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Prazdnova E.V., Chistyakov V.A., Mazanko M.S.,
Chmyhalo V.K., Churilov M.N.,
Academy of biology and biotechnology,
Southern federal university

Determination of the specificity of ROS generation by the action of platinum-based drugs in SOS-lux test

Antineoplastic drugs based on platinum compounds are widespread in anticancer therapy. High efficacy of these drugs is due to their ability to selectively affect rapidly reproducing tumor cells by damaging DNA and launching mechanisms of apoptosis [1,2].

Use of cisplatin is greatly limited by two factors. Firstly, there is a wide range of side effects. Secondly, cisplatin hasa strong genotoxic and mutagenic effect, as shown in several studies [3,4]. DNA damage induced by cisplatin can lead to secondary tumors years after chemotherapy [5]. Furthermore, the drug may cause mutations resulting in resistance to both cisplatin and other drugs [6].

Latest data show that the cytotoxic effects of these compounds may also be mediated by the generation of reactive oxygen species (ROS) and the occurrence of oxidative stress [7-11]. Apparently, ROS generation may be associated with side effects of these drugs: cisplatin nephrotoxicity, cisplatin and oxaliplatin ototoxicity and occurrence of secondary tumors[11-13].

The aim of this study was to investigate the genotoxicity of platinum-based drugs mediated by oxidative stress in a simple model system.A set of bacterial biosensors responding to oxidative stress and DNA damage was used. This technique was successfully used previously in study of prooxidant and antioxidant properties of a wide range of compounds and drugs [14], as well as physical factors [15].The theoretical possibility of determining the genotoxicity of platinum drugs in the same test was shown by our colleagues from the State Research Institute of Genetics [16].

In this study, we used biosensors reacting to various ROS to figure out if there are specific features in action of two drugs.

We used *E.coli* strains MG1655 (pKatG-lux), MG1655 (pSoxS-lux), and MG1655 (pColD-lux), containing the plasmid carrying the operon luxCDABE under control of promoters

of kat-operon, sox-operon and colD genes (cda promoter). Biosensor with the promoter PkatG registers formation of hydrogen peroxide in the cell; soxS promoter reacts to theincreasedsuperoxide-anion-radical level; biosensor with the promoter Pcda registers DNA damage(Zavilgelsky et al).Strains were provided by I. V. Manukhov (State Scientific Center Genetika, Moscow).

Cultures of strains were grown on complete medium Luria-Bertani (LB), with addition of ampicillin (100 μg / ml) for 18-20 hours at 36° C; then culture were diluted by fresh medium until it reached 0,1McFarland units, and cultured for 2 hours. Aliquots of this culture by 90 μl were transferred to the cells of a microplate(diameter 7 mm), adding 10μl of preparation solution.

Measurements were carried out every 10 min for 120 min. For evaluation of the influence of the studied factors on Sox-operon expression, the induction factor (*I*s) was calculated according to the formula:

$$I_s = {}^{L_e}/_{L_k} - 1 \qquad (1),$$

Where *L*k and *L*e are luminescence intensities of controland experimental samples, respectively.

A statistically significant excess of *L*e over *L*k estimated with the *t*-criterion was considered as a sign of significant influence on the induction effect.

Mean square deviation of SOS-response induction factors were calculated by the formula:

$$s_I = \bar{I} \times \sqrt{\left(\frac{s_{L_e}}{\overline{L_e}}\right)^2 + \left(\frac{s_{L_k}}{\overline{L_k}}\right)^2} \qquad (2),$$

where e and k are for experimental and control samples, respectively.

Calculaton of confidence intervals was made for p=0,05.

Study of cisplatin activity in bacterial biosensors.

In biosensor assay high genotoxic activity of cisplatin (fig.1A), and slight induction of superoxide anion radical (fig.1B) was shown, whereas no peroxide generation was observed (maximum induction factor was 0,59, which is statistically insignificant (data not shown)).

