Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

J. Med. Sci., 3 (4): 274-282
July-August, 2003

Subacute Toxicity Study of Malic Acid Propane 1,2-diol Copolyester

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The sub-acute toxicity study of malic acid-propane 1,2-diol copolyester (MPC) was carried out in rats. The polymer was administered intraperitoneally to the rats at a dose of 300 μg rat^{-1} day^{-1} for 21 consecutive days. The gross general observations such as changes of body weight, hematological profiles, biochemical parameters of blood and the histopathology of liver, kidney, heart, lungs and spleen were investigated both in control and experimental rats. The body weights of the rats were slightly increased. The changes of hematological and biochemical parameters were statistically insignificant. No abnormalities were found in the histopathology of the liver, kidney, heart, lung and spleen in the experimental group of rats when compared with control group of rats. From this study, it was inferred that malic acid propane 1,2-diol copolyester would be used as an enteric coating material.

Key words: Malic acid-propane 1,2-diol copolyester, sub-acute toxicity, haematological parameter, biochemical parameter, histopathology

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JMS (ISSN 1682-4474) is an international, peer-reviewed scientific journal that publish original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.

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Introduction

The invention of synthetic polymer has brought a new era in the history of modern technology and the synthesis and development of biodegradable polymers is one of the brilliant aspects of polymer science at the present time. Many of the existing biodegradable carriers are linear polymers (Heller, 1980) and are being used for specialized application such as controlled release drug formulation (Lofgren *et al.*, 1994, 1995; Gruvegard *et al.*, 1998; Rosenberg *et al.*, 1983), insecticide and pesticide carriers as well as non-toxic surgical implant materials. Poly (L-lactic acid) is a biodegradable polyester having good biocompatibility, it has been utilized as an useful biodegradable material in the medical and pharmaceutical fields. But the application scope of polyLA is limited because it is highly a crystalline polyester (Ouchi *et al.*, 2000). Ongoing research in our laboratory is directed towards the synthesis and characterization of new biodegradable, flexible materials based on aliphatic polyester for controlled and sustained drug delivery (Bakr *et al.*, 2000, 2002). Hydrolysis of labile ester linkages along the polymer backbone converts these materials into products that the human body can easily metabolize and elimate them without adverse effects. Our aim is therefore, to develop novel commercially viable polymers especially designed to degrade under controlled biological conditions and in this connection, we have attempted to synthesize MPC polymer from malic acid and propane 1,2-diol. The details of synthesis, characterization, hydrolytic degradation and drug released behaviour of MPC have already been published (Bakr *et al.*, 2000). It was reported that the MPC is a good enteric coating material. In order to develop and to establish the safety and efficacy level of a new drug, toxicity studies are very essential and no drug is used clinically without its clinical trial as well as toxicity studies. In this work, we report the sub-acute toxicity of the malic acid-propane 1,2-diol copolyester (MPC) in albino rats.

Materials and Methods

Synthesis, characterization and toxicological study of MPC on brine shrimp have been reported elsewhere (Bakr *et al.*, 2000, 2002). Subacute toxicity of MPC in albino rats is our present investigation.

Collection of experimental rats

Long Evan's rats of same sex (male) and age (adult) were collected from the Animal Resources Branch, International Center for Diarrhoeal Diseases Research, Bangladesh (ICDDRB).

Maintenance of the rats

The rats were kept properly in numbered iron cages individually and they were given ideal food (Hawk *et al.*, 1993). They were kept in a clean animal house with an optimal room temperature (25-30°C). Animals were maintained in this study way for 15 days to prior to administration of polymer and continued upto the end of the experiment.

Grouping of the rats

Rats were weighed individually and divided into two groups: group A (Average body weight

135.25 gm rat^{-1}) and group B (average body weight 150.87 g rat^{-1}), each comprising of 4 rats. Group A received vehicle only to act as control, while group B received MPC.

Administration of sample

Malic acid-propane 1,2-diol copolyester were dissolved separately in distilled water with the help of polyoxyethylene 20 sorbitan mono laurate (Tween-20) in such a way that 0.3 ml of final preparation contained 300 μg of the polyester. The MPC was administered to the rats of group B intraperitioneally at a dose 300 μg rat^{-1} day^{-1} respectively for 21 consecutive days.

Gross general observation after drug administration

The rats were observed daily very keenly to note the following features: Behaviour, CNS excitation, CNS depression, Food intake, Salivation, Diarrhoea, Muscular weakness.

