COMPARATIVE EVALUATION OF THE SERUM AND TISSUE REDOX HOMEOSTASIS IN STRESS AND CANCER, IN RAT

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Keywords: redox homeostasis; psychoemotional stress; cancer; rat

Abstract: A lot of experiments in human and animals evidenced that the exposure to various stressors; but also tumor development can alter systemic redox homeostasis by stimulation of oxidants production or/and suppression of some antioxidant/reparatory systems. Our study performed on Wistar rats aimed to investigate comparatively the influence of chronic psychoemotional stress and tumor growth on the redox balance in the serum and tissues by determining the content of thiobarbituric acid reactive substances (TBARS) and total thiol groups (-SH) and oxidase activity of ceruloplasmin (CP). Comparative evaluation of these redox parameters was based on the final results; respectively at the end of stress procedure and in the late stages of tumor evolution.

INTRODUCTION

A lot of experiments in human and animals evidenced that the exposure to various stressors (physical; chemical; infectious; emotional; etc.); but also tumor development can alter systemic redox homeostasis by stimulation of oxidants production or/and suppression of some antioxidant/reparatory systems. Thus; W.G.Yasmineh et al. (1991) have found a decreasing in catalase activity in the liver and kidney of experimental tumors bearing rats [14]. J.J.S.Freitas et al. (2001) have also showed the capacity of Walker 256 tumor to induce oxidative stress in several brain regions in rat; through the stimulation of lipid peroxidation and CuZn-superoxide dismutase activity beside the decreasing of catalase and glutathione peroxidase activity; at 14 days after subcutaneous tumor implantation [5]. Many other acute and chronic psychological stress experiments in rats have also evidenced the increasing of oxidative metabolism in various tissues at the different periods after stimulation. Redox dysregulation initiated by stress and tumor growth could be subsequently involved in the development of several complications (atherosclerosis; hypertension; immunosuppression; insulin resistance; dyslipidemia; etc.); through the dual action of oxygen metabolites as signaling versus cytotoxic molecules. Our study performed on 48 Wistar rats investigated comparatively the influence of chronic psychoemotional stress (open-field stress) and tumor development (using 2 experimental murine tumors of distinct origin and growth rate) on the redox balance in the serum and tissues by determining the content of thiobarbituric acid reactive substances (TBARS) and total thiol groups (-SH) and oxidase activity of ceruloplasmin (CP). Comparative evaluation of these redox parameters was based on the final results; respectively at the end of stress procedure and in the late stages of tumor evolution respectively. Our results revealed several similarities; but also differences of the redox
parameters between stress/cancer model. Moreover; some redox differences were even recorded between the two studied murine tumors probably due to their distinct growth rate.

MATERIAL AND METHODS

Animals: 48 male adult Wistar rats; weighing 180g; socially caged (6 animals per cage) were divided in 4 groups (12 animals/group): 1. control group; 2. stress group (including rats subjected to open-field stress); 3. tumor group (including rats grafted with fast-growing Walker 256 carcinosarcoma); 4. tumor group (including rats grafted with slow-growing RS-1 hepatocholangioma). The experiments started after 1 week acclimatization of the animals to the new environment.

Stress procedure (open-field stress): The animals were daily transferred to an open field more extended than housing cage; 5h/day for 30 days. Exposure to an open field is considered a mild psychological stressor in rat. The animals were sacrificed at 24 h after the last exposure; under ether anaesthesia.

Tumor models: Walker 256 carcinosarcoma: a fast-growing transplantable tumor in rat; spontaneously developed in the mammary gland of a pregnant female. A tumor cell suspension containing 1x10^7 cells/ml saline solution/animal was subcutaneously inoculated on the left flank in rat. The animals were sacrificed in the late stage of tumor evolution (24 days after implantation) under ether anaesthesia.

RS-1 hepatocholangioma (RS-1 hepatoma): a slow-growing transplantable tumor in rat; chemically induced by administration of 2-acetaminofluorene in food. A tumor cell suspension containing 100mg tissue/0.5ml saline solution/animal was subcutaneously inoculated on the left flank in rat. The animals were sacrificed in the late stage of tumor evolution (60 days after implantation) under ether anaesthesia.

Blood (serum) and tissue samples (liver; kidney; brain; adrenals and lung) were quickly collected from all animals. Tissue samples were homogenized in cold saline solution and the resulting supernatants were subsequently used for analyses.

