupirtine was 2, 6 dichloro3 nitropyridine. It was first synthesized in 1980 in Germany and was marked by Degussa pharma. Indirectly acting as N-methyl-D aspartate receptor antagonist by activation of k+channel (NMDA) [3]. Flupirtine is in use for the last 25years for the management of pain flowing trauma, dental extraction, surgery, pain associated with muscle spasm, cancer, degenerative joint disease [4, 5].

Tramadol

Synthetic opioids which relieves pain by opioid as well as additional mechanism. It is affinity for μ/ κ/δ recetor is low. Unlike other opioid it inhibit reuptake of NA & 5HT, increases 5HT release & thus activates monoaminergic spinal inhibition of pain. It is indicated for mild to moderate short-lasting pain due to diagnostic procedures, injury, surgery etc. but not effective in severe pain [6].

Paracetamol

One of the oldest and most used analgesics and antipyretic drug. Mediated centrally and may involve direct and indirect inhibition of central cyclo-oxygenases, but also the activation of the endocannabinoid system and spinal serotonergic pathway. The central analgesic action of paracetamol is like as aspirin. It raises the pain threshold but has weak peripheral anti-inflammatory action [7].

In this study we are trying to find out the combination of lowest effective dose (s) of Paracetamol, Tramadol hydrochloride & Flupirtine maleate to produces good and sustained analgesia as additive effects and as well as combination of these three drugs at their half of the individual dose in experimental pain management in mice.

OBJECTIVE OF THE PESENT STUDY

- To find out additive analgesic effects of Flupirtine maleate, Tramadol hydrochloride & Paracetamol in combination in their analgesic doses.
- To find out the combination of these above three drugs in their half analgesic doses.

MATERIAL AND METHODS

1. Place of the study: Department of Pharmacology, R.G. Kar Medical College and Hospital.
2. Study design: Controlled animal experimental study
3. Periods of the study: February2017-July 2018, total period of 18 months.
4. Materials: Instruments-
   - Eddy' Hot plate apparatus,
   - Tail flick apparatus (Radiant heat),
   - Stop watch,
   - Digital Weighing machine
   - Feeding cannula
   - Tuberculin syringe
   - Beakers
   - Marking pen

Animals: 80 no of Swiss albino mice (were procured from Government Licensed animal supplier).

FLOW CHART FOR EXPERIMENT

POOL OF MICE (80)

↓

Screening for sensitivity of thermal & radiant heat 36

Screened mice

↓

Mices are divided into 6 groups as follows:-

Table-1:

Group	Drugs with dose	
A	CONTROL	Distilled Water (DW)
B	FLUPIRTINE	15MG/KG
C	TRAMADOL	10MG/KG
D	PARACETAMOL	300MG/KG
E	½ (B+C+D)	(7.5+5+150)
F	B+C+D	(15+10+300)

Ethical clearance: The experiments were carried out in accordance with the guidelines of the Committee (CPCSEA,2003) and approved by Institutional Animal Ethics Committee at R.G. Kar Medical College & Hospital(IAEC-RGK Ref: RKC/1AEC/16/07 date-24/11/16)

- Methods: Healthy Swiss albino mice (weight18-22 g, average =20 g) & 2-6 months age of either sex were procured from the central animal house of the institute. They were housed in standard polypropylene cages in well ventilated room at least 10 days for acclimatization in the laboratory conditions and kept under controlled room temperature (24+/-20 C; relative humidity 60-70%) in 12 h light-dark cycle the mice were given a standard laboratory diet and water ad libitum. After proper acclimatization and then 36 mice are sorted

from pool of 80 mice with their normal response (2-3 seconds) through Hot plate & Tail flick methods both. The mice were divided into control and treatment groups, comprising on an average of 6 animals in each group. Both the groups of mice were kept on overnight fasting and on next day the fasting mice were taken out from the cage gently without giving much stress and properly weighed in the small animal weighing balance are recorded in the data sheet. With the help of a marker pen the mice were marked at a point proximal to tip of the tail for proper identification before experiment [8].

- Food was withdrawn 12h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee.
- All drugs were administered per-orally amount used as mg/kg bw by feeding cannula. Weights are taken & recorded. The reaction time of mice to thermal pain was recorded prior to administration of drugs. After that Distilled water and test drugs were administered orally and reaction time to thermal pain (55°c) was noted at 20, 60, & 90 minutes after drug administration.

Normally a cut off period of 15 seconds is allowed to prevent damage to the skin [13].The length of time until response indicates the period of greatest activity after dosing. An ED50 value is calculated at the peak time of drug activity.

