EFFECTS OF NICOTINIC ACID ON PROTEIN OXIDATIVE MODIFICATIONS IN EXPERIMENTAL CHRONIC HEART FAILURE

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Abstract

A model of chronic heart failure experimental model was induced to rats based on the toxic effects of doxorubicin. The nature of changes in the content of protein peroxidation products (neutral and basic 2,4-dinitrophenylhydrazones) was established in cardiomyocytes and hepatocytes of rats which received weekly intramuscular doxorubicin for 5 weeks in a dose of 5 mg/kg body weight (bw) together with the daily intraperitoneal administration of nicotinic acid, 10 mg/kg bw. The obtained results pointed out reduced protein peroxidation in cardiomyocytes caused by nicotinic acid, in a chronic heart failure model in rats. This allows to consider nicotinic acid as a drug with a potential cardioprotective activity.

Rezumat

Insuficiența cardiacă cronică a fost induși la șobolani într-un model experimental bazat pe efectele toxice ale doxorubicinei. S-a evaluat natura modificărilor produselor de peroxidare proteică (2,4-dinitrofenilhidrazone neutre și bazice) de la nivelul cardiomiocitelor și hepatocitelor la șobolani cărora li s-a administrat doxorubicină intramuscular săptămânal, timp de 5 săptămâni, într-o doză de 5 mg/kg corp, concomitent cu administrarea zilnică intraperitoneală de acid nicotinic, 10 mg/kg corp. Rezultatele obținute arată o peroxidare proteică redusă în cardiomiocite animalelor cu insuficiență cardiacă cronică, datorată acidului nicotinic. Aceste rezultate permit luarea în considerare a acidului nicotinic ca medicament cu potențială activitate cardioprotecțoare.

Keywords: niacin, protein oxidation, oxidative stress, chronic heart failure, doxorubicin

Introduction

Studies of protein oxidative modifications are performed due to important the role of proteins in the cell and the whole body. Quantitatively, proteins prevail over all other macromolecules in the living cell and are involved in all biological processes, performing various functions. In addition, proteins are more susceptible to free radical oxidation compared to other biomolecules and their oxidation is the earliest stable indicator of the intensity of the generation of active oxygen species that can be mediators of cell damage in cardiovascular diseases, such as ischemia, atherosclerosis and hypertension [1-5].

Studies of the protein degradation pathways which trigger the onset of pathological processes in the organism will contribute to the understanding of the mechanisms of the most common human diseases, one of which is chronic heart failure. These will generate new approaches to the development of drugs for the treatment and prevention of cardiovascular diseases.

Niacin has been used in the treatment of cardiovascular disease, although its use has largely been superseded by better-tolerated lipid-modulating therapies [6-9]. The antioxidant effects of niacin, administered to rats in doses of 10 mg/kg bw in a doxorubicin-induced chronic heart failure model, presented our previous publications, are normalization of redox process in the myocardium through inhibition of reactive oxygen species namely, superoxide anion radical and hydrogen peroxide, as well as oxidation of polyunsaturated fatty acids [10, 11]. The purpose of this study is to investigate protein oxidative modifications in cardiomyocytes and hepatocytes under the influence of nicotinic acid in a rat experimental model of chronic heart failure.

Materials and Methods

The studies were conducted on adult male Wistar rats (180 - 220 g). All procedures and experimental protocols involving animals were in compliance with the European Community guidelines (2010/63/EU) [12], as well as the provisions of the General Ethical Principles of Animal Experiments approved by the 1st National Congress on Bioethics (Kyiv, 2001) and the Law of Ukraine No. 3447-IV On the Protection of Animals from Cruelty. The animals were fed a normal, balanced diet and had free access to water in the animal house (vivarium) of the Bogomolets National Medical University (Kyiv City, Ukraine).
As a result of the study, we found a statistically significant increase in the basic aliphatic aldehyde and ketone dinitrophenylhydrazones in cardiomyocytes of the animals with experimental heart failure. Levels of basic aliphatic aldehyde and ketone dinitrophenylhydrazones in the cardiomyocytes of the animals with experimental heart failure were 2.3 times higher compared to the control group. This shows that the formation of proteins derived from carboxylates induced by doxorubicin is mainly due to the oxidation of the amino acid residues having basic properties. The content of neutral aliphatic ketone dinitrophenylhydrazones in cardiomyocytes of control animals were higher than those of keto dinitrophenylhydrazones which may indicate a process of protein fragmentation to form low molecular weight fragments.

