The study on the Antioxidation of EM-X in Liver of Rat *in vivo*

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Abstract
This experiment as a part of EM-X antioxidation series has made a rudiment study on the rats' injuries by m-dinitrobenzene (m-DNB) *in vivo,* compared with vitamin C. the results showed that EM-X obviously inhibited the production of MDA ($P<0.01$). The effect of prevention and all the dosages were much better than that of vitamin C ($P<0.01$). EM-X obviously inhibited the decrease in content to T-AOC and GSH ($P<0.01$). The effects of prevention and high- and mid-dosages were much better than that of vitamin C ($P<0.01$ or $P<0.05$). EM-X obviously inhibited the decrease of activities of CAT ($P<0.01$). The effects of prevention and high- and mid-dosages were much better than that of vitamin C ($P<0.01$). EM-X obviously inhibited the decrease of activities of CuZn-SOD, Mn-SOD and T-SOD ($P<0.01$). The effect of prevention and high- and mid- dosage were stronger than that of vitamin C ($P<0.01$ or $P<0.05$). EM-X obviously increased the activity of GSH-Px ($P<0.01$). The effect of prevention and high-mid- dosages were stronger than that of vitamin C ($P<0.01$). The research indicates that EM-X has the effects of the antioxidation, its mechanisms may inhibit lipid peroxidation through protecting or elevating the activity of organic antioxidase and/or elevating the antioxidative substances in the cell such as GSH, further improving T-AOC.

Key words: EM-X, hepatocyte membrane, CuZn-SOD, Mn-SOD, T-SOD, GSH-Px, CAT, MDA, GSH, T-AOC

Introduction

This essay will discuss the antioxidation of Properties EM-X and compare them with the traditional antioxidant Vitamin C in order to expound its mechanism of antioxidation from the subcellular level.

Methods

The Wistar rats of 180g~220g are divided into 8 groups of A (normal control, wter of 2ml/rat/day), B (model control, water of 2ml/rat/day), C (Vitamin C, 2ml/rat/day), D (EM-X control, 2ml/rat/day). E (EM-X prevention, 2ml/rat/day), F (EM-X high dosage, 3ml/rat/day), G (EM-X medium dosage 2ml/rat/day), and H (EM-X low dosage, 1ml/rat/day) at random with 10~15 rats for eacth. The

groups, B, C, E, F, G and H were treated with m-DNB liquid for 14 days by pouring them into the stomach, the group A with water and the group D with EM-X. (The group E, "prevention", was treated with EM-X prior to the administration of m-DNB in the first period and with water only on the second period.) From 15^{th} day to 35^{th} day, while the groups D, F, G and H were treated with EM-X, the group C was with Vitamin C and the groups A, B, and E were with water by pouring them into the stomach. The rats were killed by decapitation after the blood collected at 36^{th} days, and the liver were extracted quickly, and the hepatocyte membrane according to the slightly reformed methods of Wolf and so on in order to be used for the experiment.

Results

1. The effect on MDA (Table 1)

The production of MDA in the rats' hepatocyte membrane was clearly increased ($P<0.01$) after their injuries by m-DNB induction. EM-X obviously inhibited the production of MDA ($P<0.01$). The effect of prevention and all the dosages were much better than that of vitamin C ($P<0.01$). The results are shown in Table 1 as follows:

Table 1. The effect of EM-X and Vitamin C on the contents of MDA

	Group	mean ± sd (MDA mmol/mg)
A	Normal control	1.87 ± 0.30
B	Model control	4.31 ± 0.49? ?
C	Vitamin C	4.15 ± 0.47? ?
D	EM-X	1.69 ± 0.31? ?**
E	Prevention	2.14 ± 0.30? ?**
F	High dosage	2.47 ± 0.39? ? ??**
G	Medium dosage	2.89 ± 0.40? ? ??**
H	Low dosage	3.37 ± 0.43? ? ??**

Compare with Group Normal control ? P<0.05, ? ? P<0.01, with Group Model control ?P<0.05, ??P<0.01 and with Group Vitamin C*P<0.05, **P<0.01

2. The effect of GSH (Table 2)

The production of GSH in the rats' hepatocyte membrane was clearly decreased ($P<0.01$) after their injuries by m-DNB. Both EM-X and vitamin C obviously inhibited the decrease ($P<0.01$). the effect of prevention of prevention and all dosages were much better than that of vitamin C ($P<0.01$ or $P<0.05$). the results are shown in Table 2 as follows:

Table 2 The effect of EM-X and Vitamin C on the contents of GSH

	Group	Mean ± sd (GSH mmol/mg)
A	Normal control	46.30 ± 3.02
B	Model control	18.78 ± 2.74? ?
C	Vitamin C	32.23 ± 2.47? ?
D	EM-X	47.87 ± 2.38**? ?
E	Prevention	42.60 ± 2.77? ? ??**
F	High dosage	41.87 ± 3.31? ? ??**
G	Medium dosage	37.40 ± 2.00? ? ??**
H	Low dosage	35.52 ± 1.23? ? ??**

