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PLASMA LIPID PEROXIDES IN MURINE SEPSIS – SEX DIFFERENCES AND EFFECT OF ANTIOXIDATIVE/ANTI-INFLAMMATORY THERAPY

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In order to investigate the influence of antioxidative/anti-inflammatory combination therapy (AACT) with dimethyl sulfoxide (DMSO), chlorpromazine (CPZ) and vitamin E upon the activity of the inflammation, plasma lipid peroxide was measured as thiobarbituric acid reactive substance (TBARS) 12 hrs postoperatively in the modified cecal ligation sepsis model in the mouse.[1]

Significantly higher TBARS levels were found in the male control group (13.7 ± 0.7 nmol MDA/ml) than in the female control group (11.6 ± 0.6 nmol MDA/ml).

The operated male group had significantly higher TBARS levels (16.2 ± 0.6 nmol MDA/ml) than the unoperated male control group (13.7 ± 0.7 nmol MDA/ml). No increase of TBARS levels was observed in the operated female group.

Both male and female operated group, when postoperatively treated with AACT had the same TBARS level as the not operated male or female control group.

Survival curves of operated male and female group did not demonstrate any significant difference. The survival was better in an operated male and an operated female group, when postoperatively treated with AACT.

It was concluded that the applied TBARS test is too insensitive to follow the activity of the inflammation and has no predictive value for the outcome of sepsis in this model.

KEY WORDS: Thiobarbituric acid test, inflammation, antioxidants, dimethyl sulfoxide, chlorpromazine, vitamin E.

INTRODUCTION

Administration of a combination of drugs with antioxidative/anti-inflammatory properties, DMSO,[2] CPZ[3,4] and vit. E,[5] postoperatively in the modified cecum ligation sepsis model in the mouse resulted in a decrease of mortality in comparison to an untreated group.[1] The explanation of these results seems to lie in the ability of the applied antioxidative/anti-inflammatory drugs to inhibit the activity of the inflammation in the acute phase.

Excessive stimulation of the inflammation during the acute phase may lead either to generalized autodestructive inflammation followed by multiple organ failure and death,[6,7] or alternatively to excessive immunodepression followed by lifethreatening infections.[8-10]

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In order to provide evidence that the applied agents are able to inhibit the acute phase of inflammation, we determined, in the experiment described here, plasma lipid peroxide 12 hrs postoperatively in the same experimental model.

TBARS level is a reflection of auto-oxidation[11] and seems to correlate with the violence of the inflammation.[12] Plasma lipid peroxide level is increased in a variety of pathological situations, such as critical illness,[13] after near drowning,[14] rheumatoid arthritis,[12,15] abnormal pregnancy,[16,17] liver diseases,[18] cerebrovascular diseases.[19] The experiment described here was also performed to investigate whether the TBARS test is of predictive value for the outcome of sepsis.

The main reason for using drugs in combination was the expectation that this would be forming in vivo an electron/hydrogen (e^-/H^+) transport chain similarly as described in solution for glutathione – CPZ – vit. E – vit. C – NAD(P)H.[20] If such a chain for the transport of reducing ions (e^-/H^+) could be formed in vivo, the antioxidative capacity of the used combination would be considerably higher than the sum of the antioxidative effects of each single antioxidative drug. At the same time induction of oxidation-damage by oxidized anti-oxidants formed by neutralization of free radical is prevented.

The energy producing systems of the cell were supposed to be the source of the reducing ions.

MATERIALS AND METHODS

This research was carried out on Swiss mice, male and female, two months old. The modified cecal ligation sepsis model applied here has been described in detail earlier.[1] Briefly, the cecum was tightly ligated at its base without ligation of the blood vessels of the cecum itself and without causing bowel obstruction. The cecum was then punctured with a 25 gauge needle four times. After replacing the cecum in the abdomen, the abdominal incision was closed in two layers. After regaining consciousness, the mice were marked and administration of therapy was started.

All operations have been carried out in the afternoon which means at the beginning of the wake period of the mice.

For TBARS measurement, the therapy was administered only once postoperatively, immediately after regaining consciousness.

In experiments meant to judge survival, therapy was given twice daily and was continued for the next two days for a total of five doses.

Dimethyl sulfoxide (DMSO zur synthese Merck-Schuchardt) was given orally in a dosage of 3g/kg daily in a 50% aqueous solution. Chlorpromazine hydrochloride (Sigma (R)) was also given orally, in a dosage of 3 mg/kg daily mixed with DMSO. Vit. E (Alpha-Tocopheroli Acetas-Ph,Eur.) was given only once postoperatively in a dosase of 0.5 ml/kg (475 I.U.).

