INFLUENCE OF SOME BIOACTIVE PRINCIPLES IN RED WINE UPON CELLULAR OXIDATIVE PHOSPHORYLATION

I. Neacşu,[(1)] M. Niculaua[(1)], Otilia Chiriță[(2)], C.V. Zănoagă[(1)], V.V. Cotea[(2)], Cintia Colibaba[(2)]

[(1)]Enological Research Center Iaşi – Iaşi Branch of Romanian Academy
Bd. Carol I, no. 8, Iaşi, România
ccoiasi@gmail.com
[(2)]„Ion Ionescu de la Brad“ University of Agricultural Sciences and Veterinary Medicine of Iaşi
Alley M. Sadoveanu, no. 3, Iaşi, România
vcotea@uaiasi.ro

ABSTRACT

The *in vitro* influence of a Romanian red wine (Fetească neagră) upon cellular respiration and oxidative phosphorylation process of the batracian muscular and hepatic cells was studied. The oxidative phosphorylation process was appreciated by calculation of P:O ratio (oxidative phosphorylation index) values, the oxygen respiratory cellular consumption by Warburg micromanometric method and inorganic phosphate cellular content by the Bell-Doisy-Briggs method. The red wine induced certain stimulation of cellular respiration and of oxidative phosphorylation process, with amplitudes depending on the wine used concentrations (1–3%) and the cell type. The results evidence the influence of bioactive principles (especially polyphenols) in red wine upon the cellular energetic processes as well as some of theirs useful pharmacological properties.

L'influence *in vitro* d'un vin rouge roumain (Fetească neagră) sur la respiration cellulaire et les processus de phosphorylation oxydative des cellules musculaires et hépatiques batracien a été étudiée. Le processus de phosphorylation oxydative a été appréciée par le calcul de les valeurs P:O ratio (indice de phosphorylation oxydative), la consommation d'oxygène respiratoire cellulaire a été déterminé par la méthode micromanometrique Warburg et le contenu du phosphore inorganique par la méthode Bell-Doisy-Briggs. Le vin rouge induite certaine stimulation de la respiration cellulaire et du processus de phosphorylation oxydative, selon les concentrations du vin utilisé (1–3%) et de le type de cellule étudiée. Les résultats prouvés l'influence des principes bioactifs (en particulier les polyphenols) de le vin rouge sur les processus cellulaires énergétiques ainsi que certains de leurs propriétés pharmacologiques utiles.

INTRODUCTION

It is by now generally accepted the idea that some bioactive compounds of wine, polyphenols especially, evidence a series of properties known as inducing certain beneficial effects, as a result of moderate wine consumption, which also explain – among others – the so called „French paradox“ [Dudley *et al.*,2008].

Consequently, the antiradicalic, antiatherogeneic, vasodilating, anti-inflamatory, hepatoprotecting and others positive properties – in correlation with the positive effects of the bioactive principles from wine (polyphenols especially) – have been evidenced in a series of diseases, such as cardiovascular, hepatic, neurological maladies, cancer etc. [Jackson, 2008, Shan He *et al.*,2008, Cotea *et al.*, 2008, Dudley *et al.*,2008].

Previous investigations of ours [Cotea *et al.*, 2008, Neacşu *et al.*2009,] analyzed several aspects of the influence of some fractions isolated from red wine on cellular respiration and on the properties of the blood vessels of certain laboratory animals.

The present paper analyzes the effects of various concentrations of Romanian Fetească neagră red wine upon the intensity of cell respiration and of oxidative phosphorylation in muscular and hepatic frog cells.

MATERIALS AND METHOD

The experiments, performed *in vitro*, on striated muscular cells and hepatic cells taken over from frog (*Rana ridibunda*, Pall), treated with red wine obtained from Romanian Fetească neagră grapes, following cellular respiration and oxidative phosphorylation process. The studied wine had an alcoholic strength of 10.80 % v/V, FCI of 52 mg/L, TPI of 28.83 mg/L, total antocyans – 211.13 mg/L.

The experiments were performed on batches of biological preparations of sartorius muscle and liver from 10 animals. These preparations were treated with red wine in concentrations of 1, 2 and 3 mL/100mL, in a physiological Ringer solution, comparatively with untreated reference groups, incubated in normal Ringer (NR) solution.

The intensity of cellular consumption of oxygen (μL/g fresh tissue= mm^3/g tissue) was determined by the Warburg micromanometric method, at constant temperature and volume. The process of oxidative phosphorylation was appreciated through values of oxidative phosphorylation index, expressed by the P:O ratio, on the same preparations on which cellular respiration was also determined.

