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Hematological properties of cisplatin and its *Ficaria verna* Huds. extracts / β-cyclodextrin complexes in rats

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Abstract

The paper presents the hematological modifications (as hemoglobin content) in healthy rats treated with cisplatin / β-cyclodextrin complex solution and cisplatin / *Ficaria verna* Huds. extract / β-cyclodextrin ternary complex. The hemoglobin content in rats treated with cisplatin / β-cyclodextrin complex has no variation in comparison with the control (a cisplatin treatment lowered the hemoglobin content with more than 20%). Important results were obtained in the case of using of cisplatin / *Ficaria verna* Huds. extract / β-cyclodextrin ternary complexes in the treatment of rats, where the hemoglobin concentration was higher even than in the case of using β-cyclodextrin complexes without antioxidant compounds. These observations were more important in male than in female rats.

Keywords: cisplatin, *Ficaria verna* extracts, cyclodextrins, hemoglobin, *in vivo* experiments

1. Introduction

Cancer is one of the most common diseases in the world and becomes the most important cause of death in recent years. Among other techniques, different chemotherapeutic agents are used for treatment (or control) of these diseases [1-6]. The most important classes of therapeutic agents are alkylating compounds, antimetabolites, antibiotics, alkaloids, hormons, monoclonal antibodies, photodynamic therapeutic compounds, polypeptides, immunostimulating agents (interpherons), immunosuppressant agents [4,5,7,8]. Alkylating agents are most used in various treatment protocols, some of them being known from decades. Classical alkylating agents contain 2-chloroethyl moieties in their structures (such as melphalan, cyclophosphamid, or fotemustin) which chemically react with the DNA from the "wrong" cells and block the replication [5].

Transitional metal-containing compounds (including organometallic ones) are also used as alkylating agents in the treatment of cancer [9-12]. Compounds such as cisplatin and organometallic analogues have similar effects, but they interact with the vicinal purine moieties from DNA chains, furnishing a DNA-Pt$(NH_3)_2$-DNA bond; the result is the damage of the DNA replication and the cancer cell death [6,13-17].

All these cytostatic compounds, which acts as cell growth and multiplication inhibitors, have also secondary and adverse effects such as myelosuppresion, leukopenia, and thrombocytopenia for melphalan, carboplatin, cisplatin or other related compounds [6].

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Cisplatin is one of the most used anti-neoplastic compounds used in the treatment of cancer, but the adverse reactions are high for this compound: emetic effect, severe nausea and vomiting, nephrotoxicity, ototoxicity, elevated hepatic enzymes, and anemia occur [6].

Some of these adverse effects of cytostatic agents can be lowered by using various drugs or other bioactive systems. The presence of antioxidant compounds, as well as the implication of the host nanoencapsulation compounds (*i.e.* cyclodextrins) in the overall toxicity mechanism can be very important for the final results [18,19].

In this study the influence of the *Ficaria verna* Huds. extracts and its β-cyclodextrin complex on the cisplatin toxicity in rats was evaluated.

2. Material and methods

2.1. Chemicals. The cisplatin (Sinplatin) used in this study was obtained from Actavis, Inc. (1 mg/mL, Bucharest, Romania) and has a concentration of 1 mg/mL. β-Cyclodextrin used in this experiment was purchased from Fluka Chemie AG (purity >98%). The *Ficaria verna* Huds. extract / β-cyclodextrin complexes, obtained according to [20], were used in this study.

2.2. Animals. Wistar rats from "Iuliu Haţeganu" University of Medicine and Pharmacy from Cluj Napoca (Romania) of 70 days old were used in the experiments. They were *ad libitum* feeded for another 10 days in our biobase (Banat's University of Agricultural Sciences and Veterinary Medicine of Timişoara, Romania) when they were in quarantine and were monitorized their health (desinfestation was also made) until the experiment.

2.3. Animal treatments. All experiments were performed in a range time of 5 days. The average weight of the selected rats was 157 ± 15 g. Three experimental groups consist of eight rats (four males and four females) were selected as following: the first group (code "C") was used as control group where the animals were intraperitoneally injected with 1.1 mL of physiological saline solution (STADA Hemopharm SRL, Chiajna, Romania); the second group (code "CP_bCD") was injected with 1.1 mL solution containing cisplatin (concentration of 1 mg/mL) and β-cyclodextrin (concentration of 4.32 mg/mL and a CP:bCD molar ratio of 1:1); the third group (code "CP_Fv_bCD") was injected with the same volume of solution containing 1 mg/mL of CP and 4.54 mg/mL of *Ficaria verna* extract/β-cyclodextrin complex previously obtained [20]. The CP dose in all groups was 7 mg/kg body weight. The blood samples were collected after the end of the experiment from the inferior vena cava after narcosis with isofluran (Rompharm Co., Buchares, Romania); blood samples were put in 2 mL vacutainers containing K_3EDTA; up to 2 mL of blood was collected from every experimental rat. All experiments were performed with respect to the animal protection standards, which are use for research purposes or other experimental objectives, according to Directive 2010/63/EU and national laws.