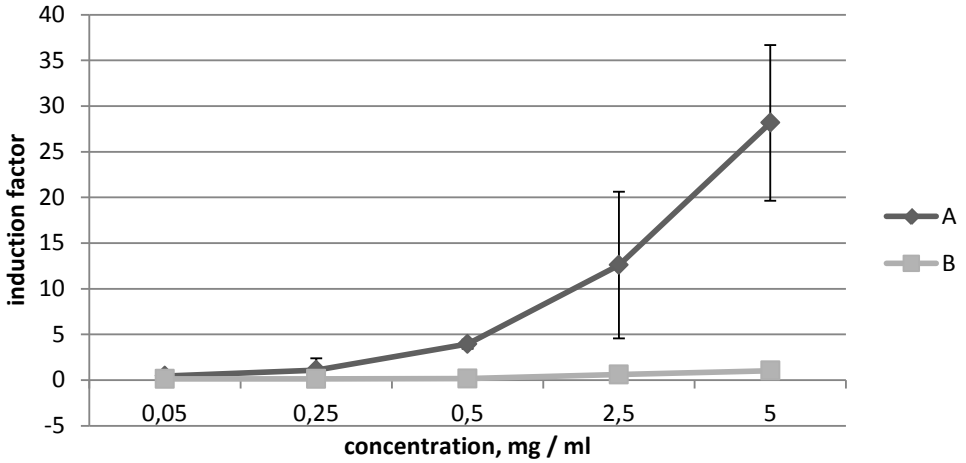

Figure 1. A: Inducton of *E.coli* MG1655 (ColD-lux) strain with cisplatinum. Active concentrations are within the range 0,005-50 mg/ml. Maximum induction factor is 95,9. B: Inducton of *E.coli* MG1655 (SoxS-lux) strain with cisplatinum. Active concentrations 0,0005 - 5 mg/ml. Maximum induction factor is 1,04.

Study of oxaliplatin activity in bacterial biosensors.

Genotoxicity of oxaliplatin in biosensor assay was similar to that of cisplatin (Fig 2A). In test with MG1655(KatG-lux) strain, which reacts to peroxide and hydroperoxides, bacterial activity was significantly higher than that of cisplatin.

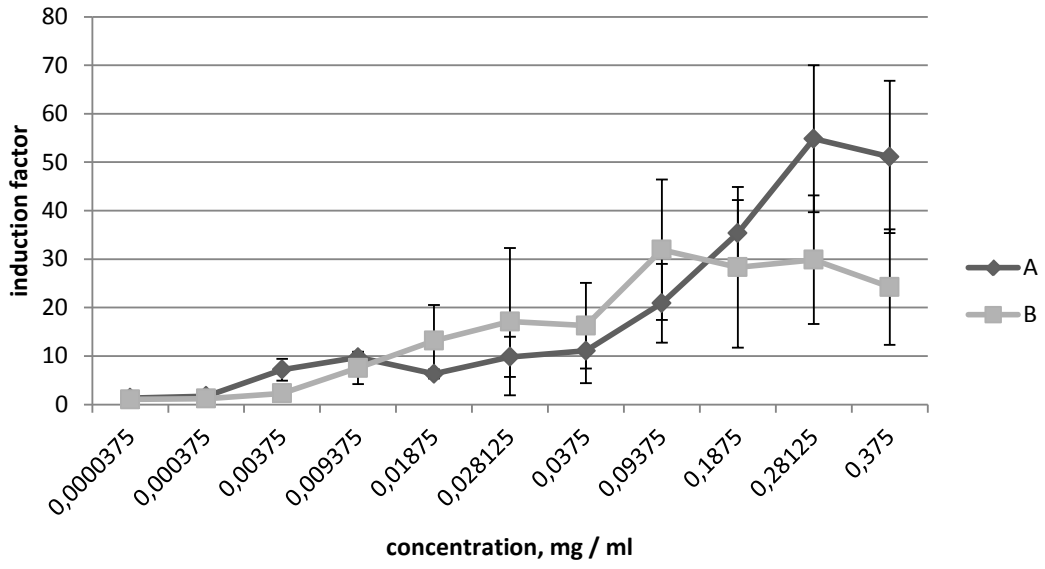

Figure 2. A: Inducton of E.coli MG1655 (ColD-lux) strain with oxaliplatin. Active concentrations - 0,0005-5 mg/ml. Maximum induction factor is 54,85. B: Induction of E.coli MG

1655 (KatG-lux) strain with oxaliplatin. Active concentrations 0,00001 – 1 mg/ml. Maximum induction factor - 31,9.