The values of the inorganic phosphate (Pi = μmol/g tissue) were represented by the difference between initial Pi content of the preparations, in the beginning of the respiration period, and the final values, recorded in the end of the experiment. The cellular Pi content was determined by the Bell-Doisy-Briggs method [Nuță, Buşneag, 1977].

The values of the oxidative phosphorylation index were calculated with relation:

$$P:O = \frac{\mu mol\ Pi/g\ tissue}{\mu atomg\ O/g\ tissue}$$

The experiments were performed at room temperature (22 ºC), for 60 minutes.

The obtained data were statistically analysed by Student test.

RESULTS AND DISCUSSION

The data recorded after a 60 minutes experiment show that the red wine obviously influences cellular processes, different values being recorded, *versus* the untreated, reference bath, both as to the intensity of cellular oxygen consumption and level of oxidative phosphorylation, expressed by the values of the P:O ratio.

In the untreated, control batch (Tab. 1), the values recorded in the end of the experimental period, for muscular cells, showed an average value of oxygen consumption of 1.309 μL/g fresh tissue (0.935 μatomg /g tissue), and an average content of inorganic phosphate (Pi) of 2.196 μmol/g fresh tissue, while the index of oxidative phosphorylation (the P:O ratio) took a value of 2.348. In the hepatic cells of the control batch, the respiration oxygen consumption was of 0.861 μL/g fresh tissue (0.615 μatomg /g tissue), the Pi content was of 1.591 μmol/g fresh tissue, and the P:O ratio – 2.587.

The data recorded for the control batch (Tab. 1), as actually those registered wine-treated ones, evidenced obvious differences between the behaviour of the muscular and respectively – hepatic cells. For example, in muscle cells, the values of the oxygen

respiratory consumption and of Pi content are higher than in the hepatic cells, however the index of oxidative phosphorylation records lower values.

Tab. 1

	Muscle cells				Liver cells			
	O_2 consumed		Pi µmol/g	P:O	O_2 consumed		Pi µmol/g	P:O
	µL/g	µatomg/g			µL/g	µatomg/g		
$\overline{X}$	1.309	0.935	2.196	2.348	0.861	0.615	1.591	2.587
ES	0.094		0.244		0.113		0.215	
CV %	17.489		27.330		28.262		22.176	

This situation indicates a more intense respiration of the muscle cells, as well as a higher level of oxidative phosphorylation at the level of hepatic cells – comparatively with the muscle ones – along with a higher energetic yield (of ATP production), which means that state 3 of processes of cellular respiration is prevailing [Brand *et al.*, 1993, Shan He *et al.*, 2008].

Such differences may be explained if considering the different morpho-physiological characteristics of the two types of cells. Thus, the striated muscular cells are of excitable type, with a contractile function, while the hepatic ones are non-excitable and play a metabolic role, being the center of numerous synthesis processes with energetic implications [Alberts *et al.*, 1998, Hăulică, 2007].

The different characteristics of the two types of cells also explain their different reactivity during the treatment with red wine. Consequently, in the cellular batches incubated in a wine-containing physiological solution (Ringer), generally, both cellular respiration and the process of oxidative phosphorylation are stimulated, although the amplitude of the phenomenon is different, as depending on the nature of the processes, type of investigated cells and applied wine concentration (Figs. 1 and 2).