Prior to sacrificing the animals, the body weights of each rat of groups A and B were measured before administration of the drug and after completion of the treatment.

Study of haematological profiles, biochemical parameters of blood and histopathology of liver, kidney, lung, heart and spleen

For haematological studies, blood was drawn from the tail veins of each rat in group A and B before the commencement of polymer administration. Blood smears were made on glass slides and stained with Leishmen reagent to perform TC, DC and platelet count. With the use of capillary tubes, blood was drawn from each rat to estimate the haemoglobin percentage by a hemocytometer. The tests were repeated on 7th, 14th and 21st days after the compound administration.

For the determination of SGOT (Serum-glutamate-oxaloacetate-transaminase), SGPT (Serum-glutamate-pyruvate-transaminase), SALP (Serum alkaline phosphatase), bilirubin, creatinine and urea, blood samples were collected separately from each of the control and experimental rat from their throat vein after sacrificing at the end of 21 days of polymer administration. The samples were then analyzed for biochemical parameters using the procedures and reagents as described in Enlehringer Mannheim GmbH Diagnostica (King and Armstrong, 1934; Reitman and Frankel, 1957; Fawcett and Scott, 1960; Coulombe and Favreau, 1963).

For histopathological studies of liver kidney, heart, lungs and spleen were collected separately, sliced into pieces, fixed in formalin (10%) for two days, processed, stained with Harris Haematoxylin and eosin reagent, mounted on glass slides with diphenyl xylene and observed under microscope at the Department of Genetics and Breeding, Rajshahi University, Bangladesh.

Results and Discussion

Gross general observation

The group A (control) and group B (experimental) rats showed no signs of tremor, convulsions and reflex abnormalities. No muscular numbness of the hind and four legs, salivation or diarrhoea was observed. The food intake per day was also found normal. However, the body

Table 1 : Effect of malic acid-propan 1, 2-diol copolyester on body weight of rats after intraperitoneal administration

Group	Dose level $\mu g\ rat^{-1}\ day^{-1}$	Body weight (in g) before drug treatment $M_1 \pm SD_1$ (n=4)	Body weight (in g) after drug treatment $M_1 \pm SD_1$ (n=4)	Percentage change	Calculated 't' value	't' value at 5% level of significant	Remark
A	300 µL of	135.0	136.5				
Control	Vehicle	137.5	139.0				
		134.5	136.0	+1.293	+2.212	2.447	NS
		134.0	136.5				
		135.25±1.34	137.00±1.17				
B		148.5	151.5				
MPC	300 µg of	152.5	154.5				
Copolyester	Copolyester	149.0	151.0	+1.491	+1.56	2.447	NS
		153.5	155.5				
		150.87±2.16	153.12±1.91				

M_1 and M_2 = Sample mean value, SD_1 and SD_2 =Standard deviations of control and experimental group respectively
N = Number of rats + = Increase, - = Decrease, NS = Not significant

Table 2: Haematological profile of group-A (Rat treated with vehicle)

Haematological parameters		Normal rats	Rats treated with vehicle only		
		Ist day $M_1 \pm SD_1$	7th day $M_1 \pm SD_1$	14th $M_1 \pm SD_1$	21th day $M_1 \pm SD_1$
		4.6	4.9	5.1	5.0
		4.8	5.2	5.2	5.2
I. Total RBC count (million/cc)		4.9	5.1	5.4	5.5
		5.1	5.3	5.5	5.4
		4.850±0.180	5.125±0.0148	5.300±0.158	5.275±0.192
ii. Total WBC count (Thousand/cc)		11.80	12.00	12.40	12.50
		12.50	12.40	12.80	12.90
		13.20	12.80	13.00	13.20
		11.60	12.20	12.40	12.80
		12.27±0.629	12.35±0.295	12.65±0.259	12.85±0.250
iii. Differential	a. Neutrophil	40	42	41	43
count of WBC		46	44	43	41
in %		38	43	41	45
		42	41	43	42
		41.5±2.958	42.50±1.118	42.00±1.000	12.85±1.479
	b. Lymphocyte	52	50	53	52
		50	53	52	54
		55	53	51	51
		54	56	55	53
		52.75±1.920	53.00±2.121	52.75±1.479	52.50±1.118
	c. Monocyte	4	2	3	2
		3	2	4	4
		4	4	3	4
		5	3	3	3
		4.00±0.707	2.75±0.829	3.25±0.433	3.25±0.825

