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A 28-DAYS SUB-ACUTE TOXICITY STUDY IN SWISS ALBINO MICE TO ASSESS NEUROTOL PLUS (GLYCEROL AND MANNITOL IN COMBINATION) TOXICITY PROFILE

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ABSTRACT

Osmotic drugs continue to be the utmost communal treatment option accessible to control intracranial pressure (ICP). The combination of glycerol and mannitol is a better alternative and is currently available as the best therapy used to increase ICP. The purpose of this analysis was to investigate the effect of repetitive doses (28 days) of the combined neurotol plus, 20% mannitol and 10% glycerol on the safety profile. A 28-days sub-acute toxicity analysis was performed at 3 various level of dose, 5 ml / kg, 10 ml / kg and 20 ml / kg. Into 4 groups of 6 animals each; mice were divided randomly. As a final evaluation point, physical and biochemical parameters as well as hematological parameters related to liver toxicity and nephrotoxicity were examined. We also perform histopathological examinations to assess the toxicity of individual organs. This study showed no or minimal changes (at high doses) in several hematological, biochemical and physical parameters between the control group and the neurotol plus group. Taken together, the statistics from this analysis show that the amalgamation of glycerol and mannitol is not related with any grave side effects and is a safe beneficial option to reduce ICP.

Keywords: neurotol plus; mannitol; glycerol; repeated dose toxicity; intracranial pressure.

INTRODUCTION

Cerebral edema is the leading reasons of augmented ICP, secondary deterioration of health, and death in stroke patients[1]. Earlier suggested treatments such as hyperventilation or barbiturates have been progressively interrogated in recent years, as they are recognized to decrease cerebral perfusion pressure by adversely affecting systemic blood pressure or by over-constricting cerebral vessels. From this perspective, hypertonic fluid therapy has a vital role in lowering ICP deprived of adversely affecting cerebral perfusion pressure. There are various studies supportive to the potential of substances such as mannitol and glycerol in reducing brain edema[2-3]. Several experimental and clinical analysis have shown that a sole dose of mannitol can significantly decrease the increase in ICP. Though, the longstanding benefit of mannitol is debatable and there is little evidence of an increase in cerebral edema following repeated mannitol therapy. Glycerol is alternative agent that has been institute to be valuable in regulation of ICP in pathological conditions and edema. In addition to their nature as a hypertonic solution, they also cause dilation of blood vessels, acting as free radical scavengers, antioxidants and activators of plasma prostaglandins. In addition, glycerol 10% can enhance energy metabolism in ischemic brain injury, as is clearly seen in the current literature. The two agents, namely glycerol and mannitol, were collective as neurotol plus to fill the gaps related with monotherapy with both agents. The mixture approach improves the back diffusion from the cerebrospinal fluid into the plasma by increasing the osmolality of plasma[4-5]. For this protective effect; two mechanisms are responsible are the redeployment of blood flow in the brain and the regional blood volume in the brain, and the reduction of focal cerebral edema. Amongst other glycerol 10% advantages can be a substitute root of energy directly through brain metabolism or through improved lipogenesis indirectly or both if glucose is not available. Our unpublished data suggest that this mixture is a good substitute to another first line medicine in patients with cerebral edema or hypertension[6]. Although glycerol and mannitol are extensively cast-off to lower high ICP and have many advantageous effects compared to other remedies, their long-term

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efficacy has not been recognized yet. Since the noxiousness profile is vital for any novel formulation to move from the preclinical to the clinical stage. Taking this fact into account, we conducted a 28 days sub-acute toxicity study to evaluate the neurotol plus (10% glycerol and 20% mannitol) safety profile as a mixture régime in Swiss albino mice.

MATERIALS AND METHODS

The healthy Swiss albino mice (15-20 g male and female) were alienated into 4 groups (one control group and three treatment groups). There are 3 male and 3 female mice in every group. Animals received typical diet (pellets) and water was administered ad libitum. At a precise room temperature of 25 ± 2 ° C; mice were kept in a polyurethane cage and at moisture level of 50.5 ± 5% and on a constant light-dark program (12 hours of light and dark).

Neurotol plus (glycerol 10% and mannitol 20%) was directed I.V at 3 level of dose, ie 5 ml / kg, 10 ml / kg and 20 ml / kg body weight in low, medium and high dose, respectively, for 28 days. Control animals were given a false saline solution. The procedure is performed once a day for 28 days. The Institutional Animal Ethics Committee has reviewed the study and accepted by it.

