WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 10, 866-873. Research Article ISSN 2277– 7105

ACUTE INTRAVENOUS TOXICITY STUDY OF DISODIUM EDTA IN SWISS ALBINO MICE

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Article Received on
07 August 2016,
Revised on 27 August 2016,
Accepted on 17 Sep. 2016
DOI: 10.20959/wjpr201610-7119

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ABSTRACT

The study was conducted to determine the acute toxicity of disodium EDTA upon slow intravenous injection in mice. The study was conducted at four dose levels and all doses were administered by slow injection in the lateral tail vein. All animals were observed upto 14 days after dosing for clinical signs of toxicity. Animals in the vehicle or disodium EDTA group showed normal gain in body weight. No clinical signs of pathological significance were recorded in this study. Mortality was not found in any group. Gross necropsy revealed that all organs appeared completely normal and were comparable to control in all the groups treated with disodium EDTA. Based on these observations, it was concluded that administration of disodium EDTA by slow intravenous injection does not produce any visible signs of toxicity upto doses of 120 mg/kg.

KEYWORDS: Disodium EDTA, Elores, Acute toxicity.

INTRODUCTION

Ethylenediaminetetraacetic acid (EDTA) is a colourless, water-soluble solid. Its conjugate base is ethylenediaminetetraacetate. Its usefulness arises because of its role as a hexadentate ("six-toothed") ligand and chelating agent, i.e., its ability to "sequester" metal ions such as Ca2+ and Fe3+. After being bound by EDTA into a metal complex, metal ions remain in solution but exhibit diminished reactivity. EDTA is produced as several salts, notably disodium EDTA and calcium disodium EDTA. EDTA is used to bind metal ions in the practice of chelation therapy, e.g., for treating mercury and lead poisoning. It is used in a similar manner to remove excess iron from the body. This therapy is used to treat the complication of repeated blood transfusions, as would be applied to treat thalassaemia. The U.S. FDA approved the use of EDTA for lead poisoning (Smith, 2013). Elores a fixed dose

combination of ceftriaxone/EDTA/sulbactam, a novel antibiotics to fight with broad-spectrum Gram-negative bacterial infections (Chaudhary and Payasi, 2012).

The use of EDTA alongwith ceftriaxone+sulbactam provides the broader spectrum of activity and reduces the toxicity of potent combination. EDTA breaks the bacterial biofilm, inhibit the nutritional uptake by bacteria and makes the bacterial cell membrane porous and vulnerable. The aim of the current study was to delineate the toxicity of disodium EDTA administered by slow intravenous injection and determine its safety profile in swiss albino mice.

MATERIALS AND METHODS

Animals

Healthy adult female swiss albino mice weighing 25-30 g were used for the experiment. Animals were acclimatized in standard animal house environmental conditions for five days before the start of the experiment. The study was approved by the institutional animal ethics committee (IAEC) of Venus Medicine Research Centre. Mice were maintained under 12 h light: dark cycle in a temperature (23±4°C) and humidity (30-70%RH) controlled room. Pelleted chow (Ashirwad Industries, Chandigarh, India) and drinking water were provided *ad libitum*. All experimental procedures were performed in accordance with the CPCSEA guidelines, Ministry of Environment, Forests and Climate Change (MoEFCC), Govt. of India, New Delhi.

Chemicals and Reagents

EDTA used was of analytical grade.

Experimental Design

Experiment was performed in accordance with Appendix I of Schedule Y (Drugs and Cosmetics Rules, 2005) with minor modifications. Female mice (nulliparous and non-pregnant) were used for the experiment. After acclimatization, the animals were randomized on the basis of their body weight into 5 groups with 6 animals each *viz.* G1-G5. All the animals were administered respective treatments as shown in Table 1.

