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Original Article

Effects of erucic acid supplemented feeding on chronic doxorubucin toxicity in rats

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Abstract: One of the undesired complications of the chemotherapy with doxorubicin is cardiotoxicity. Cardiac effect of erucic acid, which is a member of omega-9 fatty acid, is investigated on doxorubicin treatment in this study. Forty-eight rats were divided into eight groups and each group contained six rats. First group rats were fed with milk. In the third and fifth groups we fed rats with milk supplemented 0.5% and 5% erucic acid respectively. The groups 2, 4, 6 were fed as the groups 1, 3, 5 respectively; we injected 2 mg/kg twice weekly intraperitoneal doxorubicin to these groups whereas we injected isovolumous normal saline to the groups 1, 3, 5. Two other groups (groups 7 and 8) were fed with standard pellet. Group 8 received 2 mg/kg doxorubicin twice weekly; group 7 received normal saline. After 4 weeks hearts were isolated and mounted on a Langendorff apparatus perfused by modified Tyrode solution. Surviving rats were significantly less in erucic acid + doxorubicin groups at the end of the treatment period ($p<0.05$). No significant difference was found between groups for malondialdehyde, catalase, cytochrome c oxidase and isolated heart measurements. Concomitant application of erucic acid and doxorubicin showed profound toxicity.

Key words: Erucic Acid, doxorubucin, isolated perfused rat heart, cardiolipin, cytochrome c oxidase

Introduction

Erucic acid is a 22-carbon long chain monounsaturated fatty acid, which is an ingredient of rapeseed oil, mustard oil and canola oil. In vivo, erucic acid (13-cis-docosenoic acid) which is also a member of omega 9 fatty acid is metabolized to oleic acid. Dietary erucic acid is used in the management of the adrenoleukodystrophy as a gylceryl trierucate form which is commonly called as Lorenzo's oil [1]. Canola oil, mustard oil and rapeseed oil contain erucic acid in different proportions. Especially, canola oil is widely used as cooking and salad oils, for baking and in a variety of other prepared foods [2].

The anthracycline glycoside antibiotic doxorubicin is of major importance in cancer chemotherapy. However, both patients and experimental studies pointed out the cumulative dose-dependent and irreversible cardiotoxicity which limits its usefulness as a broad spectrum anticancer drug. Although the precise mechanism of this pathogenesis is not completely known yet, it has been suggested that the inhibition of long chain fatty acid oxidation in the heart by doxorubicin is an important mechanism in the development of doxorubicin related cardiotoxicity [3]. Possible mechanisms for toxicity were proposed on the basis of the interaction, since Complexes I, III and IV which are involved in mitochondrial respiratory chain are known to require cardiolipin in their environment in order to maintain maximal activity [4]. Further evidence indicates that the mitochondrial membrane could be a target responsible for cardiac toxicity: indeed, the development of cardiac failure induced by doxorubicin is correlated with the impairment of mitochondrial functions such as O_2 consumption and ATP synthesis. Cardiolipin, a phospholipid specific to the inner mitochondrial membrane, is suggested to play this role. Enzymes of the respiratory chain require cardiolipin for full activity. Among a large number

of derivatives, doxorubicin, which is known as an important cardiotoxic anthracycline compound, forms a strong complex with cardiolipin. Weak toxicity of other derivatives at this level can be correlated to a relatively weak affinity for cardiolipin [5]. Cardiolipin is essential for the activation of cytochrome c oxidase [6].

There are publications reporting an interaction between doxorubicin and cardiolipin. Other articles reporting that erucic acid also interacts with cardiolipin. In a previous report it is demonstrated that phospholipid fatty acyl composition of cardiac mitochondrial inner membrane is dynamically influenced by dietary fat [7]. Modulation caused by dietary fat is both rapid and reversible and it is associated with a change in the phosphatidylcholine/phosphatidylethanolamine ratio of cardiac mitochondrial inner membrane. Other investigations show a similar decrease in phosphatidylethanolamine with an increase in phosphatidylcholine content in heart mitochondria of rats fed with rapeseed oil [8] containing high contents of erucic acid for 10 days or for 20 weeks [9] in comparison with rats fed with peanut oil. The additional factors such as change in membrane polar-head-group content might influence dietary-fat-induced modulation of enzyme activity in vivo. Therefore, both intrinsic and extrinsic modulation of the lipid microenvironment of mitochondrial ATPase serves to control enzyme activity. It is also conceivable that many other membrane-associated enzymes are modulated in a similar way. Low-erucic acid rapeseed oil resulted in elevation of cardiac mitochondrial cardiolipin content after dietary treatment for 12 days. The rats which were fed with either high- or low-erucic acid rapeseed oil showed higher content of cardiac mitochondrial cardiolipin instead of the rats which were not fed with erucic acid supplemented diet [7]. Rapeseed oil feeding led to the changes in mitochondrial membrane phospholipid fatty acid composition. It is speculated that diet-induced changes in membrane lipid affect the activity of mitochondrial membrane associated enzymes, thus having potentially serious consequences to normal functioning of the myocardial cell [10].

