CELLULAR EFFECTS OF SOME BIOACTIVE PRINCIPLES FROM RED WINES AND MUST

ION NEACŞU[1*], PINCU ROTINBERG[2], COSMIN MIHAI[2], DANIELA GHERGHEL[2], GABRIELA CĂPRARU[2]

Keywords: red wine and must, ethanol, cell respiration, redox potential, hepatoprotecting and energizing effects

Abstract: The *in vitro* action of some samples of red wine and concentrated must on the liver and striated muscle of frog (*Rana ridibunda*, Pall) was studied, following the intensity of cellular respiration, and the redox potential (rH). Both wine (1 mL/100mL physiological solution normal Ringer – NR) and must (2 mg dry matter/100mL NR) stimulate aerobe cellular respiration (mm^3 O_2/g wet tissue) and diminish the redox potential values, comparatively with the non-treated reference sample. Also, on treating liver and muscle cells with ethanol (1 mL/100mL NR) an evident depression of cellular respiration and increase of redox potential values may be noticed, alongwith perturbation of cellular metabolic processes. The obtained results evidence possible energizing, hepatoprotecting and redox modulating properties of the bioactive principles from red wine and must.

INTRODUCTION

Various investigations have evidenced the pozitive biological effects of a moderate wine consumption, induced by some bioactive compounds – such as the polyphenols – present mainly in the red wines [Burda et Oleszek, 2001; Renaud et Lorgeril, 1992; Ursini et Sevanian, 2002].

Such compounds show anti-oxidating, anti-inflamatory, membranotrope, vasodilatatory, anti-atherogenes, anti-tumoral properties, with acting as modulators of the cell redox and bioenergetic processes, usualy mentioned for explaining the so-called “French paradox” [Renaud et Lorgeril, 1992], regarding some benefic effects of a moderate wine consumption..

The hepatoprotecting properties of such wine compounds were less studied, although the hepatotoxic effects of the ethanol present in wine, besides the principles inducing beneficial effects, have been intensely studied [Esterbauer, 1996; Naum et al., 2002; Watkins, 1977].

Based on these aspects, in the present study we investigated the effects of some red wine and concentrated must samples upon cellular respiration and on the redox potential of the frog normal liver and striated muscle cells, comparatively with the effect of ethanol and with the values registered with a control non-treated batch tissues.

MATERIAL AND METHODS

The experiments were performed *in vitro,* on the liver and sartorius striated muscle of frog (*Rana ridibunda*, Pall) incubated into a normal Ringer physiological solution (NR) containing red wine “Fetească neagră” (1mL/100mL NR), concentrated red must (2 mg dry matter/100 mL NR) or ethanol (1 mL/100 mL NR), the duration of each phase being of 1 hour.

Cellular respiration has been studied by the Warburg micromanometric method [Nuţă et Buşneag, 1977], and redox potential – by potentiometric method , the obtained values being expressed by parameter rH, calculated according the relation of Clark, correlated with the normal hydrogen electrode (factor *f*) [Ingold, 1982; Zanoagă et al, 1988]:

$$rH = \frac{E_h + 0.058 \cdot pH + f}{0.029}$$

(f = 0,248 V at 20^{o}C)

The data thus obtained have been processed statistically by Student test.

RESULTS AND DISCUSSION

Determination of the intensity of the oxygen respiratory consumption (mm^3 O_2/g wet tissue) on a normal liver and muscle, for 1 hour, at intervals of 15 minutes (Table 1) evidenced a progressive stimulation the aerobe cell respiration processes, in the presence of both wine and the concentrated must, comparatively with the control non-treated samples (100%). Generally, the stimulating effect of the must is more intense than that of the wine (Table 1), the respiratory consumption of oxygen recorded in liver after 60 minutes being 64.35% higher in the presence of must, and only 33.03% higher, respectively, in the presence of wine, comparatively with the control batch (100%), which may be explained by a possible reverse action of the ethanol of the wine [Hollman et al., 1996; Naum et al., 2002; Renaud et Lorgeril, 1992]. The corresponding values registered with muscle cells after 60 minutes of treatment, were also higher compared to the control, with 54.34% by must and with 43.19% by wine. The ethanol treatment evidenced a noxious effect, reducing the cell respiration level with 18.80% at liver and with 25.75% at muscle (Table 1).