Table 1: Grouping and allocation of animals

Group	Treatment	Dose (mg/kg)	Route of administration	Dosage regimen	No. of animals / group
G1	Vehicle	--	i.v.	*b.i.d*	6
G2	Disodium	15	i.v.		6

G3	EDTA	30	i.v.		6
G4		60	i.v.		6
G5		120	i.v.		6
Vehicle was normal saline					

7.2 Selection of dose

Maximum recommended dose of Elores in adults is 3 g to be administered twice a day by slow injtravenous injection. Each 3 g of Elores contains 74 mg of disodium EDTA. Equivalent dose of disodium EDTA in mice is 15.17 mg/kg (i.e. ~15 mg/kg). The dose levels selected in the present study represent multiples of this dose to be administered twice daily. Four dose levels selected for the present study were 15 mg/kg (1-fold equivalent to human exposure), 30 mg/kg (2-fold), 60 mg/kg (4-fold) and 120 mg/kg (8-fold). 15 mg/kg was selected as the lowest dose since this dose represents the equivalent of recommended human dose. 120 mg/kg was selected as the highest dose since upon BD administration by slow intravenous injection in a single day it represents four-fold exposure to the LD_{50} of EDTA (56 mg/kg) when administered as an intravenous bolus in mice (Lewis 2004). Intermediate doses were selected by means of geometric progression.

Description of test procedure

All the animals in groups G1, G2, G3, G4 and G5 were injected respective dose of test item dissolved in the vehicle via the tail vein by slow intravenous injection over a period of 5-6 minutes. Injection of different groups was performed in a stepwise manner so as to avoid unwanted mortality and reduce animal usage in the experiment. Subsequent dose level was administered based on the outcome of the previous dose level injected, i.e. mortality or clinical toxicity observed in previously dosed groups for 24 hrs. Accordingly, animals in groups: G1 and G2 were dosed on day 1, G3 were dosed on day 2, G4 were dosed on day 3 and G5 were dosed on day 4 of the experiment. All animals were observed upto 14 days for clinical signs of toxicity and mortality. At the end of the observation period, gross necropsy was performed on all animals to observe the effects of respective treatments. The highest dose that did not cause any mortality or clinical signs of pathological significance was considered to be the no-observed adverse effect level (NOAEL).

Selection of route of administration

Intravenous route was selected as the route of administration as it is the intended route of administration in humans for the test item.

Preparation of test item

Disodium EDTA was provided as a powder. It was reconstituted with appropriate volume of vehicle (water) immediately before administration.

OBSERVATIONS

Body weight

Body weight of all the animals was recorded on Days 0, 7 and 14.

Clinical signs

Clinical signs of all the animals were recorded immediately after injection and observations were continued intermittently upto 24 hrs. Thereafter, clinical signs were recorded once daily upto 14 days post dosing.

Mortality

Occurence of mortality was recorded twice daily upto 14 days post-dosing.

Gross necropsy

Gross necropsy was performed on all the animals either at the end of the observation period or in case of mortality before the end of observation period.

RESULTS

Effect of disodium EDTA on body weight

It was observed that disodium EDTA did not exhibit any abnormality w.r.t. body weight. Animals in the vehicle or disodium EDTA group showed normal gain in body weight at the end of the observation period. Results are shown in Table 2.

Table 2: Mean body weight of animals

Group	Treatment	Body weight		
		Day 0	Day 7	Day 14
G1	Vehicle	27.50 ± 1.43	29.31 ± 1.00	31.05 ± 1.31
G2	Disodium EDTA	27.55 ± 1.11	29.38 ± 0.70	31.10 ± 1.04
G3		27.71 ± 1.14	29.68 ± 1.46	31.16 ± 1.63
G4		28.85 ± 1.18	30.43 ± 1.05	32.67 ± 1.39
G5		29.19 ± 0.65	31.26 ± 0.76	33.12 ± 0.72
Data is shown as mean ± SD (n=6)				

Clinical signs

Clinical signs in all the animals are shown in Table 3. None of the animals in the vehicle or disodium EDTA treated groups were found to exhibit any clinical sign of pathological signficance with doses of disodium EDTA upto 120 mg/kg, *b.i.d.*