Biochemical determinations: Measurement of the thiobarbituric acid reactive substances level; as an index of lipid peroxidation (TBARS test); Measurement of the oxidase activity of ceruloplasmin (Ravin test); Measurement of total thiol groups content (Schosinsky test).

RESULTS AND DISCUSSIONS

Comparative evaluation of lipid peroxidation products (TBARS) in the serum and the tissues of stress-submitted rats and tumor bearing rats. Chronic stress and development of both murine tumors led to a stimulation of oxidative processes in the serum and the adrenals showed by increasing of TBARS level than controls (table 1). In the serum; the highest TBARS content was found in RS-1 hepatoma bearing rats (by 84%) compared with Walker 256 carcinosarcoma bearing rats (by 30%) and stressed ones (by 18%). In the adrenals; TBARS level recorded a maximum in stress-submitted rats (by 129%) by comparison with RS-1 hepatoma and Walker 256 tumor bearing rats (by 98%; respectively 61%). Surprisingly; chronic open-field stress and slow-growing RS-1 hepatoma produced a decline in TBARS formation in the liver (by 46%; respectively 40%); the kidney (by 7%; respectively 27%) and the lung (by 25%; respectively 33%) than control animals. Slow tumor evolution and the mild psychological stress may initiate in these tissues certain adaptation mechanisms of blocking/preventing the lipid peroxidation chain reactions. On the contrary; fast-growing Walker 256 carcinosarcoma stimulated TBARS production in the serum and all investigated

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tissues; mainly in the lung (by 95%) and the liver (by 80%) probably through oxidant overproduction with a concomitant overwhelming of tissue antioxidant/reparatory capacity.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>Stress</th>
<th>Walker 256 carcinosarcoma</th>
<th>RS-1 hepatoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>6.94</td>
<td>8.19 (18%)</td>
<td>9.01 (30%)</td>
<td>12.80 (84%)</td>
</tr>
<tr>
<td>Liver</td>
<td>24.82</td>
<td>13.47 (46%)</td>
<td>44.65 (80%)</td>
<td>14.87 (40%)</td>
</tr>
<tr>
<td>Kidney</td>
<td>16.77</td>
<td>15.61 (7%)</td>
<td>19.67 (17%)</td>
<td>12.19 (27%)</td>
</tr>
<tr>
<td>Brain</td>
<td>14.45</td>
<td>18.55 (28%)</td>
<td>21 (45%)</td>
<td>11.81 (18%)</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>2.86</td>
<td>6.56 (129%)</td>
<td>4.61 (61%)</td>
<td>5.67 (98%)</td>
</tr>
<tr>
<td>Lung</td>
<td>14.71</td>
<td>11 (25%)</td>
<td>28.66 (95%)</td>
<td>9.89 (33%)</td>
</tr>
</tbody>
</table>

Increased TBARS production in the serum and the adrenals of both stress-submitted rats and tumor bearing rats may have common underlying mechanisms. In the serum such a mechanism may consist in activation of circulating phagocytes by emotional and tumoral stimuli. In this way; M.J.Forlenza et al. (2002) found an activation of circulating neutrophils with a high superoxide releasing; in rats subjected to open-field stress [4]. In RS-1 hepatoma bearing rats; phagocytes stimulation could be induced by tumoral antigens of chemical origin; while in Walker 256 tumor bearing rats high plasma level of TNF-α may have the same stimulatory effect [5; 9]. They suppose that rapid activation of immune cells may represent a common response to a wide range of stressors [10]. In the adrenals; increasing of TBARS content could reflect a high biosynthesis of steroids and catecholamines; both processes being known as oxidant sources. In rat; the adrenal cortex has been found with a significant capacity of lipid peroxidation compared to other tissues [15]. In stressed and tumor bearing rats; adrenal activation evidenced by induction of prooxidant reactions could result from stimulation of stress axis (HPA axis) by the emotional and tumoral stimuli. Moreover; in tumor bearing rats adrenal hyperactivity may have beneficial effects on tumor growth; possibly by mediation of the glucocorticoids. Thus; bilateral adrenalectomy in Walker 256 carcinosarcoma bearing rats resulted in inhibition of tumor development [2].