Physical assessment (water consumption, food and body weight) and the local lesion were examined during the treatment. Death, if any, was also recorded for all groups during the treatment period. If an animal died during treatment, an autopsy was performed. At the finish of the study, biochemical (kidney and liver function tests), histological and hematological parameters were examined. The organs were removed quickly, sent for histological examination and pondered on a digital scale. The ratio of each organ body weight was documented and for H&E staining; all the tissues were processed. By cardiac puncture; blood was withdrawn. For routine hematology parameters; blood samples were analyzed. Blood counts were made on the basis of blood smears. The biochemical parameters in serum and plasma were determined. Serum glutamine oxalacetate transaminase (SGOT), SGPT, ALT, BUN, plasma sugar and protein were predicted. The animals were forfeited at the finish of the study and several organs, such as the kidneys liver, gonads and lungs were sent for histology. 10% buffered formalin was used to store the organs and histologically examined by H&E staining.

Outcomes are presented as mean ± SD. ANOVA test was applied to determine importance of the variance between the groups. If there are substantial alterations on ANOVA, Dunnet's test was applied for post hoc analysis. P less than 0.05 was taken statistically important.

RESULTS

The results of this study did not reveal any adversative changes in the physical topographies during the treatment. At the end of the treatment, there was no variation in the animals mean body weight in the neurotol plus groups related with the control group (Tables 1 and 2).

There were no substantial variations in total white blood cell count (WBC), platelet counts and red blood cells (RBC) in all treatment groups compared to the corresponding control groups (Tables 3 and 4).

DISCUSSION

The purpose of this analysis was to examine the toxicity profile of numerous doses of neurotol cast-off to decrease high ICP, frequently related with cerebral edema. Glycerol can decrease brain swelling and upsurge blood flow in the specific region of the brain. Current reports also suggest a beneficial metabolic effect on the metabolism of nerve cells[7]. A combination of the two therapeutic principles has been found to be superior to either using either agent alone. Inadequate information is accessible on the safety profile of this drug. So, we examined the toxic effects of a combination product name neurotol plus. In the case of mannitol, this effect has been repeatedly demonstrated in human and animal radiological studies. Mannitol is an unmetabolized, cell-impermeable sugar that is administered intravenously as a hypertonic solution and is often used to treat cerebral edema. Clinical concentrations of mannitol activate tyrosine and stress kinases and induce apoptosis in bovine aortic endothelial cells[8]. Therefore, the clinical use of mannitol may have a direct detrimental effect on the vascular endothelium by increasing oxidative stress. Glycerol's dilate blood vessels by acting as free radical scavengers, antioxidants and activators of plasma prostaglandins. Glycerol also increases the brain's ischemic energy metabolism and acts as an energy source for cells. A recent report suggests that glycerol infusion may normalize intracranial volume by dehydrating normal but intact brain tissue and protecting against rebound phenomena in mannitol therapy. Therefore, the associated regimen improved the safety profile of mannitol[9]. The evaluation showed that treatment with neurotol plus had no more or less unfavorable

effect on any of the groups. This may be due to the protective effect of glycerol. Neurotol plus had no effect on SGOT, SGPT and serum alkaline phosphatase activity; this established that neurotol plus no change in liver function in both sexes compared to the corresponding control group. Recent studies designate that pretreatment with 10% mannitol or glycerol has a defensive effect on late neuronal death in the gerbil's hippocampus. No variations in morphology were experiential in the brains of mice treated with neurotol plus[10-11]. Histopathological analysis showed no signs of toxicity in any organ in the treatment groups compared to neurotol plus. Therefore, the histopathological studies also confirmed the data on the safety of other physiological, biochemical and hematological parameters after treatment with neurotol plus[12-15].

CONCLUSION

It seems to be undisputed that the hypertonic regimen associated with the solution did not cause any harmful effects in mice at the two dose levels. Though, slight changes in several parameters were noted at high dose levels. This analysis delivers clinically applicable data that can be used to make a therapeutic safety decision for the current dosing regimen. Based on the results of this study, it can be concluded that neurotol plus combination therapy may be a safe alternative to ICP and cerebral edema.

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Tables

Table 1.

