UBICHINOL: STEP BY STEP TO NEW CARDIO DRUG


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Ubiquinones are a group of homologous quinones that are widely distributed in animals, plants, and microorganisms. All compounds in this group contain a 2,3-dimethoxy-5-methylbenzoquinone nucleus with a prenyl side chain in the 6-position. They differ from one another in the length of the prenyl side chain.
Coenzyme Q$_{10}$

Ubichinol (reduced coenzyme Q$_{10}$, CoQ$_{10}$H$_2$) is a free radical scavenger, prevents peroxidation damage to cell membranes, regenerates $\alpha$-tocopherol, and minimizes the effects of oxidative stress.

Ubichinon (oxidized coenzyme Q$_{10}$, CoQ$_{10}$) is a lipophilic cell membrane component and the predominant member among the coenzyme Q species in humans; functions as an electron carrier in the mitochondrial respiratory chain and as an intracellular antioxidant. It is synthesized by human cells, but exposure occurs also through the diet.
Coenzyme Q\textsubscript{10} natural sources

- Red meat (beef, chicken), heart, kidney, liver
- Oily fish such as salmon, tuna, fresh sardines and mackerel
- Fruits and vegetables: broccoli, cauliflower, oranges
- Nuts, seeds and oils: soybean, corn, olive and rapeseed oil, peanuts, pistachios, sesame seeds
Coenzyme Q_{10}

- Ubichinon (CoQ_{10})
  - CAS 303-98-0
  - C_{59}H_{90}O_{4}
  - M.w. 863.34
  - Melting point 47-49 °C
  - Crystal powder from yellow to orange color, flavourless, hydroscopic
  - Practically insoluble in water, sparingly soluble in acetone, freely soluble in hexane

- Ubichinol (CoQ_{10}H_{2})
  - CAS 992-78-9
  - C_{59}H_{92}O_{4}
  - M.w. 865.37
  - Melting point 48-50 °C
  - Crystal powder from white to pale white color, flavourless, hydroscopic
  - Practically insoluble in water, slightly soluble in ethanol, sparingly soluble in hexane
At the modern world pharmaceutical market there are a lot of drugs and food supplements on a base of oxidized form of coenzyme Q$_{10}$ (ubichinon). It is included in BP 2011, JP XV, Ph. Eur. 7, USP 34. Ubichinon has low bioavailability. Antioxidative and membrane protection action of coenzyme Q$_{10}$ is caused mainly with its reduced form. Thus development and practice application of new drugs on the base of reduced form of coenzyme Q$_{10}$ (ubichinol) is actual.
Step 1: Synthesis

Method of catalytic reduction was improved for quick synthesis of CoQ_{10}H_2 pharmaceutical substance (patent RU 2013125386, 2013).

\[ \text{CoQ}_{10} \]

\[ \text{CoQ}_{10} \text{H}_2 \]

Ascorbic acid

60 °C, 30 min, catalyst

Dehydroascorbic acid
Step 2: Substance standardization

Appearance, solubility, identification, impurities and assay tests (based on UV and HPLC methods) were offered for substance standardization.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Visual</td>
<td>Crystal powder from white to pale white color, flavourless, hydroscopic</td>
</tr>
<tr>
<td>Solubility</td>
<td>Russian Pharmacopoeia XII</td>
<td>Practically insoluble in water, slightly soluble in ethanol, sparingly soluble in hexane</td>
</tr>
<tr>
<td>Identification</td>
<td>UV</td>
<td>Maximum at 290±2</td>
</tr>
<tr>
<td></td>
<td>HPLC</td>
<td>Peak retention time relative to ubichinone</td>
</tr>
<tr>
<td>Impurities:</td>
<td>HPLC</td>
<td></td>
</tr>
<tr>
<td>Ubichinone</td>
<td></td>
<td>Not more than 5 %</td>
</tr>
<tr>
<td>Other individual impurity</td>
<td></td>
<td>Not more than 0,5 %</td>
</tr>
<tr>
<td>Total impurities</td>
<td></td>
<td>Not more than 1 %</td>
</tr>
<tr>
<td>Assay</td>
<td>UV on a base of ε</td>
<td>Not less than 95%</td>
</tr>
</tbody>
</table>
Step 3: Drug form

The form of solution for injection which provides the highest bioavailability was chosen at the third step. Through CoQ\textsubscript{10}H\textsubscript{2} is water-insoluble development of its drug in a form of 1% solution was based on addition of solubilizers and excipients which are allowed for use in injection forms:

