

The Antitumor Activity of Molecular Iodine Complexes with Lithium Halogenides and Bioorganic Ligands when Applied in Combination with Doxorubicin

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Introduction

The antitumor activity of anti-infective drugs containing iodine complex with bio-organic ligands and potassium and lithium halides (LiCl (I)-I₂-α-dextrin-peptide) has been experimentally studied.

It is shown that the combined action of the drug containing LiCl (I)-I₂-α-dextrin-peptide complexes and doxorubicin on the growth of Ehrlich ascites carcinoma (EAC) significantly enhanced the antitumor activity of doxorubicin.

Using the quantum-chemical approach at the DFT/B3PW91/6-31G** and MP2/midi level it is shown that the enhancement effect of antitumor activity of doxorubicin by molecular iodine complexes with lithium halogenides and bio-organic ligands is caused by the formation of a structure in which the nucleotide pair is interlocked with doxorubicin with stacking interaction and bound by coordination with LiCl (I)-I₂-α-dextrin complexes.

In this structure LiCl (I)-I₂-α-dextrin complexes increase the stacking interaction of doxorubicin with the nucleotide pair and become the inhibition center for the amino acid residue of arginine which is part of the active site of topo I.

Clinically applied at present is a great number of medications containing molecular iodine complexes with biopolymers and having a wide range of antiviral effect, inclusive of HIV infection. Drugs containing molecular iodine complexes with organic ligands are highly toxic, and are therefore for external use only. As part of anti-infective drugs [1-4] molecular iodine is in such an active form that when administered orally it minimizes toxic effects in humans. Toxicology research into the drugs [1-4] has shown that these can be assigned to Category IV of Globally Harmonized Classification System [5].

The distinctive feature of the drugs is that their active substance includes not only iodine-containing polymeric complex, but also potassium and lithium halogenides. Using X-ray data for iodine-α-dextrin complexes and the results of quantum chemical ab initio restricted Hartree-Fock/3-21G level calculations, a model of active complex of drugs [1-4] with anti-HIV action was proposed. It is shown that the active center contains molecular iodine located inside the α-dextrin helix and coordinated by lithium halogenides and peptides (complex LiCl (I)-I₂-α-dextrin-peptide) [6].

All components of the LiCl (I)-I₂-α-dextrin complex exhibit anticancer activity. In [7] lithium chloride is shown to inhibit the growth of carcinoid cells. A number of drugs assigned to inhibitors of topoisomerase I, contain a carbohydrate sequence attached to the DNA-intercalating chromophore. In each case, the glycoside residue plays a significant role in the interaction of the drug with the DNA double helix [8]. Also based on peptides [9] and glycopeptides [10] a vaccine was designed that has antitumor activity.

Clinical investigations of the combined effect on the growth of

Ehrlich ascites carcinoma (EAC) of a drug containing LiCl(I)-I₂-α-dextrin-peptide complexes (LiCl(I)I₂DP) and doxorubicin have shown that both abdominal and oral administration of the drug in small doses brings about a significant enhancement of antitumor activity, which is regarded as potentiation of the effect. The enhancement is more revealed when the drug containing LiCl(I)-I₂-α-dextrin-peptide complexes, and doxorubicin are introduced at the same time (Table 1). It should be noted that neither death of animals nor severe symptoms of intoxication were observed when the drugs were administered in combination using various ways.

The antitumor activity was investigated using male and female white outbred mice, weighing 22-27 gram. The test and control groups comprised eight and ten mice respectively.

Doxorubicin (Doxorubicin-Ebewe, a concentrate for the preparation of intravascular administration solutions. 5 ml vial contains 10 mg of doxorubicin hydrochloride) which is an antibiotic commonly applied for the treatment of oncology patients was used for combined therapy.

The study drug (oral solution) was administered to the mice orally and abdominally in three doses. Doxorubicin was administered abdominally in an optimal therapeutic dose. The control animals received an equivalent volume of salt solution. The preparations were administered to the animals for 6 days in 0.2-0.5 ml doses. After that the animals were left to survive. As the animals died the antitumor activity was determined based on the percentage of increased life span (ILS) of the test animals as compared to the control ones. The therapeutic effect was calculated using the equation below:

$$ILS (\%) = \frac{MLD^* \times \text{test} - MLD \text{ control}}{MLD \text{ control}}$$

where MLD* stands for mean life expectancy of animals.