In this study we examined the interactions of the erucic acid feeding and doxorubucin in rats. We intended to investigate whether erucic acid and doxorubicin have a cumulative effect or erucic acid might have a cardioprotective effect against doxorubicin cardiotoxicity.

Material and methods

Following approval of the institutional committee 48 adult male Wistar albino rats weighing 150-200 g were used in the study and divided into eight groups (n=6 for every group). All of the rats were followed up for 4 weeks. The rats in the Group 1 were fed with low fat cows milk (Nestle Mis Light), which had 1 g fat, 32 g protein and 47 g carbonhydrate per liter. The milk was supplemented with 50 g/L fat (50% corn oil and 50% soybean oil) and 50 g/L sugar. Thus the feeding milk contained ~1 Cal/mL. For each rat, 80 mL supplemented milk was supplied every day. No other feeding including water was given to these rats. The rats in the groups 3 and 5 were fed with milk, which was supplied with erucic acid (eru) 0.5% and 5% respectively. The rats in the groups 2, 4, and 6 were fed as groups 1, 3, and 5 respectively; however 2 mg/kg intraperitoneal doxorubicin (doxo) were injected to these rats twice a week. Isovolumous normal saline (0.9% NaCl) was injected twice weekly to the rats in the groups 1, 3, and 5. Two other groups of rats (Groups 7 and 8) were fed with ad libitum standard oval pellet rat diet and water. Group 8 also received however 2 mg/kg intraperitoneal doxorubicin twice weekly whereas Group 7 received normal saline intraperitoneally.

The cages were controlled every day. The rats were supplied by milk every morning and unused portions of the milk were discarded. Autopsy was performed to every rat which was found death in the cage.

Isolated heart experiments

After 4 weeks, all surviving rats were killed by cervical dislocation without the use of anaesthetic agents. The hearts were excised and immersed immediately in cold heparinized (10000 IU L^{-1}) modified Tyrode buffer solution (NaCl 128 mM, KCl 4.7 mM, $CaCl_2$ 2.36 mM, NaHCO 20 mM, NaH_2PO_4 0.36 mM, $MgCl_2$ 1 mM, glucose 10 mM), then mounted on a stainless steel cannula in the Langendorff perfusion apparatus. The perfusion performed with Minipuls 3 peristaltic pump (Gilson Medical Electronics Middleton W 1 USA) with a constant flow of 8 ml min^{-1} with modified Tyrode solution described above and the solution was

equilibrated with 95% oxygen and 5% carbon dioxide. The gas mixture was continuously bubbled in the bathing solution. To minimize evaporation the jacketed reservoir was sealed with thin paraffin sheet. A heart chamber was used to prevent drying and to keep the temperature constant. Less than 1 min elapsed between excision and aortic cannulation. The perfusate and bath temperature were kept at 37 °C by means of a heat exchanger.

Isovolumetric left ventricular pressure was continuously recorded with a pressure transducer described below connected to a thin saline filled latex balloon size 3 (Hugo Sachs Elektronik KG, Berlin, Germany) inserted into the left ventricle through the mitral valve from a cut in the left atrium. It was aimed to reach to 5 mm Hg balloon pressure at the start of the experiment. Biopac Systems (Santa Barbara, California, USA) MP 100 data acquisition system and its peripherals referred in this paragraph provided analogous digital conversion withdraw and record 100 samples per seconds in a computer with Windows 98 second edition. Pressure recordings were done by TSD104A pressure transducer using DA100B general-purpose transducer amplifier. Two electrodes were placed to the aorta and apex of the heart to monitor ECG. Realtime bipolar electrogram recordings with left ventricular pressure on a different channel were done using EL400 unipolar needle electrode via ECG100B electrocardiogram amplifier. All measurements were done after saving data to files. Derivatives of pressure curves were then produced by using Acqknowledge Software Version 3.2.6 to measure dp/dt curves.

All isolated rat hearts were subjected to same experimental protocol on Langendorff apparatus. After a stabilization period of 30 minutes, the perfusion was stopped and the hearts were subjected in normothermic total global ischemia for 15 minutes and the hearts were kept in 37 °C Tyrode solution which was filled in the heart chamber to keep the temperature constant and to prevent the hearts from drying. All hearts stopped within 1 minute when subjected to ischemia. Afterwards the hearts were reperfused with a constant flow of 8 ml min^{-1} for 20 minutes. Then the experiment was stopped and the tissue samples were stored at −70 °C until homogenization procedure.

Malondialdehyde, catalase, and cytochrome c oxidase measurements

The tissues were weighed (wet weight) and homogenized in ice-cold 0.05 M phosphate buffer (pH=7.1) to produce a 20% (w/v) homogenate. Tissue homogenate was performed with a tissue grinder fitted with a Teflon pestle. The homogenate was sonicated three times at 30 seconds intervals in 4°C, with a power output of 38 watts. The sonicated homogenates were centrifugated at 2000×g for 10 min.