- Cremophor ELP (macrogolglycerol ricinoleate)
- Tween-80 (polysorbate-80)
- Tween-20 (polysorbate-20)
- Solutol (polyethylene glycol 660 hydroxystearate)
- Polyethylene glycol (PEG) 400
- Ethanol
- Lipoid PPL 400
- Ascorbic acid
- Trilon B (sodium edetate)
- Isotonic (NaCl) and plasma replace (Hartmanns’s, Ringer’s, etc) solutions
Step 4: Drug form standardization

The new drug was standardized by all requirement parameters (appearance, identification, pH, impurities, assay, etc.). Solution is stable for a few months; studies of its stability are continued.

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<td>Appearance</td>
<td>Visual</td>
<td>Transparent or slightly opalescent liquid from almost colourless till light yellow color</td>
</tr>
<tr>
<td>Identification</td>
<td>UV</td>
<td>Maximum at 289±2</td>
</tr>
<tr>
<td></td>
<td>HPLC</td>
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</tr>
<tr>
<td>Assay</td>
<td>UV on a base of $\varepsilon$</td>
<td>9,0-11,0 mg/ml</td>
</tr>
</tbody>
</table>
Acute toxicity of the 1% ubichinol solution was studied in accordance to OECD Guidance Document on Acute Oral Toxicity, N 24, 2001
- in rats in doses 100-300 mg/kg intravenously, in doses 500-750 mg/kg intramuscularly
- and in mice in doses 50-250 mg/kg intravenously and intramuscularly. Doses were chosen on the base of the maximum volume which may be applicable to animal. Death of experimental animals was not observed, thus lethal doses weren’t determined (calculated).

*Maximum enduring dose* of the tested drug for intravenous application for rats was 300 mg/kg, for mice – 750 mg/kg, for intramuscular application for rats – 750 mg/kg, for mice – 250 mg/kg.

The drug belongs to the 5-th hazard class by OECD (Organisation for Economic Co-operation and Development) classification (300 mg/kg<LD50 i/v<700 mg/kg)
Step 6: Allergenic tests

Allergenic properties were studied in 5 tests:
On guinea pigs in doses 6.5 and 65 mg/kg:
- Reaction of general anaphylaxis;
- Reaction of active skin anaphylaxis;
- Conjunctiva test;
On mice in doses 17 and 170 mg/kg:
- Reaction of hypersensitivity of the slowed-down type;
- Reaction of an inflammation on Concanavalin A.

No anaphylaxis, allergic reactions were observed.
Step 7: Mutagenic test (AMES test)

Test was evaluated in accordance to OECD Guideline for testing chemicals, N 471, 1997 with special reagent kit AMES MPF™ 98/100/1535/1537 (Xenometrix, Switzerland)

- on Salmonella typhimurium TA98, TA100, TA1535, TA1537 (Xenometrix, Switzerland);

- with concentrations of tested drug 400 μg/ml, 40 μg/ml, 4 μg/ml, 0,4 μg/ml, 0,04 μg/ml, 0,0004 μg/ml,

- with and without metabolic activation (with/without microsomal S9 fraction).

As a result mutagenic properties were not found.

View of experimental planshet (violet – without mutations, yellow – with mutations)
Step 8: Pharmacological activity. Isolated aotra model

The influence of ubichinol solution on NO-dependent relaxation caused by Acetylcholine (ACH) was studied on the model of isolated rat’s aorta after its contraction by Phenylephrine (Ph). Experiment was done for evaluate the possible mechanism of ubichinol action.
Nitric oxide (NO) is produced from L-arginine and molecular oxygen (O₂) by endothelial nitric oxide synthase (eNOS) in a tightly “coupled” process involving tetrahydrobiopterin (BH₄) and NADPH. Oxidative stress and endothelial dysfunction results to “uncoupling” of NO production and forming the peroxynitrite (ONOO⁻).

Endothelial dysfunction was recognized as an important abnormality in chronic heart failure. Endothelial dysfunction may depend either on reduced nitric oxide synthesis, or increased nitric oxide inactivation, or both.