Compare with Group Normal control ? P<0.05, ? ? P<0.01, with Group Model control ?P<0.05, ??P<0.01 and with Group Vitamin C*P<0.05, **P<0.01

3. The effect on T-AOC (Table 3)

The T-AOC in the rats' hepatocyte membrane was clearly decreased (P<0.01) after their injuries by m-DNB. Both EM-X and vitamin C obviously inhibited the decrease (P<0.01). The effect of prevention and high- and mid- dosages were much better than that of vitamin C (P<0.01). The results are shown in Table 3 as follows:

Table 3 The effect of EM-X and Vitamin C on the T-AOC

	Group	**Mean ± sd (U/mg)**
A	Normal control	11.72 ± 1.22
B	Model control	5.14 ± 0.76? ?
C	Vitamin C	7.83 ± 0.82? ? ??
D	EM-X	12.81 ± 0.92? ? ??**
E	Prevention	10.81 ± 1.04? ??**
F	High dosage	10.57 ± 0.77? ??**
G	Medium dosage	9.15 ± 0.75? ? ??**
H	Low dosage	7.92 ± 0.76? ? ??**

Compare with Group Normal control ? P<0.05, ? ? P<0.01, with Group Model control ?P<0.05, ??P<0.01 and with Group Vitamin C*P<0.05, **P<0.01

4. The effect on CAT activity (Table 4)

The activity of CAT in the rats' hepatocyte membrane was clearly decreased (P<0.01) after their injuries by m-DNB. Both EM-X and vitamin C obviously inhibited the decrease (P<0.01). The effect of prevention and high- and mid-dosages were much better than that of vitamin C (P<0.01). The results are shown in Table 4 as follows:

Table 4 The effect of EM-X and Vitamin C on the activity of CAT

	Group	**Mean ± sd (U/mg)**
A	Normal control	7.12 ± 0.46
B	Model control	4.41 ± 0.47? ?
C	Vitamin C	4.82 ± 0.42? ? ??
D	EM-X	7.22 ± 0.52??**
E	Prevention	7.06 ± 2.44??**
F	High dosage	6.97 ± 0.57??**
G	Medium dosage	6.11 ± 0.53? ? ??**
H	Low dosage	5.22 ± 0.61? ? ??

Compare with Group Normal control ? P<0.05, ? ? P<0.01, with Group Model control ?P<0.05, ??P<0.01 and with Group Vitamin C*P<0.05, **P<0.01

5. The effect of GSH-Px activity (Table 5)

The activity of GSH-Px in the rats' hepatocyte membrane was slightly increased (P>0.05) after their injuries by m-DNB. Both EM-X and vitamin C obviously increased the activity of GSH-Px (P<0.01). The effect of prevention and high- and mid- dosage were stronger than that of vitamin C (P<0.01). The results are shown in Table 5 as follows:

Table 5 The effect of EM-X and Vitamin C on the activity of GSH-Px

	Group	Mean ± sd (U/mg)
A	Normal control	3.59 ± 0.29
B	Model control	3.66 ± 0.45
C	Vitamin C	5.19 ± 0.24? ? ??
D	EM-X	3.64 ± 0.37**
E	Prevention	6.60 ± 0.44? ? ??**
F	High dosage	6.93 ± 0.46? ? ??**
G	Medium dosage	6.10 ± 0.32? ? ??**
H	Low dosage	5.20 ± 0.41? ?

Compare with Group Normal control ? P<0.05, ? ? P<0.01, with Group Model control ?P<0.05, ??P<0.01 and with Group Vitamin C*P<0.05, **P<0.01

6. The effect on T-SOD activity (Table 6).

The activity of T-SOD in the rats' hepatocyte membrane was clearly decreased (P<0.01) after their injuries by m-DNB. Both EM-X and vitamin C obviously inhibited the decrease (P<0.01). the effect of prevention and high- and mid-dosages were stronger than that of vitamin C (P<0.01). The results are shown in Table 6 as follows:

Table 6 The effect of EM-X and Vitamin C on the activity of T-SOD

	Group	Mean ± sd (NU/mg)
A	Normal control	59.74 ± 3.03
B	Model control	42.09 ± 1.69? ?
C	Vitamin C	46.45 ± 2.53? ? ? ?
D	EM-X	62.14 ± 2.57? ? ? ?
E	Prevention	57.28 ± 2.07? ? ? ? ?
F	High dosage	55.32 ± 3.62? ? ? ? ? ?
G	Medium dosage	52.70 ± 2.23? ? ? ? ? ?
H	Low dosage	47.07 ± 1.97? ? ? ?