The results obtained were processed statistically using the Student-Fischer Method (GraphPad Prism version 3).

o. – orally, abd. – abdominally, ILE - increase in life expectancy of test animals in % as compared to controls

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Received February 24, 2014; Accepted April 23, 2014; Published April 29, 2014

Citation: Yuldasheva GA, Zhidomirov GM, Ilin AI (2014) The Antitumor Activity of Molecular Iodine Complexes with Lithium Halogenides and Bioorganic Ligands when Applied in Combination with Doxorubicin. J Antivir Antiretrovir 6: 050-053. doi:10.4172/jaa.1000096

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Animal Group	Route of Entry	Dosage	Life Expectancy (X̄±m), days	ILE (in % as compared to controls)
LiCl(I)I ₂ DP	o	5.0 ml/kg	13.0±1.7	4.0
		10.0 ml/kg	13.5±1.8	8.0
		15.0 ml/kg	14.1±2.0	12.8
Doxorubicin	abd	1.5 m/kg	36.0± 2.3	188.0*
Controls	o	15.0 ml/kg	12.5 ±1.6	-
LiCl(I)I ₂ DP + Doxorubicin, at the same time	o + abd	5.0 ml/kg + 1.5 m/kg	46.0±7.1	268.0**
		10.0 ml/kg + 1.5 m/kg	43.1±3.0	244.8**
		15.0 ml/kg + 1.5 m/kg	27.0±11.0	116.0*
LiCl(I)I ₂ DP + Doxorubicin, in 6 hours	o + abd.	5.0 ml/kg + 1.5 m/kg	40.3±7.0	222.4*
		10.0 ml/kg + 1.5 m/kg	36.5±7.2	192.0*
		15.0 ml/kg + 1.5 m/kg	27.6±9.9	120.8*
LiCl(I)I ₂ DP + Doxorubicin, in 6 hours	abd + o	1.5 m/kg + 5.0 ml/kg	43.1±5.1	244.8**
		1.5 m/kg + 10.0 ml/kg	39.5±7.7	216.0*
		1.5 m/kg + 15.0 ml/kg	32.6±11.4	160.8*
LiCl(I)I ₂ DP	abd	1.0 ml/kg	13.4±1.7	0
		2.0 ml/kg	14.5±2.8	4.3
		3.0 ml/kg	14.0± 3.2	0.7
Doxorubicin	abd	1.5 m/kg	39.6±7.2	184.9*
Controls	abd	3.0 ml/kg	13.9±1.2	-
LiCl(I)I ₂ DP + Doxorubicin at the same time	abd + abd	1.0 ml/kg + 1.5 m/kg	52.4±9.0	276.9**
		2.0 ml/kg + 1.5 m/kg	48.4 ±4.2	248.2**
		3.0 ml/kg + 1.5 m/kg	35.4±9.2	154.7*
LiCl(I)I ₂ DP + Doxorubicin, in 6 hours	abd + abd.	1.0 ml/kg + 1.5 m/kg	41.1±3.3	195.7*
LiCl(I)I ₂ DP + Doxorubicin, in 6 hours	abd + abd.	1.0 ml/kg + 1.5 m/kg	41.1±3.3	195.7*
		2.0 ml/kg + 1.5 m/kg	39.3±5.0	182.7*
		3.0 ml/kg + 1.5 m/kg	28.3±13.1	82.0*
LiCl(I)I ₂ DP + Doxorubicin, in 6 hours	abd. + abd.	1.5 m/kg + 1.0 ml/kg	42.0±8.3	202.2 *
		1.5 m/kg + 2.0 ml/kg	38.0±8.7	173.4*
		1.5 m/kg + 3.0 ml/kg	27.4±11.1	97.1*

Table 1: Effects of LiCl(I)I₂DP on the Success of Doxorubicin treatment of Mice Affected by Ehrlich Ascites Carcinoma.

*p<0.05 as compared to controls,

**p<0.05 as compared to doxorubicin.

The study drug was introduced to mice orally and abdominally. Doxorubicin which is an antibiotic widely used in oncology was applied for combined therapy.