Table-2:

Methods	No. of Mice
1. Hot plate	36
2. Tail flick	36

CHEMICAL & DRUGS used:

1. 5% Gum Acasia
2. Distrilled water
3. Paracetamol
4. Flupirtine Maleate
5. Tramadol hydrochloride

STATISTICAL ANALYSIS

Statistical analysis of data: Data collected were interpret MS Excel to create a data base. Standard statistical presentation like tabulation, proportion, percentage, mean, Standard deviation etc were used. Significance testing was done by unpaired t test, with the help of WINPEPI –statistical software, version-2.0, windows compatible-p-value less than 0.05(<0.05) was considered statistically significant.

RESULTS & OBSERVATIONS

Table-3: HOT PLATE METHOD: Group A (Control Group) vs Group E& F

Group	N	Mean (µ)	Sd
AHP20	6	2.31	0.172
AHP60	6	2.3	0.141
AHP90	6	2.316	0.132
EHP20	6	7.2	1.208
EHP60	6	9.55	1.125
EHP90	6	11.416	1.271
FHP20	6	8.116	0.515
FHP60	6	10.983	1.144
FHP90	6	13.483	0.56

HP-HOT PLATE

Table-4: TAIL FLICK METHOD: Group A (Control Group) vs Group (E & F)

Group	Mean(µ)	Sd
ATF20	2.3	0.126
ATF60	2.316	0.133
ATF90	2.316	0.183
ETF20	7.63	0.644
ETF60	9.85	0.575
ETF90	7.63	0.644
FTF20	8.433	0.7
FTF60	11.283	0.719
FTF90	13.866	0.736

TF- TAIL FLICK

Table 3 and 4 show mean reaction time increases at Group-F (Full dose) & Group-E(half dose) in relation to Group-A (Control) in Hot plate & Tail Flick method.

Table-5: HOT PLATE: Group A vs Group (B, C & D)

Group Compaired		M	Sd	t-value	p-value
AHP vs BHP	@20	6.066	0.516	16.92	0.000
	@60	8.716	0.44	34.01	0.000
	@90	10.133	0.471	33.89	0.000
AHP vs CHP	@20	6.25	1.253	7.63	0.000
	@60	7.98	0.78	17.55	0.000
	@90	10.483	0.806	23.15	0.000
AHP vs DHP	@20	5.05	0.455	13.8	0.000
	@60	7.03	0.403	27.14	0.000
	@90	9.2	0.666	22.93	0.000

Table-5 Shows highly significant p-value (<0.05) in all reading in group A with Group B, C & D at 20/60/90min in Hot plate method.

Table-6: TAIL FLICK: Group A vs Group (B, C & D)

Group Compaired		M	Sd	t-value	p-value
ATF vs BTF	@20	6.25	0.459	20.33	0.000
	@60	8.96	0.814	19.73	0.000
	@90	10.467	0.183	25.86	0.000
ATF vs CTF	@20	7.73	1.465	9.05	0.000
	@60	8.716	0.923	16.81	0.000
	@90	11.183	1.523	14.16	0.000
ATF vs DTF	@20	5.28	0.444	5.82	0.000
	@60	7.216	0.81	14.62	0.000
	@90	9.583	0.837	20.78	0.000

Table-6 Shows highly significant p-value (<0.05) in all reading in group A with Group B, C & D at 20/60/90min in Tail flick method.

Table-7: HOT PLATE: Group E vs Group (B, C & D)

Group Compaired		M	Sd	t-value	p-value
EHP vs BHP	@20	6.066	0.516	2.11	0.061
	@60	8.716	0.44	1.69	0.122
	@90	10.133	0.471	2.32	0.043
EHP vs CHP	@20	6.25	1.253	1.34	0.211
	@60	7.98	0.78	2.81	0.018
	@90	10.483	0.806	1.52	0.160
EHP vs DHP	@20	5.05	0.455	4.08	0.002
	@60	7.03	0.403	5.17	0.000
	@90	9.2	0.666	3.78	0.004

Fig-3A shows highly significant p-value (<0.05) in Group D(DHP 60) and significant on BHP90, CHP60, DHP20 & DHP 90 0F C & D group and insignificant on group BHP20, BHP60, CHP 90, CHP90 in Hot-Plate method.

Table-8: TAIL FLICK; Group E vs Group (B, C& D)

Group Compaired		M	Sd	t-value	p-value
ETF vs BTF	@20	6.25	0.459	4.27	0.002
	@60	8.96	0.814	2.19	0.054
	@90	10.467	0.75	2.7	0.022
ETF vs CTF	@20	7.73	1.465	0.15	0.881
	@60	8.716	0.923	2.55	0.029
	@90	11.183	1.523	0.75	0.471
ETF vs DTF	@20	5.28	0.444	7.36	0.000
	@60	7.216	0.81	6.5	0.000
	@90	9.583	0.837	4.38	0.001

Table-8 shows significant p-value (<0.05) in Group E(BTF 20,90,CTF60 & DTF-90) and not significant in BTF-60,CTF-20& CTF90), and highly significant in DTF-20& 60) in Tail flick method.