Coenzyme Q₁₀ can increase the production of NO and decrease its inactivation. Thus it may be the important mechanism of its action for heart diseases treatment.
Step 8: Pharmacological activity. Isolated aotra model

Ubichinol solution (in dilution $3 \times 10^{-6}$ M) increases the NO dependent relaxation caused by acetylcholine in the same degree, as NO special substrate – L-arginin. In higher concentrations tested drug is not so effective.
Step 9: Pharmacological activity. Myocardial infarction

Study of pharmacological activity of drug as one of the most important step was done in the wide range of doses (2, 10, 18, 26 and 34 mg/kg intravenously once a day during 7 days) on model of the acute myocardial infarction caused by occlusion of a coronary artery in rats.

Stages of operation:
Step 9: Pharmacological activity.
Myocardial infarction

Groups of animals (n=12):
1. **Intact** (without pathology, without treatment)
2. **Lie-operated** (operation without pathology, without treatment)
3. **Control** (treatment with placebo)
4 – 8. **Experimental** (treatment with ubichinol Q_{10} 1% solution in doses 2, 10, 18, 26 and 34 mg/kg)
9. **Positive control** (treatment with Meldonium 14,3 mg/kg; metabolic, cardio protective drug, it is used in complex therapy of acute myocardial infarction for reduce tissue necrosis and rehabilitation period).

Main types of monitoring:
- Electrocardiograms (1, 7 days)
- Biochemical blood analysis (1, 2, 7 days)
- Histology of the heart after 7-days course of the drug
Amplitude of ST-segment is the most informative parameter on electrocardiograms (ECG) for evaluate myocardial infarction in rats.

Gradation:
- 0,06 mV - normal values
- Less than 0,06 – ischemia
- More than 0,06 – myocardium destruction

Acute stage of myocardial infarction is characterized by increasing of ST amplitude, later depression of ST signal and monophase curve is formed.

Fragment of ECG
Step 9: Pharmacological activity. Myocardial infarction. Electrocardiograms

Groups of animals
1. Intact
1. Lie-operated
2. Control
4 – 8. Experimental
9. Positive control

After ECG data induction of pathology was proved and efficiency of treatment was shown. In the 1-st (acute) stage of MY tested drug was effective near to reference drug (increasing of ST amplitude). The 2-nd stage of MY (sub-acute) seems to starts earlier under treatment by both (tested, in doses 10-34 mg/kg, and reference) drugs because negative ST-signals are less expressed.
Step 9: Pharmacological activity. Myocardial infarction. Biochemical blood analysis

Creatinin kinase

* - difference from intact group
# - difference from lie-operated group
## - difference from control group
### - difference from experimental groups with tested drug application (in all doses)
Step 9: Pharmacological activity. Myocardial infarction. Biochemical blood analysis

Aspartateamino transferase

* - difference from intact group
# - difference from lie-operated group
** - difference from control group
### - difference from experimental groups with tested drug application (in all doses)
Step 9: Pharmacological activity. Myocardial infarction. Biochemical blood analysis

Myoglobin

* - difference from intact group
# - difference from lie-operated group
*- difference from control group
Step 9: Pharmacological activity.
Myocardial infarction. Biochemical blood analysis

Lactatdehydrogenase

* - difference from intact group
# - difference from lie-operated group
*- difference from control group
# - difference from experimental groups with tested drug application in dose 2 mg/kg
** - difference from experimental groups with tested drug application in dose 10 mg/kg
Step 9: Pharmacological activity. Myocardial infarction. Histology

After histological data induction of pathology was proved and efficiency of treatment was shown.
For animals with pathology special changes in heart were determined.

Place of infarction
Cardiomyocytes, connective tissue

After treatment the place of infarction was in stage of organization of new connective tissue.
Step 9: Pharmacological activity. Myocardial infarction

RESULT:
Antiischemic and cardioprotective properties of the new drug were shown in the model of acute myocardial infarction. Tested drug was more effective than reference (Meldonium in a dose 14,3 mg/kg)
Further steps of studies will include experiments:
- on chronic toxicity (intramuscularly in rats in doses 8.6; 43 and 86 mg/kg and in rabbits in doses 4.3; 21.5 and 43 mg/kg),
- specific activity in ischemia model with the subsequent reperfusiya of a myocardium at rats (in doses 2-34 mg/kg),
- pharmacokinetics, and other.
Conclusion

Thus step by step on the base of wide range preclinical investigation the new effective and safety cardio drug of coenzyme Q$_{10}$ in its reduced form is going to be developed.
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Thank you for attention!