Doxorubicin-Ebewe is a concentrate for intravascular introduction solutions. A 5 ml vial contains 10 mg of doxorubicin hydrochloride. This cytostatic agent and the drug containing LiCl (I)-I₂-α-dextrin-peptide complexes were introduced to the animals using disposable syringes and metal gastro-duodenal tubes.

Mice were selected as test models with account of all requirements of toxicological and chemotherapeutic studies.

In [6] we have shown that where the LiCl(I)-I₂-α-dextrin-peptide complex interacts with DNA, nucleotides can replace peptides and form a complex with molecular iodine, which is coordinated by lithium halogenides complexes with dextrin (LiCl(I)-I₂-α-dextrin-nucleotide complexes).

Such topoisomerase I inhibitors as, for example camptothecin and its analog topotecan [11,12], bind in one complex both onco-DNA nucleotide pairs and amino acid residues that are part of the active site of human DNA topoisomerase IB (topo I), an enzyme that changes and regulates the state of cellular DNA

The mechanism of inhibition of the active center of topo I by that

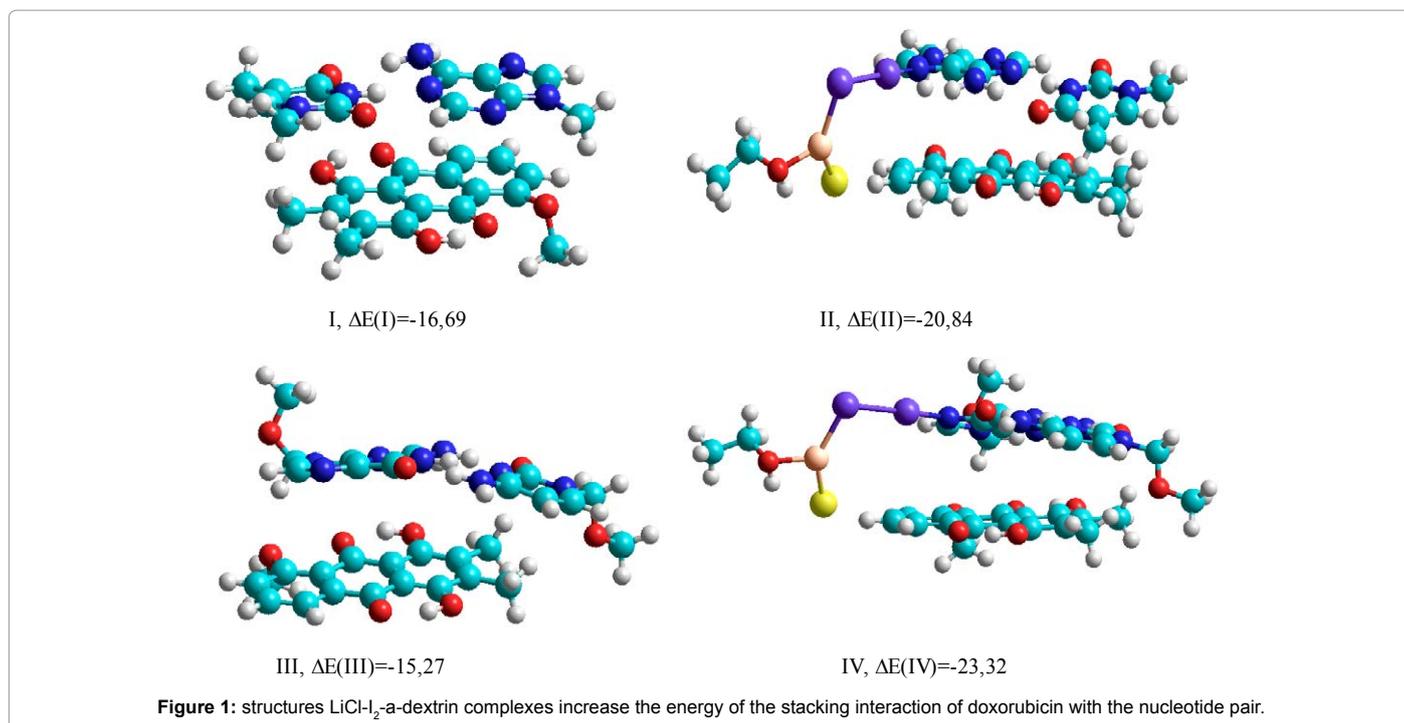
of the active center of anti-infection drugs [1-4] is proposed using the quantum-chemical approach DFT/B3PW91 level. It is shown that under the influence of the LiCl(I)-I₂-α-dextrin-peptide complexes a structure is formed in the onco- DNA in which the LiCl(I)-I₂-α-dextrin complex becomes the center of inhibition for both onco- DNA nucleotides and arginine residue that is part of the topo I active center.