Malondialdehyde (MDA), the end product of lipid peroxidation content was measured by the formation of thiobarbituric acid reacting substances (TBARS), in the collected supernatants. TBARS were determined according to a modification of the spectrophotometric procedure of Buege and Aust [11] as follows. One volume of sample is mixed thoroughly with two volumes of a stock solution of 15% w/v thiobarbituric acid, 0.375% w/v thiobarbituric acid and 0,25 N hydrochloric acid. The combination of sample and stock solution is heated in test tubes with taps for 30 minutes in a boiling water bath. After cooling, the flocculent precipitate is removed by centrifugation at 1000×g for 10 min. The absorbance of the sample is determined at 535 nm and the TBARS concentration calculated using 1.56 $\times 10^5$ M^{-1} cm^{-1} as a molar extinction coefficient. The tissue homogenates were further centrifugated at 10,000×g for 20 min. for catalase and cytochrome c oxidase activity determinations.

Catalase activity was measured by the breakdown of hydrogen peroxide catalysed by catalase in clear supernatants [12]. An approximately 5×10^{-3} M solution of hydrogen peroxide was prepared with 0.05 M phosphate buffer (pH= 7.0) as a substrate.

Cytochrome c oxidase activity was measured by the method, which depends upon spectrophotometric observation of the oxidation rate of cytochrome c oxidase added to the system supernatants [13]. Cytochrome c solution consisted of 2.5 $\times10^{-5}$ M reduced cytochrome c in 0.05 M, disodium phosphate buffer (pH= 7.1). Reduction was accomplished by adding an amount of sodium dithionite sufficient to give a concentration that is 0.001 M. The enzyme

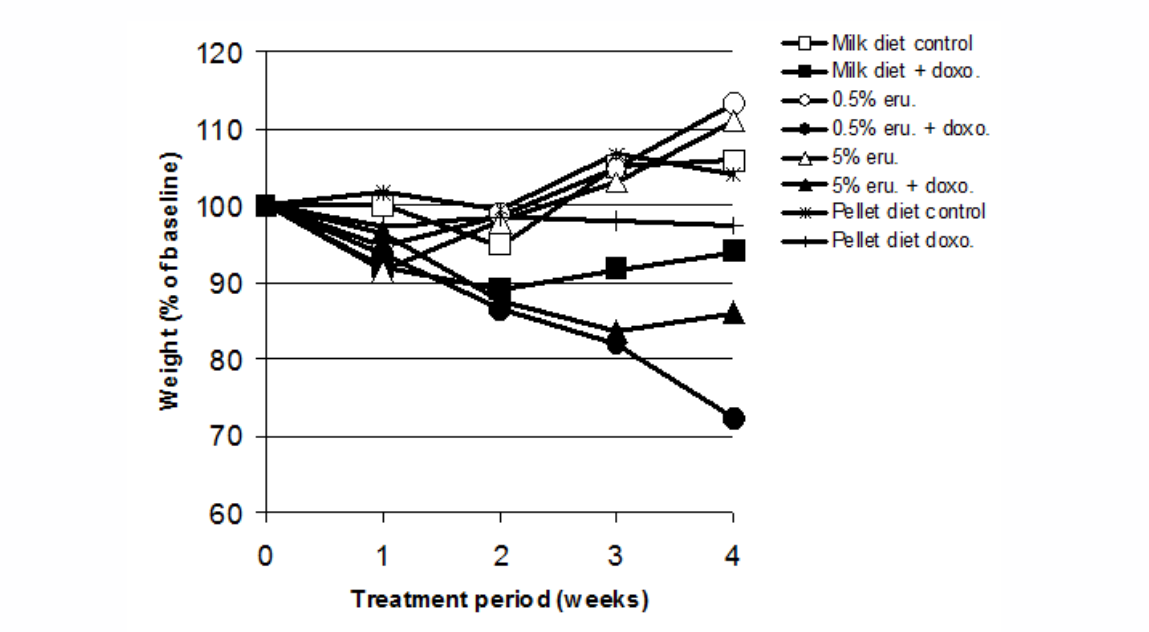

Figure 1. Body weights of the animals are represented. Compared with pellet diet control group, body weights of milk diet control +doxo groups' body weight was significantly less beginning from week 1 ($p<0.05$) and %0.5 eru +doxo and %5 eru + doxo groups' beginning from week 2 ($p<0.05$).

assays were performed at 25 °C and the results were expressed as U/g wet tissue.

Data analysis

SPSS version 11.5 was used for statistical analysis. All data are expressed as mean ± standard deviation. Statistical analysis for parametric data were performed by ANOVA post hoc Tukey for comparisons between groups, and repeated measures ANOVA post hoc Tukey for comparisons within groups between periods. Statistical analyses for non-parametric data were performed by Kruskal-Wallis non-parametric ANOVA post hoc comparisons were made by Dunn's multiple comparisons. Differences among means were considered significant when *p* is less than 0.05.