Table 1. Red wine, must and ethanol influence upon muscle (M) and liver (L) cellular respiration (mm^3 O_2/g wet tissue of frog). VC% - variability coefficient

Batch (treatment)	Tissue	Para metr.	15 min		30 min		45 min		60 min	
			mm^3 O_2/g	%	mm^3 O_2/g	%	mm^3 O_2/g	%	mm^3 O_2/g	% (±%)
CONTROL	M	$\bar{x}$	0.62	100	1.14	100	1.68	100	2.27	100
		SE	0.06		0.08		0.12		0.21	
		VC%	30.10		21.44		21.45		26.10	
	L	$\bar{x}$	0.56	100	1.11	100	1.63	100	2.12	100
		SE	0.07		0.08		0.07		0.08	
		VC%	36.09		22.21		13.49		11.40	
MUST		$\bar{x}$	0.68	108.95	1.98	173.32	2.83	168.94	3.51	154.33
		SE	0.07		0.08		0.22		0.25	(+54.34)
		VC%	31.01		26.31		22.31		28.65	
	L	$\bar{x}$	0.76	136.58	2.03	182.87	2.88	175.98	3.48	164.34
		SE	0.083		0.11		0.23		0.23	(+64.35)
		VC%	33.10		27.02		20.59		19.79	
WINE	M	$\bar{x}$	0.79	126.98	2.01	175.37	2.71	161.57	3.25	143.19
		SE	0.083		0.12		0.22		0.21	(+43.19)
		VC%	29.75		25.30		23.01		20.01	
	L	$\bar{x}$	0.97	141.98	1.94	174.76	2.64	161.54	2.82	133.03
		SE	0.13		0.12		0.17		0.20	(+33.03)
		VC%	32.07		21.87		18.57		18.31	
ETHANOL	M	$\bar{x}$	0.41	66.59	0.91	79.73	1.39	83.20	1.68	74.24
		SE	0.05		0.09		0.12		0.07	(-25.75)
		VC%	35.20		24.37		20.13		19.70	
	L	$\bar{x}$	0.44	79.49	0.85	76.97	1.47	89.86	1.72	81.19
		SE	0.06		0.08		0.097		0.08	(-18.80)
		VC%	28.76		26.32		19.31		20.35	

The intensity of respiratory response of liver cells at the polyphenolic compounds from wine and must may be correlated with the fact that hepatic cells are the headquarters of multiple metabolic processes involving oxygen consumption within the phases of the Krebs cycle of aerobe cellular respiration [Karp, 1996; Lehninger, 1987]. The values registered with the striated muscle cells evidence, however, a higher intensity of cellular respiration compared to that of hepatic cells, with both control and treated batches, which is correlated with the more intense oxygen consumption and oxidative phosporylation, creating a superior energetic balance at the muscle cells level [Karp, 1996; Lehninger, 1987].

Living cells have certain redox buffer systems [Karp, 1996; Lehninger, 1987] which may be influenced by polyphenolic compounds from wine and must, in this way they manifesting redox modulation properties. Krebs cycle include a series of redox systems having redox potential values specific for the components of each system, correlated with the respiratory oxygen consumption and mitochondrial P/O ratio, thus oxidoreduction processes being coupled with aerobe respiration, generating ATP by oxidative phosphorylation process, and, implicitly, correlated with cellular energetics [Karp, 1996; Lehninger, 1987].

Redox parameters influence the physiological processes of the living organisms, rH having an optimal value specific for each species and even for each organ [Karp, 1996;

Lehninger, 1987; Zanoagă et al, 1988], thus rH value determination being important for evidencing the specific influences of some bioactive principles upon cellular processes.

The values of the oxygen respiratory consumption by liver cells reveal the manifestation of the hepatoprotecting properties of both red wine and must. Such an effect is most probably induced by the polyphenols they contain, possessing membranotropic, stabilizing and antioxidating properties [Burda et Oleszek, 2001; Hollman et al., 1996; Renaud et Lorgeril, 1992]. Mention should be also made of the fact that the ethanol manifests hepato-toxic effects, inducing a depression of cell respiration which may be annuled, however, by the action of the polyphenolic compounds present in wine and must, owing to their anti-oxidating and anti-inflamatory properties [Hollman et al., 1996; Renaud et Lorgeril, 1992; Ursini et Sevanian, 2002; Watkins, 1977].

Thus, such polyphenol properties explain the stimulating effects of red wine upon cell respiratory processes registered in our experiments, although the wine contains about 11% v/V ethanol. The results obtained permit another important observation, too, namely that the ethanol provokes an abnormal increase of the rH values, both in the solutions (NR) and in the hepatic and muscle tissue (Table 2). Considering the value of 28.3 V as the neutral value of rH, values between 0 – 28.3 V represent the reducing domain of rH and values between 28.3 – 42.4 V represent the oxidating domain [Ingold, 1982; Ursini et Sevanian, 2002]. The obtained data permit the general observation that the red must and wine manifest an effect of normalization of redox potential, diminishing the rH values, all of them being in the reducing domain, which is actually specific to the animal organisms [Zanoagă et al, 1988]. Thus, the results reflect the redox modulation properties of polyphenols from wine and must, correlated with their anti-oxidating properties [Burda et Oleszek, 2001; Esterbauer, 1996; Renaud et Lorgeril, 1992; Ursini et Sevanian, 2002].