Table 2: Continue

Haematological parameters		Normal rats Ist day $M_1 \pm SD_1$	Rats treated with vehicle only 7th day $M_1 \pm SD_1$	14th $M_1 \pm SD_1$	21th day $M_1 \pm SD_1$
	d. Eosinophil	0	2	2	0
		2	1	1	2
		2	3	2	1
		3	1	3	3
iv. Platelet count (million/cc)		1.75±1.089	1.75±0.829	2.00±0.707	1.50±1.118
		3.50	3.60	3.50	3.70
		3.70	3.60	3.50	3.40
		3.80	3.70	3.80	3.50
		3.50	3.50	3.40	3.70
v. Haemoglobin (%)		3.625±0.129	3.600±0.707	3.550±0.150	3.575±0.129
		73	73	70	71
		70	71	72	70
		74	73	74	73
		71	72	71	72
		72.00±1.581	72.25±0.829	71.75±1.479	71.5±1.118
vi. ESR (mm/1st hour)		10	11	13	13
		13	12	14	12
		10	11	13	13
		12	11	14	14
		11.25±1.299	12.00±1.224	13.50±0.500	13.00±0.707

Table 3: Heamatological profile of group-B (Rats treated with malic acid-propane 1, 2-diol copolyster)

Haematological parameters		Normal rats Ist day $M_1 \pm SD_1$	Rats Treated with MPC only 7th day $M_1 \pm SD_1$	14th $M_1 \pm SD_1$	21th day $M_1 \pm SD_1$
I. Total RBC count (million/cc)		5.4	5.3	5.5	5.3
		5.1	5.2	5.1	5.1
		5.2	5.3	5.4	5.4
		5.3	5.5	5.6	5.2
		5.250±0.111	5.325±0.108	5.400±0.187	5.25±0.118
ii. Total WBC count (Thousand/cc)		11.50	12.00	12.80	11.90
		12.00	12.60	13.00	12.40
		12.40	12.80	13.40	12.20
		13.20	13.00	13.80	13.40
		12.275±0.621	12.600±0.374	13.250±0.384	12.475±0.562
iii. Differential count of WBC in %	a. Neutrophil	42	44	41	43
		40	43	42	44
		45	48	47	45
		43	44	42	41
		42.50±1.802	44.75±1.920	43.00±2.345	43.25±1.479
	b. Lymphocyte	55	51	54	53
		54	53	52	56
		51	50	53	52

Table 3: Continue

Haematological parameters	Normal rats Ist day $M_1 \pm SD_1$	Rats Treated with MPC only 7th day $M_1 \pm SD_1$	 14th $M_1 \pm SD_1$	 21th day $M_1 \pm SD_1$
	55	51	54	55
	53.75±1.639	51.25±1.089	53.25±0.829	54.00±1.581
c. Monocyte	4	3	2	2
	1	2	3	1
	2	4	1	3
	3	1	2	1
	2.50±1.118	2.50±1.118	2.00±0.707	1.75±0.829
	2	0	1	0
	0	2	2	1
d. Eosinophil	1	3	1	1
	2	1	3	2
	1.25±0.829	1.50±1.118	1.75±0.829	1.00±0.707
iv. Platelet count (million/cc)	3.40	3.50	3.40	3.50
	3.50	3.55	3.65	3.80
	3.70	3.65	3.75	3.65
	3.60	3.55	3.65	3.45
	3.550±1.118	3.562±0.544	3.612±1.293	3.58±0.134
v. Haemoglobin (%)	67	70	68	69
	65	66	67	70
	71	72	70	67
	72	70	69	71
	68.75±2.861	69.50±2.179	68.50±1.118	69.25±1.479
vi. ESR (mm/1st hour)	11	14	13	12
	10	13	12	15
	12	10	14	14
	13	15	14	13
	11.50±1.118	13.00±1.870	13.25±0.829	13.50±1.118

Table 4: Effect of malic acid-propane 1,2-diol copolyster on biochemical parameters of rat`s blood after i.p. administration of 300 μg $rat^{-1} day^{-1}$ for 21 consecutive days