Results

There was no significant difference among the body weights in baseline measurements. Measured body weights in each group are shown in **Figure 1**. During the follow up, rats in the groups which received doxorubicin showed reduction in body weights beginning from first week ($p<0.05$).

Postmortem examination was performed for all spontaneously died rats. Signs of congestive heart failure such as pulmonary congestion and edema hepatomegaly were evident in one rat in the fourth group whereas the other three showed small and contracted heart. Miliary pneumonia of both lungs was observed through the examination of the unique death seen in the 5th group (%5 eru). Two deaths were seen in the 6th group (%5 eru + doxo), one rat showed signs of congestive heart failure. Neither spontaneously died rats nor the rats that were included the heart isolation experiments showed any other gross pathologic organ change. Number of the surviving rats in the treatment period is shown in the **Figure 2**. Number of the surviving rats was significantly less in %0.5 eru + doxo and %5 eru + doxo groups to the end of the treatment period ($p<0.05$).

Table 1-3 shows the results of the heart isolation study of the animals which were still alive to the end of the treatment period. No significant difference was found between groups for these results for all parameters except for $-dp/dt_{max}$ parameter measured at baseline; it was significantly less in standard oval pellet

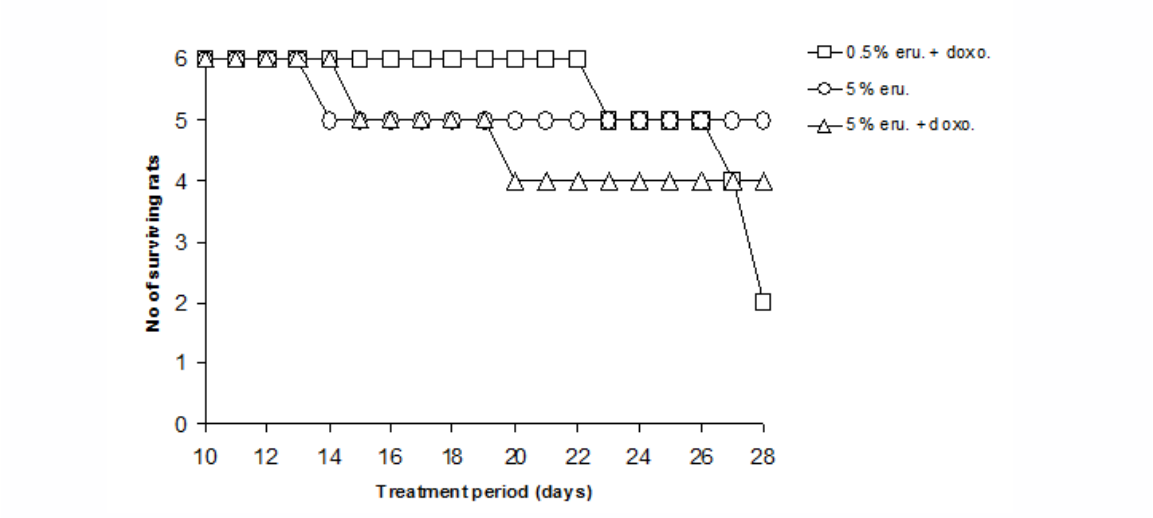

Figure 2. Number of surviving rats in the treatment period. None of the rats died until 14th day. Two, four and five rats survived in groups that were treated with 0.5% erucic acid and doxo, 5% erucic acid and doxo, and 5% erucic acid, respectively to the end of the experiment. None of rats died in other groups and data were not shown in the figure.

rat diet control + doxorubicin group in comparison with 0.5% eru group ($p<0.05$).

Table 4 shows the tissue levels of MDA, catalase, and cytochrome c oxidase. No significant difference was found between groups for these results.

Discussion

The hypothesized interaction between erucic acid and doxorubicin can be explained by the final convergence of individual effects over cardiolipin, an acidic phospholipid which is required for cytochrome c oxidase activity. Both agents are shown to possess high affinity to cardiolipin. The question of whether erucic acid is cardioprotective or cardiotoxic emerges. In the present study, additionally, it is evaluated whether erucic acid prevents or augments doxorubicin cardiotoxicity. Male rats are chosen intentionally due to the sex-related physiologic factors that render them more vulnerable to rapeseed oil-induced myocardial lesions [10].

In our study the rats fed with erucic acid showed an increase in doxorubicin toxicity. That is to say, erucic acid converted heart vulnerable to doxorubicin toxicity. Mechanical functions of the rats in the 4th and 6th groups have the lower mean values for all measured criteria, particularly in the reperfusion period. Speculatively since the animals with severe cardiac injury died, significant mechanical dysfunction in perfusion experiments may not be shown statistically in our study. Moreover, the greatest numbers of the deaths were observed in the group of the rats which were fed by low erucic acid diet. Doxorubicin especially inhibits beta-oxidation of long-chain fatty acids in cardiac tissues [3], but high dose erucic acid may induce peroxisomal beta-oxidation of fatty acids via peroxisome proliferator activated receptors (PPARs) especially for PPAR-delta/hNUC1 [14, 15], thus energy production which is inhibited by doxorubicin might somewhat continue in the heart. Although the cardiac damage comes true, mechanical functions may be protected, but low dose erucic acid does not seem as effective as high dose erucic acid.