Table 2. Influence of red wine and must and of ethanol upon rH values (V) of NR+agent solutions and upon hepatic and muscle tissue of frog (NR – normal Ringer physiological solution).

Agent	NR + agent			Hepatic tissue			Muscle tissue		
	rH (V)	%	Effect (± %)	rH (V)	%	Effect (±%)	rH (V)	%	Effect (±%)
NR (control)	28.27	100	0	22.33	100	0	23.47	100	0
NR + Ethanol	28.31	100.14	+0.14	23.24	104.07	+4.07	24.83	105.79	+5.79
NR + Must	26.96	95.37	–4.63	22.4	100.31	+0.31	23.14	98.59	-1.41
NR + Wine	27.17	96.11	–3.89	22.15	99.19	–0.81	23.61	100.60	+0.60

The values of the oxygen consumption intensity registered in our experiments reflect the global effect of red wine and must, as well as of ethanol, upon cellular aerobe respiration. The stimulatory effect of red wine and must upon cellular oxygen consumption highlights implications of polyphenolic compounds from wine and must in the developing of Krebs cycle processes and in the redox reactions, having important energo-modulating and redox-modulating properties, too [Karp, 1996; Lehninger, 1987].

All these experimental results evidence multiple cellular influences and positive effects of the bioactive principles present in red wine and must.

CONCLUSIONS

The results of the study indicate the positive effects of the active principles present in wine and must – the red ones, especially – expressed by intensification of the processes of aerobe cellular respiration and by the normalization of the redox potential of the hepatocytes and muscle cells intoxicated with ethanol.

In this way, some energizing and hepatoprotecting actions as well as redox modulation and energo-modulation properties of these bioactive principles are put into evidence, which support the idea of some benefic effects of a moderate wine consumption, depending, however, on the individual physiological particularities.

REFERENCES

Burda S.,Oleszek W., 2001, *Antioxidant and antiradical activities of flavonoids,* J. Agric. Food. Chem. 49, 2774-2779

Esterbauer H., 1996, *Estimation of peroxide damage*, Path. Biol., 44, 25-28

Hollman P.C.H., Hertog M.G.L., Kata M.B., 1996. – *Analysis of health effects of flavonoids*, Food. Chem., 57, 43 – 46

Ingold W. 1982, *Redox measurements principles and problems*, INGOLD, Urdorf, Switzerland

Karp G., 1996, *Cellular and molecular biology,* John Willey and Sons Inc., New York, Chichester, Brisbane, Toronto, Singapore, 177 – 205, 675

6.Lehninger A.L.,1987, *Biochimie,* vol II, Ed. Tehnică, Bucureşti, 473 – 573

Naum E., Maftei A., Neacşu I., Maniu C.,2002, *Disturbance of redox processes determined by the action of ethanol at the cellular level*, Sci. Anal. Univ. Agric. Sci. Vet. Med. Ion Ionescu de la Brad Iaşi, Horticulture, **XLV,** 97 –102

Neacşu I., Zănoagă C.V., Niculaua M. 2006, *Hepatoprotective effects of some anthocyanic vegetal extracts*, St. Cercet. Biol. Univ. Bacău (Romania), 11, 141 –145

Nuță G., Buşneag C. 1977, *Investigații biochimice,* Ed. Didactică şi pedagogică, Bucureşti, 283 –286

Renaud S., Lorgeril M., 1992, *Wine, alcohol, platelet and French paradox for coronary disease*, Lancet, 339, 1523 –1526

Ursini F., Sevanian A., 2002, *Wine polyphenols and optimal nutrition*, Annals N.Y. Acad. Sci., 957, 202 –209

Zănoagă C.V., Neacşu I., Zănoagă M., 1988, *Considerații asupra tehnicii de determinare a rH-ului unor probe biologice*, St. Cerc. Biochim., **31**, 1, 53 –58

Watkins T.R., 1977, *Wine-nutritional and therapeutic benefits*, ACS Symposium Series 661, American Chemical Society, Washington D.C.

1 Faculty of Biology, „Al.I.Cuza” University, Bd. Carol I, nr. 20A, 700506, Iasi, Romania
2 Biological Research Institute, Bd. Carol I, nr. 20A, 700506, Iasi, Romania
* ineacsu@uaic.ro