Biochemical parameters	Group-A, (n=4) $M_1 \pm SD_1$	Group-B,(n=a) $M_1 \pm SD_1$	Percentage change	Calculated 't' value	't' value at 5% level of significant	Remarks
Serum Glutamate	10	11				
Oxalocetate	11	10				
Transaminase(SGOT)	9	9	+2.439	+0.359	2.447	NS
(IUL)	11	12				
	10.25±0.829	10.50±1.118				
Serum Glutamate	14	13				
pyruvate Trans-	11	12				
aminase (SGPT)	12	12	+2.127	+0.305	2.447	NS
(IU/L)	10	11				
	11.75±1.479	12.00±0.707				

Table 4: Continue

Biochemical parameters	Group-A, n=4 $M_1 \pm SD_1$	Group-B, n=a $M_1 \pm SD_1$	Percentage change	Calculated 't' value	't' value at 5% level of significant	Remarks
Serum Alkaline	45	47				
phosphatase(SALP)	46	45				
(IU/L)	48	48	+2.162	+1.089	2.447	NS
	46	49				
	46.25±1.089	47.25±1.479				
Serum bilirubin	0.32	0.35				
(mg dl^{-1})	034	0.37				
	0.35	0.33	+1.408	+0.252	2.447	NS
	0.41	0.39				
	0.355±0.033	0.360±0.022				
Creatinine	0.90	0.95				
(mg dl^{-1})	1.05	1.05				
	1.20	1.20	+3.342	+0.275	+2.447	NS
	1.40	1.50				
	1.137±0.184	1.175±0.207				
Urea (mg dl^{-1})	35	37				
	44	42				
	39	40	+2.500	+0.447	+2.447	NS
	42	45				
	40.00±3.390	41.00±2.915				

M_1 and M_2 = Sample mean value; SD_1 and SD_2 = Standard deviations; n = Number of rats; + = Increase; - = Decrease; NS = Non significant, S = Significance

Table 5: Effect of malic acid-propane 1,2-diol copolyster on histopathology of rat's kidney, hearts, lung, lover and spleen tissue after I-p administration of 300 µg rat^{-1} day^{-1} for 21 consecutive days

		Histopathological change observed				
Group	Dose (i.p.)	Kidney	Heart	Lung	Liver	Spleen
A	µL rat^{-1} day^{-1}(Vehicle)	NAD	NAD	NAD	NAD	NAD
A	µL rat^{-1} day^{-1} (MPC)	NAD	NAD	NAD	NAD	NAD

NAD = No abnormality detected

weights of all the rats were increased after administration of MPC and the changes of body weights were found to be statistically insignificant which are shown in Table 1.

Haematological profiles

The haematological profiles of the experimental rats were studied after intraperitoneal administration of the polymer to check the haematological disorders. Haematological profiles like total counts of RBC and WBC, differential count of WBC, platelet count and haemoglobin percentage were found normal before treatment and after 7th, 14th and 21st days of treatment. No detectable changes were observed in the values of these parameters compared to that of the control groups. The results are shown in Table 2 and 3.

Monitoring the biochemical parameters

Biochemical parameters of blood e.g. SGOT, SGPT, SALP, serum bilirubin, serum creatinine, urea of both experimental and control rats were determined to check any change of these parameters due to the administration of polymer (MPC) with respect to control rats. The results are presented in Table 4. It was found that most of the parameters were slightly increased with respect to that of the control groups but remained within the normal range.

From the Table 4, it was found that the changes are also statistically insignificant. These results indicated that the compound has no adverse effects on liver and kidney functioning.

Histopathological studies

The histopathological studies of liver, kidney, heart, lung and spleen of both control and experimental rats were performed after intraperitoneal administration of the drugs for 21 consecutive days (Table 5). No detectable differences in the histopathology of these organs of control and drug treated rats were observed when viewed under oil immersion objective. This indicates that the tested polymer MPC has no effect on cellular structures, i.e., the polymer does not cause degeneration of the cells of these organs.

Acknowledgments

The authors would like to thank the Ministry of National Science and Technology, Bangladesh and the authority of the university of Rajshahi, Bangladesh for providing financial assistance to carry out the project. They also express heartfelt thanks to M. Asadul Islam, Lecturer, Department of Genetics and Breeding, Rajshahi University and to Dr. M. Anwar Habib, M.B.B.S., Lecturer, Department of Pharmacology, Rajshahi medical College and Ph.D. Fellow, Department of Pharmacy, Rajshahi University for their full cooperation in performing the toxicological tests of the research samples.

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