For TBARS measurements the blood was taken 12 hrs postoperatively by heart puncture from anesthetized mice, heparinized and processed in anaerobic conditions by use of mineral oil. The method to measure TBARS was essentially as described:[13] plasma was diluted tenfold with distilled water. To 0,5 ml diluted plasma 0,2 ml 7% (w/v) sodium dodecylsulfate followed by 2 ml 0.1 N HCl, 0.3 ml 10% (w/v) phosphotungstic acid and 1 ml 0.5% (w/v) thiobarbituric acid was added. The tubes were heated to 95°C for a period of 90 minutes. After cooling 5 ml n-butanol was added. The mixture was mixed thoroughly and then was centrifuged 10 mintues at approxim-

ately 3000 g. The butanol layer was measured fluorimetrically. Wavelength for excitation is 505 nm, emission: 548 and 600 nm. The difference of fluorescent intensity was used. Malondialdehyde was used as standard.

TBARS level was determined in 6 groups of mice. The number of animals in each group varied from 8–9. Group data were expressed as mean ± SEM

Survival data were determined in four groups of mice, each consisting of 36 animals and presented as percentage. Half of these data originate from previous experiments[1] and have now only been divided in a male and a female group.

Statistical significance was determined by One Way Analysis of Variance. Probability values less than 0.05 were considered significant.

RESULTS

TBARS level determined in healthy mice was significantly higher ($p < 0.04$) in the male group (13.7 ± 0.7 nmol MDA/ml) than in the female group (11.6 ± 0.6 nmol MDA/ml) (Figure 1).

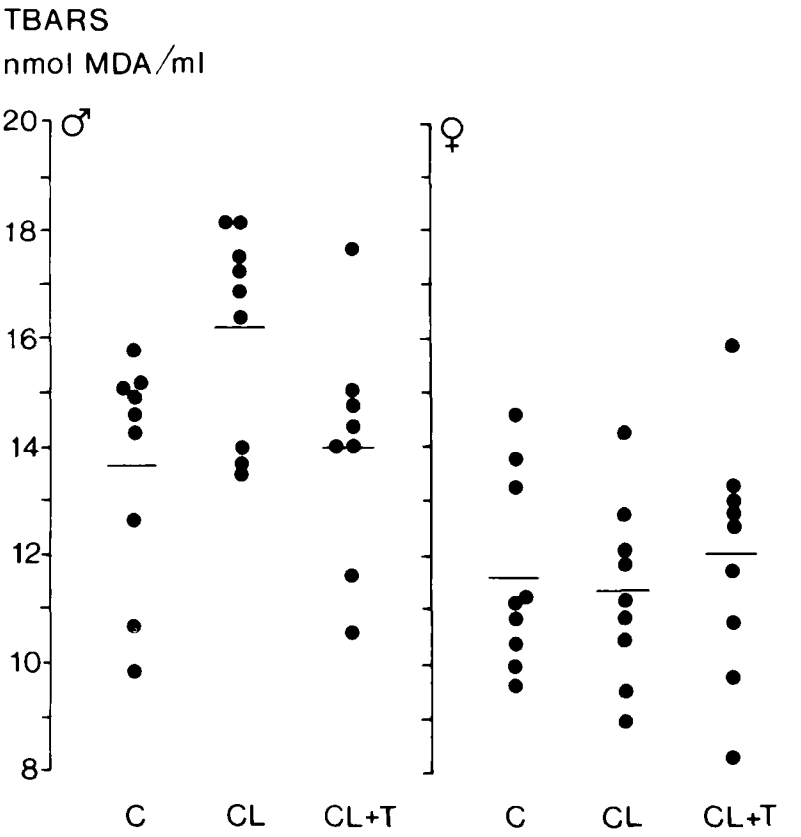

FIGURE 1 Plasma TBARS levels of male and female mice: healthy control group (C), cecal ligation group (CL) and cecal ligation group postoperatively treated with AACT (CL + T).

