Evaluation of Hydroxypropyl-beta-Cyclodextrin (HPβCD) as formulation vehicle for use in general toxicity studies in mice

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Introduction

The standardization of vehicle use across the industry is hindered by the varied physicochemical properties of new chemical entities in development and tendency of a sponsor to use vehicles with which they have previous experience (1). Moreover, a survey of the pharmaceutical industry revealed highly divergent vehicle use (2).
Since unexpected vehicle-related toxicities can be difficult to segregate from NCE-related toxicities, there is a need for critical assessment of the vehicle suitability prior to use in safety studies.
HPβCD is a commonly used pharmaceutical excipient, well tolerated in humans and considered non-toxic following oral administration (3, 4). However, limited data is available to support use of HPβCD in toxicology studies in mice.

Objective

The aim of the present study was to evaluate the usefulness of HPβCD as a vehicle in repeat-dose preclinical safety studies in mice.

Materials and Methods

1000 mg/kg of HPβCD (Roquette, Italia S.p.A.) in purified water (10% w/v, pH3) has been administered by the oral route (gavage) to male CD1 mice for 5 days, UID or BID. Animals were sacrificed at D6.

Clinical Observations

On D1, between 30 and 90 minutes after administration, and on D5 the animals were submitted to a full clinical examination outside the housing cage, including functional and neurobehavioral tests.
Animals were weighed on D1 and D5.

Clinical Biochemistry

Activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), as well as concentrations of total bilirubin and total cholesterol were determined in serum using Olympus AU400 Clinical Chemistry Analyser.

Histopathology

Livers were weighed, fixed in formaldehyde, paraffin embedded and stained with hematoxiline-eosine.

Results

Clinical Observations

Mortality / Morbidity: No death occured during the study
Clinical signs: No clinical signs were observed during the study

Results

Clinical Biochemistry

Formulations containing HPβCD induced severe increase in serum aspartate- (AST) and alanine (ALT) aminotransferase activity compared to saline control. More pronounced effects were observed in animals dosed twice daily (40-fold increase in AST activity and 15-fold increase in the mean ALT activity).
There were no changes in the mean total bilirubin and cholesterol concentrations in serum of animals treated with HPβCD as compared to saline control.

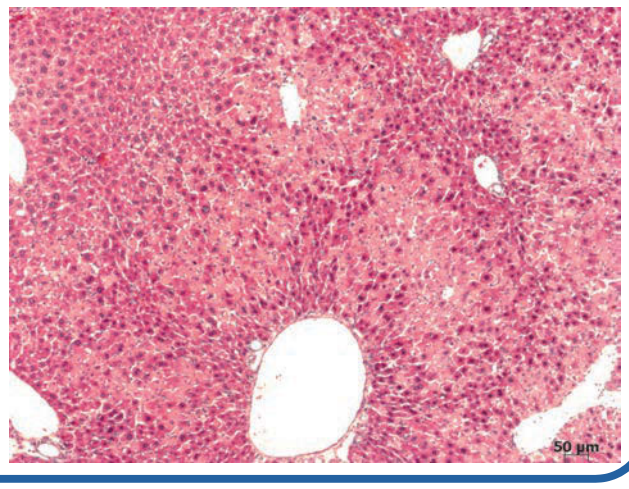
		SALINE	HPBCD 1000 mg/kg BID		HPBCD 1000 mg/kg UID	
ALT U/L	MEAN	57,1	2392,2	**	446,7	**
	SD	37,9	2206,8		223,4	
	N	5	5		5	
AST U/L	MEAN	113,6	1788,8	*	297,8	*
	SD	33,0	1679,9		80,6	
	N	5	5		5	
ALP U/L	MEAN	92,2	83,1		98,6	
	SD	17,6	19,9		18,8	
	N	5	5		5	
Total bilirubin µmol/L	MEAN	4,14	5,28		4,02	
	SD	0,50	2,06		0,92	
	N	5	5		5	
Cholesterol mmol/L	MEAN	3,68	4,58		4,00	
	SD	0,62	1,03		0,80	
	N	5	5		5	

*/** *p<0.05 /p<0.01* vs. saline; Mann Whitney test

Results

Histopathology

No significant increase in liver weight was observed.
In both groups treated with HPβCD moderate to severe necrosis was noted in hepatic acinar zone 2 and acinar zone 3.
Atrophic hepatocytes in acinar zone 2 were recorded in animals treated with HPβCD once daily.



Discussion

In order to evaluate the use of HPβCD as a pharmaceutical excipient, numerous toxicological studies on laboratory animals have been performed. It has been noted, that in laboratory rodents oral HPβCD administration at a dose of ≥1000 mg/kg/day can induce elevation of liver enzymes in plasma. In male CD1 mice receiving 1000 mg/kg HPβCD for 13 weeks, ALT activity in serum was elevated, but this finding was not accompanied by alterations in liver morphology (1). In rats, HPβCD applied orally for 7 days at a dose of 4500 mg/kg/day 45% w/v and 14 days at a dose of 2250 mg/kg/day 45% w/v, induced elevation of liver enzyme levels in plasma (ALT, AST, GLDH) without any histopathological changes (5).

Conclusion

It is suggested, based on the presented results, that formulations containing HPβCD should be used with caution in mice since HPβCD may induce hepatocyte necrosis accompanied by severe elevation of serum transaminase activity. These findings could be of critical importance for interpretation of data in preclinical safety studies in mice.

References

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