DRUGS EXPTL. CLIN. RES **XXIV** (5/6) 277-280 (1998)

# COMPARATIVE *IN VITRO* STUDY OF THE EFFECTS OF THE NEW ANTITUMOR DRUG UKRAIN AND SEVERAL CYTOSTATIC AGENTS ON THE THIOL GROUPS IN THE TISSUE OF GUERIN CARCINOMA AND ITS RESISTANCE TO CISPLATIN VARIANT

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**Summary:** *A comparative* in vitro *study between the effects of Ukrain (a new synthetic thiophosphoric acid derivative of great celandine alkaloids) and alkylating antitumor drugs cyclophosphamid and cisplatin on total thiol content in Guerin carcinoma, Guerin/cis-DDP carcinoma, and in animal livers was carried out. It is shown that Ukrain action on thiol groups in Guerin carcinoma tissue does not differ from that of cyclophospamid and cisplatin to both of which Guerin carcinoma is very sensitive. Once tumor resistance to cisplatin has developed, cisplatin does not react with tumor thiol groups and cyclophosphamid reactions with tumor thiol groups decrease. Reactions of Ukrain with thiol groups of cisplatin resistant tumors increase. This indirectly indicates the increase of tumor sensitivity to this drug. Therefore, the cytotoxicity of Ukrain is similar to that of known antitumor drugs and can probably overcome the cisplatin resistance of the tumor.*

## Introduction

Ukrain is a new semisynthetic derivative of thiophosphoric acid and chelidonin, an alkaloid isolated from *Chelidonium majus L.* It possesses antitumor and immunomodulating properties (1, 2).

Ukrain is produced on the basis of the well-known antitumor drug thiophosphamide and the alkaloid chelidonin. In contrast to thiophosphamide, this conjugate has antitumor properties and is not hematotoxic; moreover, it exerts a positive immunomodulatory influence (3, 4). In this light, the study of the possible differences in the mechanisms of action of these two drugs seems to be of great importance.

As was shown in our earlier research, the mechanism of antitumor and toxic action of several cytostatic agents such as thiophosphamide, cisplatin and doxorubicin relies predominantly on their interaction with tissue SH-groups (5). The degree of decreasing SH-groups in tumor tissue *in vitro* was shown to correlate with antitumor action of these drugs *in vivo*.

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The data presented here strongly suggests that the higher the antitumor activity of the drug *in vivo,* the higher is the relative decrease of total content of thiol groups in the tissue of the same tumor under the influence of the drug *in vitro*.

In order to analyze further the peculiarities of the mechanism of action of Ukrain, a comparative study of the interaction of Ukrain, thiophosphamide and cis-DDP with thiol groups in the tissue of Guerin carcinoma (Ca Guerin/wild) and its variant with acquired resistance to cis-DDP (Ca Guerin/Cis-DDP) was carried out.

## Materials and methods

The experiments were performed on Wistar rats (from breeding farm of Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kiev, Ukraine). Animals were given standard food and water *ad libitum*. Ca Guerin/wild and Ca Guerin/Cis-DDP were transplanted intercutaneously, $10^6$ living cells per animal. The rats were killed on the 12th to 14th day after transplantation and homogenates of tumor tissue and liver were prepared.

Upon determination of the total thiol group content, the equitoxic doses of the drugs Ukrain (Nowicky Pharma, Vienna, Austria), thio-TEPA 'Lederle' (Cyanamid GmbH, Germany) or cis-DDP (Cisplatin, Lilly France F.A., Saint-Cloud, France) were added to the homogenates. The homogenates were incubated with the drugs for 1 h at 37 °C. The difference in SH-group content prior to and after incubation with the drug expressed in percentages served as the measure of the level of SH-group inactivation. Total SH-group content was