Table 3: Clinical signs in the control and disodium EDTA treated animals

Animal No.	Group	Min			Hours					Days												
		5	15	30	1	2	4	8	24	2	3	4	5	6	7	8	9	10	11	12	13	14
1	G1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	G2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	G3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	G4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25	G5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
26		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
28		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
29		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

* The clinical signs were recorded as per the scores given below:

1	No abnormality	15	Dyspnea	29	Apnea	43	paraplegia
2	Found Dead	16	Edema	30	Paralysis	44	quadriplegia
3	Abdominal Breathing	17	Emaciation	31	Piloerection	45	kyphosis
4	Alopecia	18	Enophthalmos	32	Ptosis	46	conjuctivitis
5	Ataxia	19	Epiphora	33	Tonic convulsion	47	blepharospasm
6	Catalepsy	20	Erythema	34	Tremors	48	nystagmus
7	Cataract	21	External Genitalia	35	Bradycardia	49	vasodilation
8	Chemosis	22	Exophthalmos	36	Tachypnea	50	keratitis
9	Chromodacryorrhea	23	Gasping	37	nostil discharges	51	vasoconstriction
10	Clonic convulsion	24	Hematuria	38	somnolence	52	Hunching
11	Corneal (eyelid closure)	25	Lacrimation	39	Ear-abnormal	53	Moribund
12	Cyanosis	26	Miosis	40	prostration		
13	Diarrhea	27	Mydriasis	41	hemiplegia		
14	Anuria	28	Tachycardia	42	corneal opacity		

Mortality

No mortality was observed in any group. All the animals were found to survive until their scheduled period of necropsy.

Gross necropsy

No pathological lesions could be identified in any animal upon gross examination. Attention was paid to vital organs like heart, lungs, liver, spleen, brain, kidneys and ovaries. Tissues were found to be completely normal and no difference was observed between tissues from control and disodium EDTA treated animals.

DISCUSSION

It has been reported in the literature that disodium EDTA upon intravenous bolus injection can cause sudden death in rodents and humans (Lewis, 2004). In fact, there are clinical reports showing that accidental injection of disodium EDTA to humans have led to death of the patients in the clinic (Brown et al, 2006; Baxter and Krenzelok, 2008; Cosmetic

ingredient review panel, 2002). Intravenous bolus injection of disodium EDTA is reported to have an LD_{50} value of 56 mg/kg in mice (Lewis, 2004). However, disodium EDTA can be safely administered if it is given by slow intravenous injection. It has been reported that intravenous injection of disodium EDTA, at rates slower than 20 mg/min, does not produce any adverse effects and can be administered safely (Foreman, 1963). Thus, the present study aimed to determine the acute toxicity of disodium EDTA administered by slow intravenous injection in mice. Maximum exposure of Elores in adult human is recommended to be 3 g administered by slow intravenous injection. Each 3 g of Elores contains 74 mg of disodium EDTA. Equivalent dose of disodium EDTA is 15.17 mg/kg. Based on this dose, four dose levels were selected for the present study. The lowest dose level reflected equivalent dose of disodium EDTA. Dose levels higher than 15 mg/kg were chosen to determine the acute toxic dose of disodium EDTA upon slow intravenous injection. Based on the results of the study, it was found that dose levels upto 120 mg/kg (*b.i.d*) did not produce any visible signs of toxicity. No mortality or pathological signs of clinical significance were observed in the present study. Gross observations revealed that all organs appeared normal and no difference was observed between control and treated groups. Based on these observations it was conlcuded that when disodium EDTA is administered by slow intravenous injection, no toxic effects can be observed upto a dose level of 120 mg/kg.

CONCLUSION

It can be further concluded that doses upto 120 mg/kg represent no-observed adverse effect level (NOAEL) upon slow intravenous injection for atleast five minutes.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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