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Original

Marked survival prolongation of mice bearing a transplantable colon adenocarcinoma by treatment with radioactive platinum–[^{125}I]histamine complex. Preliminary report

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Abstract

BACKGROUND: Recently, a new $PtCl_2$-histamine complex, and its radioactive analogues labelled with I-131 and I-125 have been synthesised and investigated both *in vitro* and *in vivo*. In this preliminary report the survival rate of radioactive platinum-[^{125}I] histamine therapy in tumour-bearing mice is demonstrated.
MATERIAL AND METHODS: A murine model of transplantable colon adenocarcinoma (C38) in C57BL/6 mice (15 days post-implantation) was used for the experiment. Three groups of animals were treated every 2–3 days with five intraperitoneal injections of the following preparations: $PtCl_2$Hist (total dose of Pt — 125 μmol/kg), $PtCl_2$[^{125}I]Hist (total dose of I-125 — 4.2 MBq; Pt — 13 μmol/kg), and „Active/Cold" — $PtCl_2$[^{125}I]Hist/$PtCl_2$Hist (I-125 — 4.2 MBq; Pt — 125 μmol/kg). A solution of 15% dimethylformamide in saline was applied to the control group. A survival analysis with the Kaplan-Meier estimation of survival curves and a statistical comparison by a log-rank test was applied to evaluate the anticancer activity of the tested preparations.
RESULTS: Treatment of the animals with platinum-histamine preparations resulted in a significant prolongation of survivals, especially if the radioactive complex with carrier-added $PtCl_2$Hist ($p < 0.005$) was applied. The highest, almost a 60% prolongation of survival was observed in the Active/Cold group (MS_{tr}/ /MS_{con} ratio = 1.58, 95% CI 1.22–1.93). For this group there was the lowest risk of death (hazard ratio HR = 0.29), whereas HR = 0.45 and 0.47 were found in the animals treated with unattended $PtCl_2$Hist and ^{125}I-labelled complex, respectively.
CONCLUSION: The significant enhancement of *in vivo* anti-cancer activity by a concomitant combination of the therapeutic factors, *i.e.* cytotoxic/cytostatic activity of the platinum(II)-histamine and the Auger electrons effects generated by the attached I-125 radionuclide, was found on the murine model of transplantable colon adenocarcinoma.

Key words: Pt(II)Cl_2-histamine complex, iodine-125, transplantable colon adenocarcinoma (C38), tumour-bearing C57BL/6/C38 mice, survival analysis

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Introduction

Platinum-based anticancer drugs are known to be excellent radiosensitizers. Thus, in combination with external irradiation or with low-dose continuous internal radiotherapy these chemotherapeutics produce significant supra-additive treatment effects towards several tumour cells [1–3]. Several radioactive analogues of cisplatin, carboplatin and iproplatin, etc., have been synthesised using platinum radionuclides such as: ^{195m}Pt (4.02 d; IT, e^-),

^{193m}Pt (4.33 d; IT, e$^-$) and ^{191}Pt (2.9 d; EC, γ). Their utilities for the determination of the pharmacokinetics of platinum-cytostatics, and the therapeutic efficacy of internal radio-chemotherapy have been extensively studied [4–6].

Considering the growing role and benefits of a strategy of employing a concomitant combination of ionising radiation and chemotherapeutic agents for combating cancer, new radioactive platinum-[*I]histamine complexes containing Pt(II) core and radiotherapeutic isotopes *i.e.* I-131 (β-emitter) or I-125 (prolific emitter of Auger electrons), the moieties of a plausible synergistic anticancer potency have been synthesised and evaluated [7–10]. *In vitro* experiments have shown that the Pt(II)Cl_2Histamine complex produced cytostatic and cytotoxic activity against human mammary adenocarcinoma MCF-7 cells and against human colon cancer COLO-205 cells. However, it was slightly less potent than cisplatin [8, 10]. The results of the previous *in vitro* investigation, as well as biodistribution and pharmacokinetic studies [8–10] suggest the potential usefulness of platinum-[*I]histamine complexes for radio-chemotherapy of solid tumours.

In this report the survival rate of radioactive platinum--[^{125}I]histamine therapy in the murine model of transplantable cancer is demonstrated.