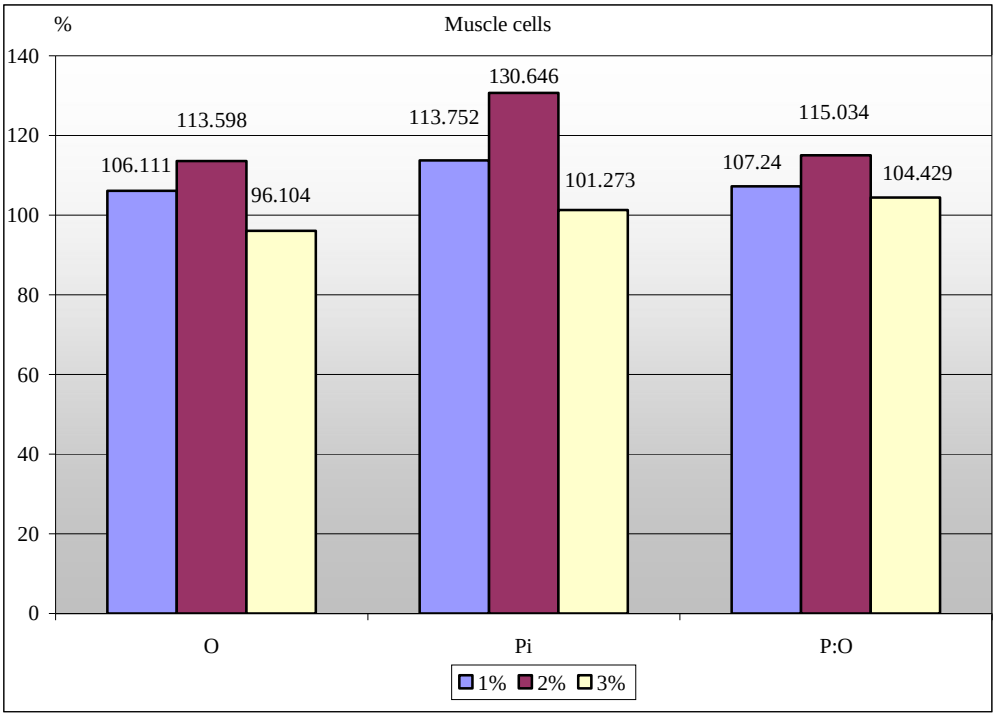

Fig. 1

Similarly with the control batch, the treated ones register – in the muscle cells – higher values of oxygen consumption and content of inorganic phosphate, comparatively with the

values evidenced by hepatic cells, while the oxidative phosphorylation index (P:O) takes lower values.

An 1 % red wine concentration induces, in muscular cells (Fig. 1), an increase of the respiration oxygen consumption up to 106.111 % *versus* the value of the reference batch (100 %), along with an increase of the Pi content up to 113.752 %, as well as a higher value of P:O ratio, up to 107.240 %. In hepatic cells (Fig. 2), an increase of the values of such parameters should be also noticed, the oxygen consumption increasing up to 110.337 %, the Pi content – up to 115.713 %, and the P:O ratio – up to 104.948 %, comparatively with the values registered in the control batch.

On may therefore observe that, at an 1 % wine concentration, oxidative phosphorylation is more intense in the hepatic (P:O = 2.715) than in the muscle (P:O = 2.518) cells, yet, comparatively with the values of the reference batch, the efficiency of the process is lower (with 2.292 %) in the hepatic than in the muscle cells, although the values oxygen consumption and of Pi are higher than those of the muscle cells.

The treatment with 2% red wine concentration evidences the strongest stimulation action of the cellular processes under investigation, when the highest values of the parameter involved are recorded (Figs. 1 and 2). Thus, comparatively with the values of the reference group, the muscular cells recorded an increase of respiratory oxygen consumption up to 113.598 %, of the Pi content – up to 130.646 %, and the P:O ratio, respectively, up to 115.034 %. In the hepatic cells, higher values than in the reference batch are also recorded, up to 137.282 % (respiratory oxygen), 148.837 % (Pi), and 108.465 %, respectively (P:O ratio).

