BIOFYSICAL INTERACTION STUDY OF DNA WITH PLATINUM CYTOSTATICS

carboplatin  oxaliplatin  cisplatin

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Introduction - platinum cytostatics

The biological activity of the first platinum-based cytostatics (cisplatin), which is still one of the most widely used cytotoxic agent, was discovered in 1965. Platinum drug (second generation) carboplatin was developed in the 1980s as a less toxic alternative to cisplatin and showing fewer side effects. Another often used platinum-based cytostatic is oxaliplatin, which was designed in the 1990s as a third generation platinum drug to overcome resistance to cisplatin and carboplatin.
Heptaplatin - results indicate that molecular mechanisms of heptaplatin effective against cisplatin-resistant gastric cancer sublines is at least in part due to the less involvement of MT in heptaplatin resistance as well as its attenuation of MT induction.

lobaplatin is a platinum drug, which overcomes some forms of cisplatin resistance in preclinical tumour models. Several potential clinical applications remain unexplored, such as its use in relapsed testicular cancer and in combination with other cancer chemotherapeutic agents and ionising radiation.

Nedaplatin (marketed under the tradename Aqupla) is a platinum compound which is used for cancer chemotherapy. It produces less nausea, vomiting and nephrotoxicity than other platinum-containing drugs.
Satraplatin is a platinum-based antineoplastic agent that is currently under investigation as one treatment of patients with advanced prostate cancer who have failed previous chemotherapy. It has not yet received approval from the U.S. Food and Drug Administration (2010). First mentioned in the medical literature in 1993, satraplatin is the first orally active platinum-based chemotherapeutic drug; other available platinum analogues - cisplatin, carboplatin, and oxaliplatin - must be given intravenously.

**Lipoplatin** (Liposomal cisplatin) is a nanoparticle of 110 nm average diameter composed of lipids and cisplatin. Lipoplatin evades immune surveillance thus escaping clearance from macrophages, circulates for long periods in body fluids after intravenous administration with a half-life of ~120 h. The clinical development of Lipoplatin in adenocarcinomas establishes this drug as the most active platinum drug with significantly lower side effects.

Further tested cytostatics: **picoplatin, ProLindac**
apoferitin part
peptides

apoferitin part
peptides

apoferritin part
peptides

A

B

C

target therapy

fluorescent marker

MS quantification of urinary biomarkers

cancer cell

cancer

bladder

drugs (cytostatic)

fluorescent marker (quantum dot)

nanoparticles (paramagnetic)

8 nm
Effect of cis-Pt in the somatic cell

- Entry of cis-Pt is allowed by passive diffusion or transporter CTR1
- DNA damage caused by cis-Pt activates the ATR kinase
- Objective of the ATR is a tumor suppressor protein p53, which is phosphorylated by the ATR kinase
- P53 initiates transcription of the gene for the protein p21, which inhibits cyclin dependent kinases leading to end of cell cycle
- P53 induces expression of proapoptotic family Bcl-2 members, that are responsible for activating the mitochondrial apoptosis pathways
- The main determinant of response to DNA damage caused by cisplatin is the proapoptotic and antiapoptotic proteins ratio
Aim of the work

The aim of the experiments was to study the interaction of cytostatics with DNA

a) amplification and isolation of DNA fragments: create copies of DNA fragments using polymerase chain reaction

b) spectrophotometry: changes in absorption spectrum and melting temperatures of nucleic acids

c) electrochemistry: changes in the oxidation signals of bases of nucleic acids

d) gel electrophoresis: changes in electrophoretic mobility of the fragment
Sample preparation and distribution

- PCR cycler
- SPECORD 210
- Amicon Ultra – 0.5 ml 3K
- Centrifuge

1. a) amplification and isolation of the DNA fragment
2. b) spectrophotometry
3. c) electrochemistry
4. d) gel electrophoresis
Platinum cytostatics - change of the absorption spectrum (200 – 600 nm)

- **cisplatin (µM)**
- **carboplatin (µM)**

- **oxaliplatin (µM)**
Comparison of melting temperature for cisplatin, carboplatin and oxaliplatin

A

Temperature (°C) vs. Applied dose of cytostatics (µM)

B

Temperature (°C) vs. Applied dose of cytostatics (µM)
Electrochemistry

\[ y = 0.2174x + 0.1323 \quad R^2 = 0.9899 \]

\[ y = 0.0778x - 0.2819 \quad R^2 = 0.9648 \]

\[ y = 0.0548x - 0.6718 \quad R^2 = 0.9943 \]

Gel electrophoresis

Applied dose cytostatics (µM)

Bound cytostatics (µM)

cisplatin (µM)

carboplatin (µM)

oxaliplatin (µM)
Conclusion

- Basic biophysical interaction studies of cytostatics with DNA was performed.
- The melting temperature is significantly influenced by the cisplatin, in contrast to the carboplatin and the oxaliplatin.
- There were stronger interactions between the cisplatine and DNA proved, in contrast to the carboplatine and the oxaliplatine.

Further research

- Validation of the results by electrochemical methods
- Research of the interaction of nanoparticles with DNA
- Research of the interaction of cystostatics with another DNA fragment
- Research of the interaction of doxorubicin and other cytostatics with DNA
- Research of the interaction of doxorubicin and other cytotoxic drugs with DNA
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Thank you for your attention