Comparative evaluation of ceruloplasmin oxidase activity (CP) in the serum and the tissues of stress-submitted rats and tumor bearing rats

Chronic stress produced a decreasing of CP activity in the serum and the most examined tissues (except the adrenals). The lowest enzymatic activity was noticed in the liver (by 83%); the kidney (by 63%) and the lung (by 61%) – table 2. Development of RS-1 hepatoma and Walker 256 carcinosarcoma caused a stimulation of CP activity in the serum (by 174%; respectively 61%); the kidney (by 99%; respectively 111%) and the adrenals (by 479%; respectively 536%). On the other hand; the two murine tumors had opposite effects on CP activity in the liver, the brain and the lung namely; Walker 256 tumor declined enzymatic activity in these tissues while RS-1 hepatoma enhanced it. Actually; RS-1 hepatoma growth stimulated CP activity in the serum and all examined tissues (particularly in the adrenals).

Our results also revealed an intense activity of CP in the adrenals both of stress-subjected rats and tumor bearing rats (more significant in the latter). The enhanced CP activity beside the increased TBARS production could reflect an adrenal hyperfunction both in stress and cancer. In this tissue; high CP may function as a copper supplier for increased catecholamine biosynthesis (in the adrenal medulla) or; in exchange; adrenal CP may be
involved by its amine oxidase activity in removing excess catecholamine with a potential role in oxidative stress generation [1].

Table 2
Ceruloplasmin oxidase activity (CP) in the serum and tissues of stress-submitted rats and tumor bearing rats (UI)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>Stress</th>
<th>Walker 256 carcinosarcoma</th>
<th>RS-1 hepatoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>125.75</td>
<td>82 (35%)</td>
<td>203 (61%)</td>
<td>344 (174%)</td>
</tr>
<tr>
<td>Liver</td>
<td>17.83</td>
<td>3 (83%)</td>
<td>15 (16%)</td>
<td>18.62 (4%)</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.75</td>
<td>5 (63%)</td>
<td>29 (111%)</td>
<td>27.37 (99%)</td>
</tr>
<tr>
<td>Brain</td>
<td>7.25</td>
<td>4 (45%)</td>
<td>6 (17%)</td>
<td>12.62 (74%)</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>2.2</td>
<td>5 (127%)</td>
<td>14 (536%)</td>
<td>12.75 (479%)</td>
</tr>
<tr>
<td>Lung</td>
<td>25.75</td>
<td>10 (61%)</td>
<td>15 (42%)</td>
<td>29 (12%)</td>
</tr>
</tbody>
</table>

Decline of CP activity in the serum and most investigated tissues of stressed rats could result from the reduction of protein-enzyme synthesis in the liver which is recognized as a major source of body CP. Some studies showed that hepatic production of CP is controlled by the adrenal glands so that adrenal insufficiency/exhaustion produced by chronic stress or other causes (toxic; infectious; nutritional; congenital) may be involved in decreasing of CP synthesis by the liver with a subsequent copper accumulation in the body tissues where exerts cytotoxic (degenerative) effects [3]. In our experiment; chronic psychological stress of low intensity; but persistent; may be responsible for the adrenal exhaustion with second influences on CP production by the liver. It is worth reminding the difficulties in interpretation of serum and tissue CP fluctuations taking into account the multiple properties of CP (antioxidant; angiogenic; copper vehicle; acute-phase reactant etc.) on the one hand; and the dual prooxidant/antioxidant behaviour of CP (depending on the microenvironment; protein-enzyme concentration and structural integrity; etc.); on the other hand.

Comparative evaluation of total thiol groups (-SH) in the serum and the tissues of stress-submitted rats and tumor bearing rats. Chronic stress and development of both murine tumors caused a decreasing of total thiols content in the serum; but an increasing in the adrenals (table 3). In the serum; total thiols level declined by 45% in stressed rats; by 42% and 33% in Walker 256 tumor and RS-1 hepatoma bearing rats; respectively. In the adrenals; total thiols recorded an increasing by 19% in stressed rats; by 20% and 29% in Walker 256 tumor and RS-1 hepatoma bearing rats; respectively. Both tumors enhanced thiols level in the liver by 14% (Walker 256 tumor) and by 44% (RS-1 hepatoma); while chronic stress had an opposite effect by a decreasing of 10% in hepatic thiols.