**Table 1**

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Neutral products, optical units/mg of protein</th>
<th>Basic products, optical units/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group 1)</td>
<td>0.65 ± 0.04</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>Experimental chronic heart failure (Group 2)</td>
<td>0.67 ± 0.06</td>
<td>0.85 ± 0.07*</td>
</tr>
<tr>
<td>Experimental chronic heart failure + niacin (Group 3)</td>
<td>0.66 ± 0.05</td>
<td>0.68 ± 0.05*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation, p < 0.05 compared to the control group, *p < 0.05 compared to the group with experimental chronic heart failure.
induced by doxorubicin are due to the protein
in the levels of basic ketone dinitrophenylhydrazones
induced oxidation processes. These significant changes
are due to the protein oxidative modifications compared to the
control animals were registered.

The administration of nicotinic acid caused a statistically
significant reduction in the content of dinitrophenyl-
hydrazones determined at wavelengths of 370, 430
and 530 nm when compared to group 2 of animals.
Values of neutral aliphatic aldehyde dinitrophenyl-
hydrazones in the cardiomyocytes did not differ in
all treatment groups. The predominance of aliphatic
aldehyde dinitrophenylhydrazones in cardiomyocytes
suggests that oxidative stress does not reach its
developmental stage and appears to be reversible.

Table II

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Neutral products, optical units/mg of protein</th>
<th>Neutral products, optical units/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>356 nm</td>
<td>370 nm</td>
</tr>
<tr>
<td>Control (Group 1)</td>
<td>0.92 ± 0.08</td>
<td>1.06 ± 0.09</td>
</tr>
<tr>
<td>Experimental chronic heart failure (Group 2)</td>
<td>1.49 ± 0.13</td>
<td>1.75 ± 0.14</td>
</tr>
<tr>
<td>Experimental chronic heart failure + niacin (Group 3)</td>
<td>1.25 ± 0.11</td>
<td>1.52 ± 0.11</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation, *p < 0.05 compared to the control group

In the experimental conditions, of induced chronic
heart failure, in rat hepatocytes (Table II) the increase
to different extents in the content of neutral and basic
aliphatic aldehyde and ketone dinitrophenylhydrazones
was observed when compared to the group of control
animals. This fact indicates an increase in the intensity
of the oxidized protein degradation under doxorubicin-
induced oxidative stress and suggests that the
doxorubicin targets in hepatocytes are both basic and
neutral amino acid residues.

The fact that ketone dinitrophenylhydrazones are
superior to aldehyde dinitrophenylhydrazones indicates
that oxidative stress has reached its irreversible stage.
The administration of nicotinic acid in the presence
of doxorubicin acid did not result in a statistically
significant reduction in the content of dinitrophenyl-
hydrazones in animal hepatocytes measured at wave-
lengths of 356, 370, 430 and 530 nm when compared
to group 2 of animals, whilst we observed a decrease
in the intensity of oxidative stress in cardiomyocytes
after nicotinic acid administration in the experimental
chronic heart failure model used.

The parameters of the studied protein oxidative
modifications products in rat hepatocytes from group
3 were significantly different from the values of the
control group.

These data indicate a deep oxidative damage to
proteins in the hepatocytes in this experimental model.
The prevalence of secondary markers of oxidative stress,
aliphatic ketone dinitrophenylhydrazones, in animal
hepatocytes from group 3 indicates the active
transition of the primary markers of oxidative stress
to the secondary ones and aggravation of doxorubicin-
induced oxidation processes. These significant changes
in the levels of basic ketone dinitrophenylhydrazones
induced by doxorubicin are due to the protein

glycosylation which is a typical marker of stress. It
was shown that protein glycosylation was closely
connected with free-radical processes and the content
of the products of protein non-enzymatic glycosylation
in oxidative stress was increased.

Conclusions

In the context of experimentally induced CHF with
doxorubicin, deep oxidative damage to proteins is
observed in cardiomyocytes and hepatocytes in
experimental animals. The administration of nicotinic
acid in animals with experimental heart failure
significantly reduced the intensity of protein oxidative
modification in cardiomyocytes. The results suggest
that further studies of nicotinic acid and its derivatives
as drugs reducing cardiotoxic effects of doxorubicin
are necessary.

References

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