We expected the beneficial effect of low-dose erucic acid with regard to the publications that indicated the effect of low dose on cardiolipin, via enhancing it and supporting mitochondria [7], however we concluded that it deteriorated cardiac function. Surprisingly, survivals of the rats were affected negatively by low dose erucic acid. This observation could partially be explained by the presence of a toxic metabo-

Table 1. Peak systolic pressure, +dp/dt$_{max}$, and -dp/dt$_{max}$ values

	Peak systolic pressure (mm Hg)				
	Initial	Reperfusion period (min)			
		5th min	10th min	15th min	20th min
Standard oval pellet rat diet fed animals					
Control1	52 ± 22	43 ± 13	52 ± 16	59 ± 19	59 ± 28
Control1 + doxo.	45 ± 20	42 ± 11	50 ± 10	57 ± 13	57 ± 11
Milk diet fed animals					
Control2	61 ± 21	33 ± 25	55 ± 19	63 ± 17	72 ± 17
Control2 + doxo.	75 ± 19	40 ± 17	61 ± 30	68 ± 29	69 ± 27
0.5% eru.	80 ± 15	32 ± 22	45 ± 23	46 ± 25	50 ± 24
0.5% eru. + doxo.	54 ± 40	46 ± 25	59 ± 36	64 ± 45	61 ± 42
5% eru.	64 ± 26	34 ± 20	43 ± 20	56 ± 16	60 ± 18
5% eru. + doxo.	44 ± 27	30 ± 6	37 ± 9	44 ± 12	49 ± 15
p	0.135	0.846	0.685	0.673	0.738
	+dp/dt$_{max}$ (mm Hg · s^{-1})				
Standard oval pellet rat diet fed animals					
Control1	977 ± 356	765 ± 217	920 ± 230	1047 ± 290	1041 ± 460
Control1 + doxo.	773 ± 352	701 ± 185	874 ± 169	979 ± 261	994 ± 304
Milk diet fed animals					
Control2	1220 ± 423	600 ± 491	976 ± 398	1149 ± 401	1318 ± 425
Control2 + doxo.	1359 ± 429	742 ± 300	1159 ± 523	1239 ± 494	1249 ± 529
0.5% eru.	1543 ± 338	578 ± 386	749 ± 464	840 ± 480	927 ± 485
0.5% eru. + doxo.	915 ± 625	787 ± 392	1035 ± 623	1064 ± 674	1082 ± 723
5% eru.	1105 ± 416	621 ± 276	722 ± 263	964 ± 237	1039 ± 315
5% eru. + doxo.	865 ± 438	588 ± 123	706 ± 201	849 ± 285	967 ± 395
p	0.069	0.933	0.496	0.715	0.821
	-dp/dt$_{max}$ (mm Hg · s^{-1})				
Standard oval pellet rat diet fed animals					
Control1	769 ± 243	537 ± 307	2100 ± 3663	758 ± 368	753 ± 458
Control1 + doxo.	610 ± 254	526 ± 103	686 ± 127	771 ± 181	841 ± 372
Milk diet fed animals					
Control2	1165 ± 474	636 ± 490	1027 ± 394	1226 ± 383	1411 ± 424
Control2 + doxo.	1267 ± 531	720 ± 314	1172 ± 502	1237 ± 473	1267 ± 432
0.5% eru.	1386 ± 390	519 ± 308	763 ± 463	852 ± 471	951 ± 468
0.5% eru. + doxo.	668 ± 510	608 ± 431	849 ± 668	878 ± 744	895 ± 790
5% eru.	989 ± 283	567 ± 258	696 ± 307	899 ± 211	972 ± 262
5% eru. + doxo.	819 ± 384	597 ± 106	709 ± 200	869 ± 312	1001 ± 436
p	0.023*	0.966	0.789	0.279	0.157

For *p* values under the columns represent one way ANOVA results of columns and none of them were significant (doxo., doxorubicin; eru., erucic acid). n= 6 for control1, control1 + doxo, control2, control2 + doxo., and 0.5% eru groups, n= 2 for 0.5% eru + doxo group, n= 5 for 5% eru group, n=4 for 5% eru. + doxo. group. *P* <0.05 was considered statistically significant.

lite of erucic acid acting peculiarly when is in lower concentration, while the useful counterpart alters mitochondrial function at times when higher concentrations are achieved.