Material and methods

Tumour model

The murine model of transplantable colon adenocarcinoma (C38) in C57BL/6 mice was used for this experiment. The tumour was developed in 5–7 week old male mice (16–18 g) by subcutaneous (s.c.) injection (second passage) of 0.2 ml of colon adenocarcinoma (C38) cell suspension (33% v/v in Hank's balanced salt solution) into area of the dorsal region of healthy mice. The tumour-bearing animals were purchased from the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences in Wrocław.

The therapy was started 15 days after tumour inoculation, when tumours reached ca 0.5–0.7 cm in diameter. In the course of the experiment, the mice were kept in an area maintained at a standardised temperature of 22 ± 1°C and a 12 h light/dark cycle, and had access to rodent chow and water *ad libitum*.

The animal experiment was approved by The IVth Local Animal Ethics Committee in Warsaw (the authorisation number ZT/27/2002), and was carried out in accordance with the principles of good laboratory practice.

Tested preparations of platinum-histamine complexes

The Pt(II)Cl_2-histamine and Pt(II)Cl_2-[^{125}I]histamine complexes were synthesised and purified according to the developed procedures [7]. Stock solutions of the platinum-histamine complexes were prepared in dimethylformamide (DMF). The radiochemical purity of the $PtCl_2$[^{125}I]histamine complex was determined by RP-HPLC and paper radioelectrophoresis, and was consistently over 95%.

Three preparations of the platinum complexes were composed just before each scheduled administration into C57BL/6/C38 mice, *i.e.*: "Cold", "Active", and "Active/Cold". Table 1 shows a description of preparations and total dosages applied employing the transplantable colon adenocarcinoma model. Finally, each preparation contained 15% of DMF diluted in physiological (0.9%) saline.

Table 1. Description of tested preparations in C57BL6/C38/s.c. tumour-bearing mice

Tested groups	Applied preparations	Total dosage* applied
Control (n = 14)	Solution of 15% DMF in saline	5 injections (0.1 ml)
Cold (n = 9)	Solution of "cold" Pt(II)Cl_2Histamine complex	5 injections Σ_D 125 μmol/kg
Active (n = 10)	Solution of radioactive Pt(II)Cl_2[^{125}I]Hist (specific activity ca. 17 MBq/μmol)	5 injections Σ_D 13 μmol/kg Σ_A 4.2 MBq
Active/Cold (n = 9)	Solution of radioactive Pt(II)Cl_2[^{125}I]Hist with carrier-added Pt(II)Cl_2Histamine complex	5 injections Σ_D 125 μmol/kg Σ_A 4.2 MBq

* the single maximum tolerated dose (MTD) of $PtCl_2$Histamine = 75 μmol/kg (ca. 28 mg/kg)

Evaluation of anti-cancer activity

Prior to the treatment the C57BL/6/C38/s.c. mice were randomised into four groups. Each group consisted of at least 9 animals. Five intraperitoneal injections of tested preparations every two-three days were applied. A solution of 15% DMF in saline was applied to a control group.

The anticancer activity of the tested preparations was evaluated by means of the survival prolongation of tumour-bearing animals due to the treatments. The Kaplan-Meier method was applied to estimating the survival curves, and a comparison of the survival curves between each treatment and the control group was performed with a log-rank test. Based on the applied methodology, the common estimates of the differences between the survival curves were calculated, i.e.: the median survivals (MS), $MS_{treated}/MS_{control}$ ratios, and the hazard ratios (HR — the death risk in a treated group in the relation to the death risk in a control group). A survival analysis and 95% confidence intervals (95% CI) for the estimated parameters were performed with the help of GraphPad Prism computer software (Version 4.0, GraphPad Software Inc., San Diego, CA, United States 2003).

Results and discussion

Figure 1 shows the Kaplan-Meier survival curves estimated for the treated tumour-bearing animals. Because of the very aggressive type of transplantable colon cancer (C38) used, the control group showed a high mortality rate within one-month of the post tumour implantation. The median survival time for the control animals was 26 days post tumour implantation, and 11 days in relation to the beginning of the experiment only. The treatment of animals with platinum-histamine preparations resulted in a significant prolongation of survivals, especially if the radioactive complex with carrier-added $PtCl_2$-histamine (Active/Cold) was applied (Figure 1). As presented in Table 2, a statistical analysis employing the log-rank test pointed out significant differences between the K-M curves of the treated animals with Cold and Active/Cold preparations in comparison to the survival curve estimated for the control group. A slightly worse result (p = 0.0539) was observed