Table-9: HOT PLATE: Group F vs Group (B,C & D)

Group Compaired		M	Sd	t-value	p-value
FHP vs BHP	@20	6.066	0.516	4.89	0.001
	@60	8.716	0.44	4.53	0.001
	@90	10.133	0.471	11.210	0.000
FHP vs CHP	@20	6.25	1.253	3.37	0.007
	@60	7.98	0.78	5.31	0.000
	@90	10.483	0.806	7.490	0.000
FHP vs DHP	@20	5.05	0.455	9.32	0.000
	@60	7.03	0.403	7.98	0.000
	@90	9.2	0.666	12.060	0.000

Table-9 shows significant p value (<0.05) in compaired group BHP-(20 & 60,) &CHP-20 but highly significant in BHP-90, CHP (60 & 90) & DHP (20/60/90) in Hot plate method.

Table-10: TAIL FLICK: Compaired Group-FTF vs Group-B,C & D)

Group Compaired		M	Sd	t-value	p-value
FTF vs BTF	@20	6.25	0.459	6.39	0.000
	@60	8.96	0.814	5.24	0.000
	@90	10.467	0.75	7.920	0.000
FTF vs CTF	@20	7.73	1.465	1.06	0.314
	@60	8.716	0.923	5.37	0.000
	@90	11.183	1.523	3.890	0.003
FTF vs DTF	@20	5.28	0.444	9.32	0.000
	@60	7.216	0.81	9.2	0.000
	@90	9.583	0.837	9.410	0,000

Table-10 Shows not significant p value (<0.05) in compaired group CTF--(20) significant in group-CTF-20 but highly significant in BTF (20/60/90), CTF (60 & 90) in DTF (20/60/90) in Tail flick method.

DISCUSSION

Direct activation by intense pressure & consequent cell damage induces lower pH (H+) and leads to release of potassium (K+) and to synthesis of prostaglandins (PG) and bradykinin (BK) , prostaglandin (PG) are increase the sensitivity of the terminal to bradykinin (BK) & other pain producing substances [9].

In secondary activation impulses are generated in the stimulated terminal propagate not only to the spinal cord but also into other terminal branches where they include the release of peptides, substance-Pthat causes vasodilation and neurogenic oedema with further accumulation of bradykinin (BK) and it also cause the release of Histamin (H) from mast cells and serotonin (5HT) from platelets [10].

Opioids receptor activation reduces intracellur cAMP formation & opens K + channels (mainly μ & δ) or closes voltage gated N type Ca+ channel (κ). This results in neuronal hyperpolarization and reduced availability of intracellular Ca+…→ decreased neurotransmitter release by cerebral, spinal and myenteric neurons(eg-glutamate & neuropeptides from primary nociceptive afferent). Activationof these receptors results in K+ channel opening, hyperpolarization & neuronal inhibition. Synergism can be additive-the effect of the two drugs is in the same direction and simply adds up effect of drugs A+B=effect of drug A+ effect of drug B or The effect of combination is greater than the individual effects of the components. Effect of drug A + B > effect of drug A + B= Supraadditive or Potentiation.

Flupitrine being a K+ channel opener increases pain threshold of pain sensation.Tramadol, a synthetic opioid analgesic also reduces pain sensation. Paracetamol reduces prostaglanding synthesis in CNS and incerases pain threshold. So, in our study their combination has supraadditive analgesic effect. In another study Naser *et al*., also got the same supra-additive analgesic effect of the combination of tramadol and flupitrine maleate [11].

In another stusy Adis *et al*., also got the additive analgesic affect of fixed dose combination of tramadol and paracetamol, which establishes our finding in the above animal experiment [12].

The interaction may take place at pharmacokinetic or pharmacodynamic level. In hot plate method flupirtine, tramadol & paracetamol received mice showed significant increase in mean reaction time compared to the control group, at 20, 60 & 90 minutes. There was a significant increase in (p<0.05) reaction time in group E (Combination of ½ individual dose) than control group (A) & in group F (Combination of full individual dose).

CONCLUSION

The present study is able to reestablish the analgesic role of Flupirtine maleate, Tramadol hydrochloride and & Paracetamol individually & more in combindly.

The present work has shown that flupirtin maleate+tramadol hydrochloride+paracetamol has greater analgesic effect compared to individual drug alone. So, we can conclude that flupitrine, tramadol and paracetamol combination may be an effective analgesic with their supra additive effects.

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