Among the combined results of the study the causes of the deaths should be elucidated. Although, we failed to show cardiac toxicity, we demonstrated increased toxicity with doxorubicin and erucic acid supplemented diet treatment. Moreover, erucic acid is not only shown to be cardiotoxic in English medical literaure. In a study conducted by Badawy at al, toxic effects on hepatocytes and renal cells were shown histopathologically in rats fed with erucic acid [16]. Doxorubicin was also shown to cause renal toxicity in previous studies. In this manner nephrotoxicity could be another reason for the deaths. In our study separate application of erucic acid (either low or high-

Table 2. Heart rate, and pressure rate product, and mean perfusion pressure values

	Heart rate (bpm)				
	Initial	Reperfusion period (min)			
		5th min	10th min	15th min	20th min
Standard oval pellet rat diet fed animals					
Control1	279 ± 57	224 ± 51	237 ± 57	238 ± 59	244 ± 56
Control1 + doxo.	257 ± 21	244 ± 43	262 ± 43	260 ± 39	274 ± 40
Milk diet fed animals					
Control2	285 ± 39	309 ± 112	249 ± 31	261 ± 31	267 ± 35
Control2 + doxo.	250 ± 35	322 ± 102	279 ± 55	275 ± 56	276 ± 51
0.5% eru.	261 ± 82	257 ± 86	266 ± 55	260 ± 49	274 ± 54
0.5% eru. + doxo.	203 ± 25	189 ± 30	188 ± 3	180 ± 28	183 ± 25
5% eru.	229 ± 58	325 ± 64	291 ± 92	244 ± 95	247 ± 90
5% eru. + doxo.	306 ± 36	302 ± 50	282 ± 49	287 ± 48	288 ± 46
p	0.217	0.126	0.433	0.521	0.481
	Pressure rate product (mm Hg · s^{-1} × bpm)				
Standard oval pellet rat diet fed animals					
Control1	270931 ± 99351	169049 ± 50637	207430 ± 16150	236093 ± 16296	241088 ± 73603
Control1 + doxo.	198595 ± 91885	166208 ± 35492	228455 ± 59564	255701 ± 86993	270706 ± 80150
Milk diet fed animals					
Control2	353299 ± 132983	165131 ± 138757	248909 ± 115373	306125 ± 125345	357593 ± 138022
Control2 + doxo.	349783 ± 154663	228154 ± 77824	307417 ± 101917	327254 ± 105356	335751 ± 126170
0.5% eru.	418307 ± 193828	141897 ± 97187	188340 ± 106174	208824 ± 108579	244385 ± 121654
0.5% eru. + doxo.	193023 ± 149224	154560 ± 97411	195367 ± 120043	200960 ± 151377	206408 ± 158664
5% eru.	242763 ± 83750	189632 ± 49503	191914 ± 38971	218079 ± 33175	235288 ± 42441
5% eru. + doxo.	254249 ± 108572	174778 ± 30307	193775 ± 44562	240729 ± 86550	280987 ± 135126
p	0.124	0.798	0.282	0.360	0.419
	Mean perfusion pressure (mm Hg)				
Standard oval pellet rat diet fed animals					
Control1	110 ± 34	75 ± 37	81 ± 36	93 ± 31	86 ± 21
Control1 + doxo.	95 ± 25	59 ± 12	63 ± 10	75 ± 15	72 ± 17
Milk diet fed animals					
Control2	95 ± 33	67 ± 8	65 ± 9	69 ± 12	72 ± 14
Control2 + doxo.	75 ± 45	63 ± 17	62 ± 20	66 ± 23	69 ± 26
0.5% eru.	93 ± 31	74 ± 16	75 ± 17	77 ± 18	79 ± 20
0.5% eru. + doxo.	86 ± 25	61 ± 11	61 ± 10	64 ± 6	66 ± 4
5% eru.	86 ± 16	60 ± 21	63 ± 20	67 ± 23	69 ± 24
5% eru. + doxo.	77 ± 46	59 ± 23	60 ± 23	61 ± 25	63 ± 27
p	0.761	0.835	0.693	0.376	0.642

p values under the columns represent one way ANOVA results of columns and none of them were significant (doxo., doxorubicin; eru., erucic acid). n= 6 for control1, control1 + doxo, control2, control2 + doxo., and 0.5% eru groups, n= 2 for 0.5% eru + doxo group, n= 5 for 5% eru group, n=4 for 5% eru. + doxo. group. $P < 0.05$ was considered statistically significant.