Table 3
Total thiols content (-SH) in the serum and tissues of stress-submitted rats and tumor bearing rats (mmoli/l)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>Stress</th>
<th>Walker 256 carcinosarcoma</th>
<th>RS-1 hepatoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>279</td>
<td>153 (45%)</td>
<td>162 (42%)</td>
<td>186 (33%)</td>
</tr>
<tr>
<td>Liver</td>
<td>641</td>
<td>576 (10%)</td>
<td>731 (14%)</td>
<td>924 (44%)</td>
</tr>
<tr>
<td>Kidney</td>
<td>363</td>
<td>345 (5%)</td>
<td>319 (12%)</td>
<td>490 (35%)</td>
</tr>
<tr>
<td>Brain</td>
<td>319</td>
<td>233 (27%)</td>
<td>201 (37%)</td>
<td>359 (12%)</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>187</td>
<td>223 (19%)</td>
<td>225 (20%)</td>
<td>241 (29%)</td>
</tr>
<tr>
<td>Lung</td>
<td>601</td>
<td>439 (27%)</td>
<td>470 (22%)</td>
<td>976 (62%)</td>
</tr>
</tbody>
</table>
We also found that RS-1 hepatoma produced the growth of total thiols in all examined tissues (more obvious in the lung and the liver); while chronic stress led to their reduction mainly in the lung and the brain; except the adrenals (table 3).

The decreasing of serum thiols; both in stress and cancer; may originate from the lowering of albumin that is recognized as the major source of serum thiol groups. Moreover; albumin decline may rely on several mechanisms consisting in: reduced albumin synthesis in the liver; intense oxidative consumption of albumin or accelerated serum albumin degradation probably under the influence of stress hormones (glucocorticoids) produced by the adrenals [7;12;13]. The increasing of total thiols in the adrenals; both in stress and cancer; may result from activation of stress axis (HPA axis) by the emotional and tumoral stimuli. In this way; it was reported that corticotropin (ACTH) administration in a single small doses rised considerably the adrenal glutathione; in rat [6]. Rat adrenals also distinguished by a very high glutathione and selenium content compared to other tissues [15].

However; our results demonstrated that neither high thiols content nor high ceruloplasmin activity were efficacious in reducing of TBARS formation in rat adrenals; under stress and cancer conditions. The increasing of total thiols in the liver of tumor bearing rats could reflect an acute phase response triggered by the tumoral signals. This response may consist in stimulation of thiols synthesis and may be controlled by the adrenal glucocorticoids [8]. On the contrary; the decreasing of liver thiols by 10% in stressed rats may be mediated by catecholamine (adrenaline) which was found able to promote the depletion of liver thiols through the releasing of glutathione in the biliary space [11]. In mouse; it was reported that adrenaline decreased hepatic glutathione by about 30% at 3h after single intraperitoneal injection [11]. Therefore; neuroendocrine; immune/inflammatory and other disorders could influence; more or less; the serum and tissue redox homeostasis; both in stress and cancer.

CONCLUSIONS

- Chronic open-field stress and development of both murine tumors stimulated TBARS production (lipid peroxidation) in the serum and the adrenal glands; Walker 256 carcinosarcoma increased TBARS content in the serum and all examined tissues while chronic open-field stress and RS-1 hepatoma; surprisingly; declined TBARS formation in the liver; the kidney and the lung;
- Chronic open-field stress and development of both murine tumors stimulated oxidase activity of ceruloplasmin (CP) in the adrenal glands (more obvious in tumor bearing rats); Chronic open-field stress produced the decreasing of CP activity in the serum and most examined tissues (except the adrenals); RS-1 hepatoma enhanced CP activity in the serum and all investigated tissues while Walker 256 carcinosarcoma reduced the enzymatic activity in the liver; the brain and the lung;
- Chronic open-field stress and development of both murine tumors induced the decreasing of total thiols content in the serum; but the increasing in the adrenal glands; Chronic open-field stress declined thiols content in the serum and most investigated tissues (except the adrenals); RS-1 hepatoma rose thiols level in all examined tissues while Walker 256 carcinosarcoma declined the thiols in the kidney; the brain and the lung; however; both tumors enhanced total thiols content in the liver;
- Integrate analysis of redox parameters (TBARS; ceruloplasmin and total thiols) reflected the generation of redox disturbance (oxidative stress) mainly in the serum and the brain (in stress-submitted rats); in the lung and the brain (in Walker 256 tumor bearing rats) and in the serum (in RS-1 hepatoma bearing rats);
The clearest similarities between stress and cancer condition were noticed in the adrenal glands and consisted in the increasing of TBARS production; CP activity and total thiols content; this suggests that both stress and tumor evolution can induce an adrenal hyperfunction; moreover; stress hormones produced by the adrenals (catecholamine; glucocorticoids) could influence; to a certain degree; serum and tissue redox homeostasis.

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