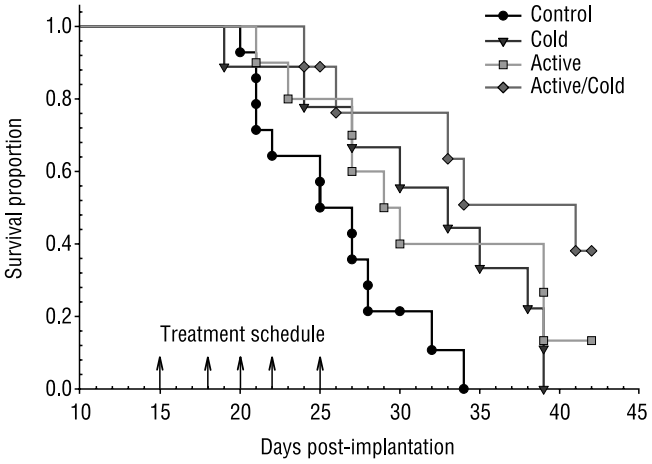

Figure 1. Kaplan-Meier survival curves for the C57Bl/6/C38 animals treated with platinum-histamine complexes.

in the survival curve obtained from animals treated with the Active preparation, which contained low amounts of carrier $PtCl_2$-histamine complex.

Comparing the survival estimates calculated (Table 2), it is obvious that marked and statistically significant anti-cancer activity was gained in the group treated with the radioactive complex with the concomitant carrier $PtCl_2$-histamine. The highest, and almost a 60-percent prolongation of survival (MS_{tr}/MS_{con} ratio = 1.58) was observed in the Active/Cold group. The hazard ratio was the lowest in this group (HR = 0.29), and considering the calculated 95% confidence interval of HR, means at least ca. a 40% lower risk of death in comparison to the control group. Slightly lower survival rate was observed in the Cold group, in spite of an equal amount of the platinum(II) complex being applied as in the Active/ /Cold group. Nevertheless, both the survival prolongation and the hazard ratio found in this group were more favorable than in the animals treated mostly with radiation (the Active group). Therefore, it is evident that the concomitant combination of chemical and Auger electrons effects obtained with $PtCl_2$-histamine and iodine-125 (Active/Cold preparation) resulted in a significant enhancement of anticancer activity. This might be due to radiosensitisation of the cells [11], or the inhibition of the intracellular repair mechanism arising from molecular interaction between the drug and radiation [12]. However, further studies are needed to explain the mechanism of co-action between the $PtCl_2$-histamine complex and the Auger electrons.

Finally, considering that the treatment was begun at an advanced stage of tumour development (start 15 days post-implantation), almost 240% of the relative survival rate was noted for Active/Cold preparation (Table 2) which suggests a highly potent anti-cancer effectiveness of the radioactive platinum-histamine complex and promises its clinical usefulness.

Conclusion

The significant enhancement of anticancer activity by a concomitant combination of the cytotoxic/cytostatic platinum(II)-histamine complex and the radiotoxic Auger electrons generated by the attached I-125 radionuclide ($t_{1/2}$ 59.6d; 25 Auger electrons per decay) was found in the murine model of transplantable colon adenocarcinoma (C57BL/6/C38/s.c.). In this preliminary report, the marked survival rate of tumour-bearing animals from treatment with radioactive platinum-[^{125}I]histamine was demonstrated. The encouraging results of the study justify further investigation of the internal radio-chemotherapy of solid cancers using the developed radioactive platinum complexes.

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Table 2. Summary of survival analysis due to treatment using the murine model of transplantable colon adenocarcinoma (C57BL6/C38/s.c.) with platinum(II)-[*I]histamine preparations

Preparations	Log-rank test p value	Median survival, MS (post tumour inoculation)	Ratio MS_{Tr}/MS_{Con} (95% CI of ratio)	Hazard ratio (95%CI of hazard ratio)	Survival rate* due to treatment
Control	–	26 d	–	–	–
Cold	p = 0.029	33 d	1.27 (0.84–1.70)	0.45 (0.12–0.89)	164%
Active	p = 0.0539	29.5 d	1.14 (0.72–1.55)	0.47 (0.15–1.02)	132%
Active/Cold	p = 0.0047	41 d	1.58 (1.22–1.93)	0.29 (0.08–0.63)	236%

*Survival rate due to treatment — survival prolongation in relation to the beginning of treatment — ratio (%) of the median survivals (counted from the beginning of treatment) of treated vs. the control animals

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