Parameters	Organ	Control	Neurotol plus (5 ml/kg)	Neurotol plus (10 ml/kg)	Neurotol plus (20 ml/kg)
Food Intake/day (g)		3.2 ± 0.3	3.1 ± 0.6	3.1 ± 0.4	3.4 ± 0.6
Body weight (g)		27.88 ± 1.63	24.98 ± 1.43	25.22 ± 2.25	25.92 ± 1.97
Water Intake/day (ml)		4.2 ± 0.5	4.4 ± 0.3	4.0 ± 0.5	4.0 ± 0.8
Organ body weight ratio (%)	Liver (g%)	9.71 ± 2.18	10.31 ± 2.46	10.40 ± 3.19	9.87 ± 2.55
	Kidney (g%)	1.88 ± 0.27	2.04 ± 0.29	2.00 ± 0.31	2.02 ± 0.22
	Heart (g%)	0.90 ± 0.20	0.92 ± 0.21	0.89 ± 0.18	0.96 ± 0.17
Organ weight (g)	Liver (g)	2.64 ± 0.56	2.63 ± 0.64	2.71 ± 0.89	2.59 ± 0.59
	Kidney (g)	0.57 ± 0.13	0.58 ± 0.15	0.58 ± 0.13	0.59 ± 0.12
	Heart (g)	0.31 ± 0.12	0.30 ± 0.12	0.30 ± 0.11	0.32 ± 0.11

Table 2.

Parameters	Organ	Control	Neurotol plus (5 ml/kg)	Neurotol plus (10 ml/kg)	Neurotol plus (20 ml/kg)
Food Intake (g/day)		3.2 ± 0.5	3.0 ± 0.7	3.3 ± 0.5	3.4 ± 0.5
Body weight (g)		26.05 ± 1.49	23.93 ± 2.72	23.98 ± 1.71	24.43 ± 2.43
Water Intake (ml/day)		3.6 ± 0.7	3.7 ± 0.5	3.4 ± 0.8	3.9 ± 0.6
Organ body weight ratio (%)	Liver (g%)	8.06 ± 2.75	12.16 ± 3.39	9.62 ± 2.70	10.98 ± 2.34
	Kidney (g%)	1.97 ± 0.23	2.24 ± 0.28	2.16 ± 0.21	2.07 ± 0.41
	Heart (g%)	0.97 ± 0.17	0.96 ± 0.23	1.07 ± 0.19	1.05 ± 0.24
Organ weight (g)	Liver (g)	2.16 ± 0.77	2.92 ± 0.65	2.36 ± 0.67	2.65 ± 0.55
	Kidney (g)	0.58 ± 0.13	0.61 ± 0.12	0.59 ± 0.12	0.58 ± 0.15
	Heart (g)	0.33 ± 0.12	0.30 ± 0.12	0.33 ± 0.12	0.33 ± 0.13

Table 3.

Parameters		Control	Neurotol plus (5 ml/kg)	Neurotol plus (10 ml/kg)	Neurotol plus (20 ml/kg)
Platelets (× 10^5/cmm)		8.57 ± 0.98	9.08 ± 0.63	8.95 ± 0.66	8.03 ± 0.88
Total WBC (× 10^3/cmm)		5.47 ± 0.94	6.57 ± 0.71	6.25 ± 1.01	5.73 ± 0.58
Haemoglobin (g%)		17.23 ± 1.78	15.77 ± 1.35	13.95 ± 1.20[a]	12.30 ± 0.85[a]
Total RBC (× 10^6/cmm)		5.45 ± 0.89	6.12 ± 0.66	6.80 ± 0.55	6.90 ± 0.57
Differential %	N	20.77 ± 2.90	19.43 ± 3.04	19.93 ± 2.42	18.60 ± 3.18
	L	76.60 ± 3.18	77.77 ± 2.98	77.77 ± 2.17	78.60 ± 4.09
	E	2.27 ± 0.85	2.37 ± 0.85	2.20 ± 0.85	2.25 ± 0.85
	M	0.77 ± 0.92	0.93 ± 0.85	0.43 ± 0.62	0.93 ± 0.85

Table 4.

Parameters		Control	Neurotol plus (5 ml/kg)	Neurotol plus (10 ml/kg)	Neurotol plus (20 ml/kg)
Platelets (×105/cmm)		8.57 ± 0.73	8.50 ± 0.50	7.68 ± 0.83	8.27 ± 0.85
Total WBC (×103/cmm)		5.90 ± 0.59	6.03 ± 0.56	6.47 ± 1.03	6.22 ± 1.02
Haemoglobin (g%)		16.73 ± 0.88	15.38 ± 0.95	13.77 ± 1.17a	11.82 ± 0.91a
Total RBC (×106/cmm)		5.35 ± 0.90	6.17 ± 0.98	6.10 ± 0.82	5.73 ± 0.86
Differential %	N	19.50 ± 2.43	20.00 ± 4.34	18.17 ± 2.71	19.00 ± 2.37
	L	77.33 ± 3.01	77.50 ± 4.81	79.00 ± 3.85	78.17 ± 2.64
	E	2.33 ± 0.82	2.00 ± 0.89	1.83 ± 0.98	2.33 ± 0.82
	M	0.83 ± 0.75	0.50 ± 0.55	1.00 ± 0.89	0.50 ± 0.55