No Lux-operon expression of *E.coli* MG1655(SoxS-lux) under the influence of the drug was observed (data not shown).

In addition to direct interaction with DNA, mutagenic and cytotoxic properties of cisplatin can be performed through induction of oxidative stress and ROS generation [7,8]. This point of view is supported by the fact that certain antioxidants are able to reduce the mutagenic potential of cisplatin[17]. Oxidative stress and the inhibition of antioxidant defense mechanisms is also known to be the main mechanism of cisplatin-induced nephrotoxicity *in vitro* and *in vivo* [1,18-19].

Oxaliplatinis often used as an alternative option to cisplatin.Being transformed *in vivo*, oxaliplatin forms hydrated derivatives, which interact with DNA and interfere with its synthesis. The drug is structurally similar to cisplatin, but contains a non-leaving carrier ligand (DACH) and a hydrolysable oxalate ligand. This modification enhances the anti-tumor activity but alters the side-effect profile of the drug [20].

It is known that oxaliplatin increases ROS production in a dose-dependent manner, and H_2O_2 generated by oxaliplatin can increase the alteration of DNA structure. Moreover, platinum-induced oxidative stress involves oxidation of cellular components and depletion of intracellular antioxidants [8].

It was suggested that one of the causes of peripheral neuropathy in oxaliplatintreatment is precisely the generation of ROS [21]. As in case of cisplatin, there is evidence [13], that natural antioxidants - silibinin and α-tocopherolreduce neurotoxicity of the drug without affecting its antineoplastic activity.

Recent data also indicate the role of ROS in the development of resistance of tumors to treatment with oxaliplatin[22], and it was shown recently that antioxidants may reduce the development of resistance to chemotherapy [13].

According to our experiments, the spectrum and intensity of ROS generation in these two drugs differ. Consequently, side effects can also develop via different mechanisms.

As can be seen in the data, both drugs show a significant genotoxicity in rapid diagnostic tests with bacterial biosensors. Therefore, this test allows to register the main type of activity of the compounds of platinum - DNA damage.

However, in tests with biosensors reacting to oxidative stress, the specificity of action is observed: cisplatin did not cause any significant induction in biosensor registering hydroperoxides (Figure 3), or biosensor that responds to superoxide (Figure 2). Oksaliaplatin, on the other hand, caused induction of the catalase operon (Figure 5), but not of superoxide

dismutase genes, which leads to the conclusion about the significant role of the intracellular generation of hydrogen peroxide and its derivatives in the implementation of the genotoxic effect of this drug. With regard to cisplatin apparently role of ROS in the implementation of its effect is not significant.

The distinctions in spectrum of ROS generated in the cell after treatment of cisplatin and oxaliplatin allow us to conclude that the search strategies for additional medicines to eliminate side effects of such drugs should also be different. Since the effects of oxaliplatin are significantly associated with oxidative mechanisms, attention should be paid to the existing antioxidants, particularly mitochondria-targeted.

It should be also noted that the genotoxicity, demonstrated on bacterial models, makes us wonder about the possible mutagenicity of this drug for pathogenic microorganisms, which could acquire resistance to antimicrobial agents under the influence of the drug, making it difficult for traditional treatment. Antioxidants, in addition to reducing the side effects, could reduce the risk of such complications. Study of spectrum of mutations associated with oxidative stress in microorganisms under the treatment with platinum-based drugs, will be the goal of our further research.

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