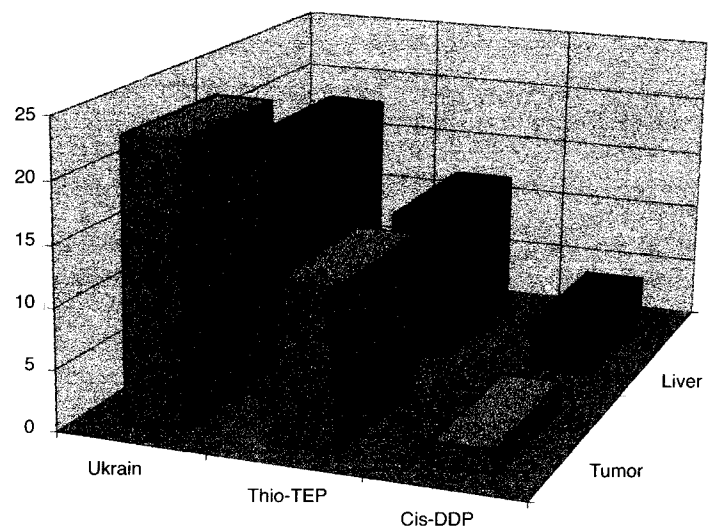


**Fig. 1** SH-group content in tumor and normal liver tissue of rats after *in vitro* exposure to Ukrain, thio-TEPA and cisplatin.

**Table I** *Decrease of SH-group content in tumor and liver tissue of rats with Ca Guerin/wild, induced by* in vitro *exposure to Ukrain, Thio-TEP and Cis-DDP*

| Preparation | Tumor | Liver |
|---|---|---|
| Ukrain | 22.9 ± 3.5 | 17.6 ± 1.2 |
| Thio-TEP | 12.85 ± 1.7 | 12.1 ± 1.3 |
| Cis-DDP | 1.7 ± 0.7 | 3.5 ± 1.1 |

**Table II** *Decrease of SH-group content in tumor and liver tissue of rats with Ca Guerin/Cis-DDP, induced by* in vitro *exposure to Ukrain, Thio-TEP and Cis-DDP*

| Preparation | Tumor | Liver |
|---|---|---|
| Ukrain | 19.5 ± 3.0 | 3.9 ± 0.91 |
| Thio-TEP | 22.97 ± 3.47 | 12.41 ± 2.46 |
| Cis-DDP | 21.7 ± 3.02 | 12.63 ± 2.99 |

measured spectrophotometrically by Elman's technique, as described by Veryovkina *et al.* (6). Each experiment was performed on not less than 10 animals.

## Results

The SH-group content in tumor and normal liver tissue of rats after *in vitro* exposure to Ukrain, thio-TEPA, and cis-platin is shown in Figure 1. The interaction of Ukrain with SH-groups of Ca Guerin/wild (Table I) was shown to be 19.5%. This value is characteristic of such active cytostatic agents as thio-TEP and cis-DDP, the latter inhibiting the growth of Ca Guerin/wild by 95-98%. It is worth mentioning the high selectivity of Ukrain interaction with tumor tissue thiol groups compared with thiol groups of normal liver tissue. At the same time, the degree of interaction of the liver tissue thiol groups with thio-TEP and cis-DDP was significantly higher than with Ukrain, amounting to 10-12%.

The data presented here shows that thiol groups of the tumor tissue with acquired resistance to cis-DDP (Ca Guerin/cis-DDP) (Table II) do not interact with this drug. Their interaction with thio-TEP was also lowered, while the degree of thiol group interaction of this tumor with Ukrain exceeded the degree of interaction with thiol groups characteristic of the wild variant of this tumor.

Nevertheless, the selectivity of Ukrain action against the tumor decreased due to a higher level of interaction with thiol groups in the liver. At the same time the reactivity of cis-DDP with liver thiol groups of the host bearing the tumor resistant to this drug was rather low.

The interaction of thio-TEP with liver thiols did not depend on the sensitivity of the tumor to cis-DDP. If the degree of drug interaction with tissue thiols of the tumors is assumed as the index of tumor sensitivity to the drugs, the data obtained suggest some cross-resistance of tumors resistant to cis-DDP to thio-TEP while the cross-resistance towards Ukrain is absent.

To sum up, the data presented here demonstrates that the cytotoxic activity of Ukrain is close to that of known antitumor drugs. Moreover, this drug appears to overcome resistance to cis-DDP. Final conclusions, however, will only be possible after further investigations, both *in vitro* and *in vivo*.

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