Table 3. Contraction time, time to peak contraction, and ejection time values

	Initial	Reperfusion period (min)			
		5th min	10th min	15th min	20th min
Contraction time (ms)					
Standard oval pellet rat diet fed animals					
Control1	200 ± 36	233 ± 67	220 ± 31	215 ± 37	222 ± 47
Control1 + doxo.	230 ± 20	240 ± 24	218 ± 16	217 ± 21	215 ± 20
Milk diet fed animals					
Control2	204 ± 42	196 ± 78	216 ± 55	202 ± 44	201 ± 41
Control2 + doxo.	220 ± 40	182 ± 25	200 ± 54	208 ± 43	218 ± 44
0.5% eru.	202 ± 29	200 ± 27	201 ± 48	190 ± 20	189 ± 11
0.5% eru. + doxo.	215 ± 21	225 ± 35	205 ± 21	220 ± 42	220 ± 42
5% eru.	218 ± 33	212 ± 59	226 ± 69	234 ± 51	218 ± 50
5% eru. + doxo.	198 ± 22	210 ± 41	223 ± 21	218 ± 24	218 ± 28
p	0.691	0.585	0.973	0.730	0.836
Time to peak contraction (ms)					
Standard oval pellet rat diet fed animals					
Control1	97 ± 25	113 ± 39	107 ± 29	107 ± 27	112 ± 37
Control1 + doxo.	110 ± 15	110 ± 20	102 ± 10	107 ± 16	105 ± 17
Milk diet fed animals					
Control2	102 ± 28	116 ± 72	122 ± 52	120 ± 46	116 ± 41
Control2 + doxo.	120 ± 41	90 ± 23	110 ± 51	116 ± 45	128 ± 40
0.5% eru.	100 ± 29	106 ± 23	112 ± 33	102 ± 22	101 ± 15
0.5% eru. + doxo.	95 ± 7	90 ± 0	85 ± 7	95 ± 7	95 ± 7
5% eru.	118 ± 37	112 ± 52	130 ± 60	136 ± 48	126 ± 47
5% eru. + doxo.	108 ± 17	115 ± 30	135 ± 17	130 ± 24	135 ± 24
p	0.827	0.958	0.752	0.670	0.700
Ejection time (ms)					
Standard oval pellet rat diet fed animals					
Control1	133 ± 21	142 ± 17	143 ± 12	138 ± 12	143 ± 15
Control1 + doxo.	152 ± 19	155 ± 16	152 ± 16	152 ± 12	155 ± 11
Milk diet fed animals					
Control2	136 ± 24	150 ± 69	164 ± 60	154 ± 42	151 ± 44
Control2 + doxo.	158 ± 41	128 ± 25	150 ± 54	152 ± 49	166 ± 43
0.5% eru.	138 ± 25	142 ± 22	147 ± 41	139 ± 22	139 ± 16
0.5% eru. + doxo.	145 ± 7	135 ± 7	140 ± 0	140 ± 14	135 ± 7
5% eru.	162 ± 29	150 ± 58	170 ± 67	178 ± 46	170 ± 46
5% eru. + doxo.	145 ± 24	150 ± 28	165 ± 25	165 ± 19	165 ± 24
p	0.576	0.959	0.953	0.502	0.606

p values under the columns represent one way ANOVA results of columns and none of them were significant (doxo., doxorubicin; eru., erucic acid). n= 6 control1, control1 + doxo, control2, control2 + doxo., and 0.5% eru groups, n= 2 for 0.5% eru + doxo group, n= 5 for 5% eru group, n=4 for 5% eru. + doxo. group. $P < 0.05$ was considered statistically significant.

doses) and doxorubicin did not result in death or any significant cardiac function loss except for an isolated death due to pneumonia in the 5th group. Additionally, piglets fed formulas with 100% canola oil had lower platelet counts than piglets fed with formula soybean oil [2]. Hematological toxicity could be other reason for increased toxicity.

Cardiolipin, a major phospholipid component of the inner mitochondrial membrane is rich in polyunsaturated fatty acids. Cardiolipin damage results in inactivation of the enzymes which are required for mithochondrial respiratory chain, particularly cytochrome c oxidase [6]. In many studies it is shown that erucic acid had a high affinity to cardiolipin of rat heart

Table 4. Tissue levels of MDA, catalase, and cytochrome c oxidase

	MDA ($\mu mol \cdot g\ tissue^{-1}$)	Catalase ($U \cdot g\ tissue^{-1}$)	Cytochrome c oxidase ($\mu mol \cdot g\ tissue^{-1} \cdot min^{-1}$)
Standard oval pellet rat diet fed animals			
Control1	5.8 ± 0.6	0.178 ± 0.061	10.66 ± 4.02
Control1 + doxo.	6.8 ± 1.1	0.226 ± 0.132	11.18 ± 4.17
Milk diet fed animals			
Control2	9.6 ± 3.1	0.195 ± 0.034	12.53 ± 2.96
Control2 + doxo.	10.0 ± 2.8	0.183 ± 0.035	12.90 ± 5.14
0.5% eru.	6.1 ± 2.7	0.173 ± 0.039	7.12 ± 1.96
0.5% eru. + doxo.	12.0 ± 1.8	0.138 ± 0.003	6.11 ± 0.92
5% eru.	7.1 ± 2.9	0.200 ± 0.020	8.39 ± 2.28
5% eru. + doxo.	9.1 ± 3.4	0.081 ± 0.017	14.65 ± 5.23
p	0.012*	0.176	0.066

p values under the columns represent one way ANOVA results of columns (doxo., doxorubicin; eru., erucic acid). n= 6 control1, control1 + doxo, control2, control2 + doxo., and 0.5% eru groups, n= 2 for 0.5% eru + doxo group, n= 5 for 5% eru group, n=4 for 5% eru. + doxo. group. $P < 0.05$ was considered statistically significant. * There was no significant difference in post hoc Tukey comparison between groups.