Compare with Group Normal control ? P<0.05, ? ? P<0.01, with Group Model control ?P<0.05, ??P<0.01 and with Group Vitamin C*P<0.05, **P<0.01

7. The effect on CuZn-SOD activity (Table 7)

The activity of CuZn-SOD in the rats' hepatocyte membrane was clearly decreased (P<0.01) after their injuries by m-DBN. Both EM-X and vitamin C obviously inhibited the decrease (P<0.01). The effect of prevention and high- and mid- dosages were stronger than that of vitamin C (P<0.01). the results are shown in Table 7 as follows:

Table 7 The effect of EM-X and Vitamin C on the activity of CuZn-SOD

	Group	Mean ± sd (NU/mg)
A	Normal control	57.40 ± 2.88
B	Model control	40.97 ± 1.83? ?
C	Vitamin C	44.81 ± 2.72? ? ??
D	EM-X	59.41 ± 2.38??**
E	Prevention	54.99 ± 1.95? ? ??**
F	High dosage	53.23 ± 3.41? ? ??**
G	Medium dosage	50.70 ± 2.40? ? ??**
H	Low dosage	45.34 ± 2.04? ? ??

Compare with Group Normal control ? P<0.05, ? ? P<0.01, with Group Model control ?P<0.05, ??P<0.01 and with Group Vitamin C*P<0.05, **P<0.01

8. The effect on Mn-SOD activity (Table 8)
The activity of Mn-SOD in the rats' hepatocyte membrane was clearly decreased ($P<0.01$) after their injuries by m-DNB. Both EM-X and vitamin C obviously inhibited the decrease ($P<0.01$). The effect of prevention and high dosage were stronger than that of vitamin C ($P<0.05$). The results are shown in Table 8 as follows:

Table 8 The effect of EM-X and Vitamin C on the activity of Mn-SOD

	Group	**Mean ± sd (NU/mg)**
A	Normal control	2.34 ± 0.39
B	Model control	1.13 ± 0.36? ?
C	Vitamin C	1.64 ± 0.47? ? ??
D	EM-X	2.73 ± 0.65? ??**
E	Prevention	2.29 ± 0.41??**
F	High dosage	2.19 ± 0.37?? *
G	Medium dosage	1.99 ± 0.34??
H	Low dosage	1.73 ± 0.33? ??

Compare with Group Normal control ? $P<0.05$, ? ? $P<0.01$, with Group Model control ?$P<0.05$, ??$P<0.01$ and with Group Vitamin C*$P<0.05$, **$P<0.01$

Discussion

The latest research showed that the injury form free radicals had a direct relationship with the occurrence and development of many diseases such as cancer, decrepitude and injury by radiation. The key to the injury was a series of injury processes as the free radicals produced by the live bodies injured biomembranes, enzymes, nucleic acid and so on, for example, by producing pilid peroxides (LPO). However, the natural protection system of anti-injury on free radical that existed in the body effectively cleared the free radical in order to protect the body. The protection system consists of antioxidant system including Vitamin E, Vitamin C, and GSH, and antioxidase system including SOD, GSH-Px, and CAT.

This paper examines deeply the antioxidation mechanisms of EM-X with the results revealing that they significantly inhibited either the enhancement of lipid peroxidative reaction caused by oxidation or the decrease of activity of antioxidases *in vivo*. Therefore, we can conclude that the action against injuries of free radicals by EM-X probably results form the protection and improvement of activity of antioxidase (CuZn-SOD, Mn-SOD, GSH-Px and CAT) system and/or elevating the antioxidative substances in the cell such as GSH, further improving T-AOC and reducing the production of lipid peroxides.

Conclusion

The experiment results indicate that EM-X exerts strong antioxidant competence in live membrane, which shows in the following respects:

1. Inhibiting of MDA production
2. Improving the activity of antioxidant enzymes (T-SOD, CuZn-SOD, CAT and GSH-Px).

3. Raising antioxidant substances such as GSH content in the cell, further raising the total antioxidant competence (T-AOC).
4. EM-X's effect is much stronger than that of Vitamin C, which has a close dependent relation with its dosage.
5. The above EM-X's antioxidant effects will be better if administered long term.

Methodology

1. Experimental Materials and animals

a. EM-X: EM Research Organization, Japan, licensed number: OB0005264132
b. Vitamin C: 12.5g/100ml Chemical Test Material Whole-sale Station, Harbin Medicine Company, licensed number: 1999413
c. m-dinitrobenzene (m-DNB): produced by Shanghai No. 3 Chemical Test Material Plant, licensed number: 991130
d. Malonaldehyde (MDA) Test kits: Nanjing Jiancheng Bio-engineering Research Institute, licensed number:20010313
e. Glutathione (GSH) Test Kits: Nanjing Jiancheng Bio-engineering Research Institute, licensed number: 20010308
f. Total antioxidant competence (T-AOC) Test Kits: Nanjing Jiancheng Bio-engineering Research Institute, licensed number:20010313
g. Catalase (CAT) Test Kits: Nanjing Jiancheng Bio-engineering Research Institute, licensed number: 20010314
h. Glutathione peroxidase (GSH-Px) Test Kits: Nanjing jiancheng Bio-engineering Reserch Institute, licensed number:20010313
i. Superoxide dismutase (SOD) Test Kits: Nanjing Jiancheng Bio-engineering Research institute, licensed number:20010313
j. Wistar rats are provided by Animal Experiment Center, Qiqihar Medical Collage.