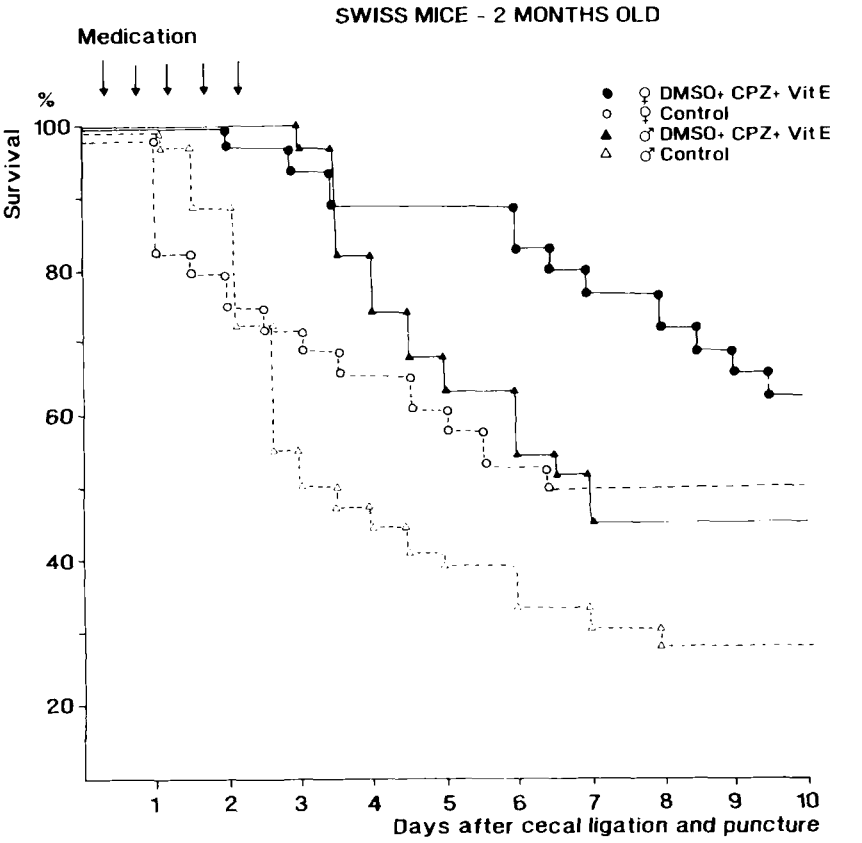

FIGURE 2 Survival of groups of Swiss male and female mice in the cecal ligation sepsis model: nontreated control groups and postoperatively with AACT of DMSO + CPZ + Vit. E treated groups.

In the operated untreated male group the TBARS level (16.2 ± 0.6 nmol MDA/ml) increased significantly ($p < 0.02$) in comparison to the healthy control group (13.7 ± 0.7 nmol MDA/ml). In the operated female group no increase of TBARS level (11.3 ± 0.5 nmol MDA/ml) was observed in comparison to the healthy control group (11.6 ± 0.6 nmol MDA/ml).

Both male and female operated group, postoperatively treated with AACT, had the same TBARS levels (14.0 ± 0.8 and 12.0 ± 0.7 nmol MDA/ml respectively) as the unoperated male and female control group (13.7 ± 0.7 and 11.6 ± 0.6 nmol MDA/ml respectively). The difference between the TBARS level in the operated male group and in the operated male group postoperatively treated with AACT was significant ($p < 0.04$).

Survival in the operated and postoperatively with AACT treated separate male and female group was higher in comparison with the corresponding operated untreated male and female group (Figure 2). Comparison of these separate male and female groups was statistically insignificant ($p > 0.05$). However the difference between united male and female groups was significant ($p < 0.001$).

DISCUSSION

After trauma or infection the organism reacts with inflammation. The intensity of this process depends on many factors among which the extent of the lesion and the number and virulence of present microorganisms are important.[21-23]

The activation of the cellular component of the immune system during this process may be accompanied by a production of toxic products which can act as mediators for vicious aggravation of the inflammation. If the production of these toxic, mainly oxidation products, exceeds the detoxification capacity of the defence system, oxidation of available substrate (lipids, amino-acids, carbohydrates)[11,24] and damage of the normal tissues may occur.[6,7,25-28]

In order to obtain better orientation about the influence of the antioxidative/anti-inflammatory therapy on inflammation, plasma TBARS level has been determined. TBARS level is a reflection of auto-oxidation[11] and seems to correlate at the same time with the violence of the inflammation.[12]

The difference in TBARS level between the healthy male and female group (Figure 1) is in accordance with the results in man.[18]

A rise of the TBARS level postoperatively only in the male group but not in the female group (Figure 1) is difficult to explain, but points towards the fact that males have an incresed sensitivity to auto-oxidation.

The finding of the same TBARS values in the not operated control group as in the operated male group treated with AACT (Figure 1) points at the ability of the applied therapy to inhibit the excessive auto-oxidation induced by the inflammation in this model.

The difference of TBARS level between the male groups and the lack of difference between the female groups is not in accordance with the survival, where both corresponding male and female AACT treated groups showed increase in survival in comparison with the untreated groups.

These results suggest that the TBARS test is too insensitive to follow the activity of the inflammation and that it has no predictive value for the outcome of sepsis in this model.

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