2.4. Hematological parameters. Hematological parameters were determined by using an Abacus Junior Vet hematological multiparametric analyzer by using Para 12®Extend N control serum for the internal quality control.

2.5. Statistical analysis. Classical statistical analysis of the data obtained from hematological analysis was performed. Thus, the mean values and their standard deviations were determined for the hematological parameter values in every experimental group.

3. Results and discussion

The toxicity of cisplatin used in the treatment of various cancer forms is well known and the amelioration of its side and adverse effects is a continuous goal of the scientists. From different methods used in this way, molecular encapsulation of cisplatin in cyclodextrins [21], alone or together with other protecting compounds can reduce the cisplatin toxicity.

It is well known that cisplatin treatment of various cancer forms, using different treatment protocols, conduct to a decrease of the hemoglobin lower than 12 mg/dL [22,23], even at values of 6-8 mg/dL. These analyses were not presented here. Different methods to maintain or increase of the hemoglobin concentration were used (*e.g.* erythropoietin administration [23]).

A new method to reduce the toxic effect of cisplatin is the administration of cisplatin/cyclodextrin complex (CP_bCD) solution instead cisplatin alone, and further by using a ternary complex of cisplatin / antioxidant / cyclodextrin (the cisplatin / *Ficaria*

verna Huds. extract / β-cyclodextrin complex – code "CP_Fv_bCD" – was used). In all cases, the hemoglobin concentration was higher than the known values in cases of the treatment with cisplatin only (<12 mg/dL). Thus, the treatment of rats with CP_bCD complex conduct to similar values to the control group (14.7 mg/dL and 14.9 mg/dL, respectively), while the treatment of rats with the ternary CP_Fv_bCD complex conduct to a higher value for hemoglobin (15.55 mg/dL) (Table 1).

Table 1. Hemoglobin analysis in rats treated with cisplatin/β-cyclodextrin and cisplatin/*Ficaria verna* Huds. extract/β-cyclodextrin complexes; *n* – the number of replicates

N°	Code*	Hemoglobin (g/dL)
1	C	14.90 ± 0.52 (*n* = 8)
2	CP_bCD	14.68 ± 0.95 (*n* = 8)
3	CP_Fv_bCD	15.55 ± 0.62 (*n* = 10)

* C – control group, CP_bCD – cisplatin/β-cyclodextrin complex, CP_Fv_bCD – cisplatin/*Ficaria verna* Huds. extract/β-cyclodextrin complex

Interesting results were obtained for the rat female and male subgroups. The treatment with CP_bCD complex conduct to approximate the same values for hemoglobin content in female and male subgroups (14.63 mg/dL and 14.73 mg/dL, respectively; Table 2), which were close to the hemoglobin content in control subgroups (~14.9 mg/dL for both cases; Table 2). Higher hemoglobin contents were obtained in the case of subgroups treated with the ternary CP_Fv_bCD complex, especially in the case of male subgroup, ~16 mg/dL (in comparison with the female subgroup with a hemoglobin content of 15.1 mg/dL; Table 2).

Table 2. Hemoglobin analysis in female and male rats treated with cisplatin/β-cyclodextrin and cisplatin/*Ficaria verna* Huds. extract/β-cyclodextrin complexes; *n* – the number of replicates

N°	Code	Hemoglobin (g/dL)
Female		
1	C	14.93 ± 0.68 (*n* = 4)
2	CP_bCD	14.63 ± 1.10 (*n* = 4)
3	CP_Fv_bCD	15.12 ± 0.57 (*n* = 5)
Male		
1	C	14.88 ± 0.40 (*n* = 4)
2	CP_bCD	14.73 ± 0.93 (*n* = 4)
3	CP_Fv_bCD	15.98 ± 0.26 (*n* = 5)

4. Conclusion

The following conclusions can be drawn among the hematological properties (hemoglobin) of cisplatin / β-cyclodextrin and cisplatin / *Ficaria verna* Huds. extract / β-cyclodextrin complexes in rats: (1) β-cyclodextrin complexes of cisplatin reduce the adverse effect of this cytostatic compound; (2) the presence of antioxidant compounds in the cisplatin / β-cyclodextrin complex (a ternary complex containig flavonoids from *Ficaria verna* Huds. extract - antioxidant compounds – among the cisplatin and β-cyclodextrin molecules) increases significantly the protection capacity of β-cyclodextrin against the lowering of the hemoglobin content in rats treated with cisplatin; (3) the protection effect of the *Ficaria verna* Huds. extract / β-cyclodextrin complex against cisplatin adverse effects, evaluated as the hemoglobin content in rats, is more efficient in male than in female cases.

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