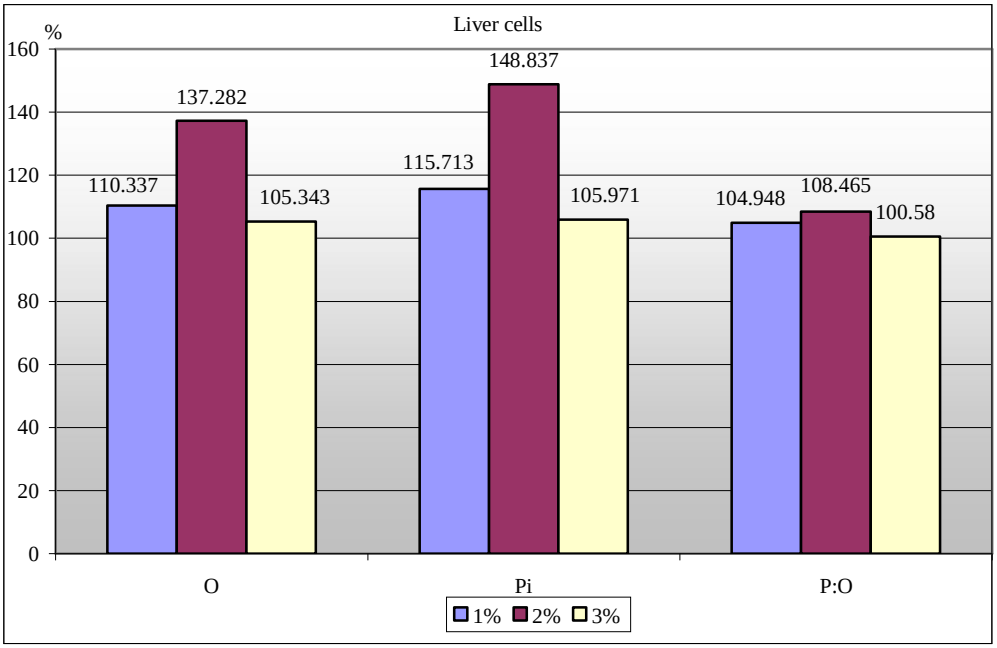

Fig. 2

Similarly with the treatment with 1 % red wine concentration, in the hepatic cells, the respiratory oxygen consumption and Pi values are lower than in the muscular cells, and the increase of the P:O ratio is also more reduced than in the muscular cells (108.465% compared with 115.034 %), which indicate a weaker stimulation of oxidative phosphorylation by 2 % wine concentration.

A 3 % concentration of red wine (Figs. 1 and 2) generally induces weaker effects than those determined by lower concentrations, which is probably due to a higher ethanol ratio

in the incubation medium, known as having its own physiological effects of oxidative phosphorylation depression [Cunningham, Bailey, 2001].

In muscular cells (Fig 1), under the action of such a concentration, respiratory oxygen consumption attains a lower value than in the reference batch (96.104 %), the Pi content remains practically unchanged (100.273 %), while the P:O ratio registers only a slight increase (104.273 %), comparatively with the control. The hepatic cells (Fig. 2) evidence higher values than the muscular ones, yet much more reduced than those attained with lower wine concentrations, as follows: respiratory oxygen consumption – 105.343 % *versus* the control, Pi – 105.971 %, and the P:O ratio – 100.580 %.

As generally known, the intensity of cellular respiration is directly correlated with the mitochondrial processes characterized by respiratory oxygen consumption and release of energy, deposited in the macroergic structure of the ATP molecules [Alberts *et al.*, 1998, Lehninger, 1987]. Production of ATP at mitochondrial level involves phosphorylation of cellular ADP ($ADP + Pi \rightarrow ATP$), a process correlated with the chain of electron carriers and with the mitochondrial enzymatic systems within the Krebs cycle, the final electron acceptor being the oxygen taken over through breathing. Within such processes, for 1 mol of glucose, which represent the metabolic substrate, 3 moles of ATP are generated – if the first carrier of electron chain is NADH, or 2 moles of ATP if the succinate, oxidated through $FADH_2$ intervenes, and only 1 mol ATP if cytochrome c belonging to the chain of electron carriers is oxidated. Therefore, such processes involve the P:O ratio, which represent the index of oxidative phosphorylation expressing the coupling degree of ADP phosphorylation with the oxygen consumed through respiration, taking part in oxido-reduction processes from which ATP results [Alberts *et al.*, 1998, Lehninger, 1987]. At a maximum phosphorylation degree, the respiration processes occurs in state 3, at a P:O ratio of 3 value, complete oxidation of glucose thus occurring; on the contrary, at a minimum (zero) phosphorylation, cellular respiration is in state 4, glucose being incompletely oxidated [Brand *et al.*1993, Lehninger, 1987], and the P:O ratio records its minimum value.

An important observation to be made is that the active principles from wine (polyphenols, especially) cause an increase in the enzymatic activity of the mitochondrial complexes, thus stimulating oxidative phosphorylation and ATP synthesis in cardiac cells, inducing cardioprotecting effects [Dudley *et al.*,2008].Various investigations also evidenced the antiradicalic properties of wine (the red wine, mainly) and the positive effects in other affections, characterized by disorders of the cellular oxidative and energetic processes [Burda, Oleszek, 2001Chiriță *et al.*, 2009, Cotea, 2008, Jackson, 2008, Shan He *et al.*, 2008].

The results of the present study, evidencing that red wine leads to high values, close to 3, of the P:O ratio, indicate a high degree of oxidative phosphorylation, with production of ATP and no generation of free radicals, which highlights the antiradicalic properties of the Fetească neagră wine, already evidenced in some other researches [Chiriță *et al.*, 2009], and also its energetic effects.

CONCLUSIONS

The Fetească neagră red wine stimulates cellular aerobe respiration and oxidative phosphorylation in both muscular and hepatic cells, the amplitude of its effect being dependent on the wine concentration applied and on the cell type.

Respiratory oxygen consumption is more intensely stimulated in hepatic cells, while the index of oxidative phosphorylation records higher values in muscular cells.

The obtained results attest the positive influence of the bioactive principles in red wine upon cellular energetic processes, and evidence some useful pharmacological properties of these principles.

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