mithochondria after feeding with rapeseed oil [17]. It is claimed that dietary erucic acid might influence fatty acid composition and function of cardiolipin [18]. Additionally, dietary erucic acid increases mitochondrial cardiolipin content [7]. However, we did not find any statistically significant difference in cytochrome c oxidase tissue levels and other biochemical parameters (MDA, catalase) between groups.

Chronic cardiotoxicity of doxorubicin which may be severe is characterized by congestive heart failure. Major pathological lesions observed in myocytes are sarcoplasmic vacuolization, myocytolysis and hyaline necrosis [19]. The mitochondrial membrane appears to be the most likely target of doxorubicin toxicity at intracellular level. Cardiolipin is susceptible to peroxidative injury by harmful radicals produced by redox cycle such as those produced by anthracyclines. This, in turn leads to the inactivation of key enzymes in the mitochondrial respiratory chain [20]. It is reported that enhancement of cardiolipin in mitochondrias of the hearts, which are forced to work in hyperdynamic condition, is parallel with the enhancement of the incorporation of dietary erucic acid into this phospholipid [21]. The consistence of a very stable complex between cardiolipin and doxorubicin is shown to inhibit numerous mitochondrial enzymes whose activities depend on the presence of cardiolipin [22].

A study on rats was performed by Badawy et al., shows that low erucic acid fed rats gained more weight with respect to high erucic acid fed group [16]. This result is also consistent with our observations. In our study, we assume that the increment on cardiolipin due to erucic acid would help on energy balance of injured hearts. However, in our experiments, low erucic acid fed rats lost more weight when doxorubicin is simultaneously applied.

This study is not the first study that points potential cardiotoxicity of erucic acid. Pasini et al., show decline in peak systolic pressure from the hearts of the rats which were fed 10% erucic acid enriched diet [23]. Light and electron-microscopic examination of the hearts of the rats fed with diets including erucic acid reveal myocardial lipidosis as early lesions, and later on focal myocardial necrosis, macrophage invasion and fibrosis follow [19].

While the dietary fish oil (omega-3 fatty acid) is also known to have beneficial effects on the development of cardiovascular diseases, the highest mortality rate and the lowest cardiac performance with both doxorubicin and diet rich in fish oil (containing 10 % fish oil) is reported too [24]. This state is partly explained by reduced antioxidant defences and accelerated susceptibility of the myocardium to lipid peroxidation in rats under doxorubicin treatment [24]. In this context, dietary erucic acid may accelerate susceptibility of myocyte cel-

lular membranes to lipid peroxidation as high dietary fish oil does. This mechanism could explain increased mortality with erucic acid diet and doxorubicin treatment.

Altered pharmacokinetics of doxorubicin with erucic acid could be an alternative explanation for increased mortality. However, supplementation of n-3 fatty acids did not affect doxorubicin pharmacokinetics in a recent study [25]. Up to our knowledge, there is no study comparing n-3 and n-9 fatty acids about their effects on doxorubicin pharmacokinetics. Further studies need to determine the effects of the dietary fatty acids on the pharmacokinetics of chemotherapeutic drugs.

Erucic acid may also be a cardiotoxic agent for human beings. Thus, the consumption of the oils that contain erucic acid (22:1 omega-9) deserves attention. At the present time, canola oil containing no more than 2% fatty acids as erucic acid is permitted in foods for adults and children [2]. In particular, the group of the rats, which received doxorubicin treatment and at the same time fed with diet containing 0.5% erucic acid, exhibited the lowest survival rates. Interestingly, 0.5% erucic acid content exhibits similarities with the erucic acid contents of the canola oil hence its widely consumption awakes concern.

Conclusion

In conclusion, erucic acid diet increases doxorubicin toxicity. It may seem as a speculation but the rats, in which cardiotoxicity was profound, died spontaneously therefore, we could not prove cardiotoxicity in isolated preparations. Additionally, both erucic acid diet and doxorubicin administration can lead to profound renal toxicity or hematological toxicity. Consequently, renal and hematological toxicities can be the possible mechanisms which were related decreased survival. Unfortunately, we did not perform any further evaluation of renal toxicity and hematological toxicity. The major limitation of our study is that we could not show the cardiotoxicity, possibly due to small sample size. When applied alone, neither doxorubicin nor erucic acid shows negative effect on survival and contractility, while concomitant use shows profound toxicity. Finally, our findings are noteworthy and further studies are needed to elucidate the potential mechanisms of decreased survival with concomitant administration of doxorubicin and erucic acid diet.

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