According to the literature the ability of doxorubicin to inhibit topo II proved in experimental studies. However, results of experimental research in vitro have shown that doxorubicin inhibits and human DNA topoisomerase I [13].

Doxorubicin is an anticancer drug of the anthracycline group of antibiotics. Chemotherapeutic effect of the antibiotic doxorubicin is due to its ability to intercalate into DNA structure. According to the results of experimental studies the energy of stacking interactions of the antibiotic with a pair of nucleotide bases correlates with its anticancer activity [14].

The ability of doxorubicin to intercalate into DNA and form a structure in which the molecule of doxorubicin may be located parallel to the nucleotide pair and bounded to it through stacking interaction is investigated using the quantum-chemical approach at the RHF / midi and MP2/midi level.

The RHF / midi method was chosen to optimize the geometry of doxorubicin –adenine-thimine (I) and doxorubicin – guanine-cytosine (III) structures, and their complexes with LiCl-I₂-α-dextrin (II,IV). In



the II, IV-structures molecular iodine forms a coordination bond both with the nitrogen atom of the five-member cycle of adenosine (II) or guanosine (IV), and the LiCl- α -dextrin complex.

The sugar-phosphate moiety and the cyclohexane ring of doxorubicin may limit the overlap of the aromatic rings of the nucleotide pairs, and doxorubicin. To account for the effect of the steric effect of these moieties in the I-IV structure the sugar-phosphate moiety is replaced by the -OCH₃ group, while the cyclohexane ring in doxorubicin is replaced by two methyl groups.

With the geometry retained and the O-CH₃ groups in the nucleotide pairs and the CH₃-groups in doxorubicin replaced with hydrogen atoms the MR2/midi was used to calculate the total energy of structures I-IV.

The results of calculations have shown that the enhancement effect of anticancer activity of doxorubicin by molecular iodine complexes with lithium halogenides and bio-organic ligands is caused by the formation of a structure in which the nucleotide pair is interlocked with doxorubicin with staking interaction and bound by coordination with LiCl (I)- I₂- α -dextrin. In these structures LiCl (I)- I₂- α -dextrin complexes increase the energy of the stacking interaction of doxorubicin with the nucleotide pair (Figure 1).

(Figure 1) Structures in which the molecule of doxorubicin is located parallel to the nucleotide pairs (I, III) and complexes of nucleotide pairs with LiCl-I₂- α -dextrin (II, IV). Stacking interaction energy (ΔE , kcal/mol) doxorubicin with nucleotide pairs and complexes of nucleotide pairs with LiCl-I₂- α -dextrin.

In the figure: blue balls-carbon atoms, dark blue balls – nitrogen atoms, red balls – oxygen atoms, violet balls – iodine atoms, yellow ball - chlorine.

ΔE was calculated as:

$$\Delta E = E^{\text{tot}}_1 - E^{\text{tot}}_2 - E^{\text{tot}}_3 \quad (1)$$

E^{tot}_1 –total energy of structures (I-IV),

$E^{\text{nonh.}}_2$ – total energy of doxorubicin molecular,

$E^{\text{nonh.}}_3$ – total energy of nucleotide pair in structures (I-III) and complex nucleotide pair with LiCl - I₂- α -dextrin in structures (II-IV).

Thus, our research have shown that the strengthening of anticancer activity of doxorubicin by complexes of molecular iodine with lithium halogenides and bio-organic ligands is due to the formation of a structure in which the nucleotide pair is bounded by the stacking interaction to doxorubicin and coordinated by the LiCl- α -I₂-dextrin complex. In this structure the LiCl- α -I₂-dextrin complex increases the stacking interaction of doxorubicin with the nucleotide pair and becomes the center of inhibition for the amino acid residue of arginine, which is part of the active site of topo I.

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