Eur J Cancer Clin Oncol, Vol. 19, No. 5, pp. 695-699, 1983.
Printed in Great Britain.

0277-5379/83/050695-04$03.00/0

Letter to the Editor

Treatment of Primary Embryo-derived Teratocarcinomas in Mice with *cis*-Diamminedichloroplatinum*

JAN W. OOSTERHUIS†‡ and IVAN DAMJANOV§

†*The Department of Pathology, University of Groningen, Oostersingel* 63, 9713 *EZ Groningen, The Netherlands and* § *The Department of Pathology, Hahnemann Medical College,* 230 *North Broad Street, Philadelphia, PA* 19102, *U.S.A.*

CHEMOTHERAPY of disseminated non-seminomatous germ cell tumors (NSGCT) often results in residual metastatic tumor tissue with the histologic appearance of mature (fully differentiated or adult) teratoma [1–3]. It is a matter of debate whether this mature tissue is the end result of selective killing of embryonal carcinoma (EC) cells or the undifferentiated tumor stem cells, or *de novo* induction of their differentiation into benign somatic tissues by chemotherapy [2, 3].

Generally speaking, the treatment strategy is to render the NSGCT patients disease-free by surgical removal of the residual tumor whenever possible [4]. Consequently, few patient data are available on the malignant potential of the histologically benign tumor masses found in metastatic sites after chemotherapy.

Murine embryo-derived teratocarcinomas are a useful replica of their human counterparts [5] and could be used to study the aforementioned problems. We thus chose this murine model and treated the tumor-bearing animals with *cis*-diamminedichloroplatinum (CDDP), the most effective single cytotoxic drug used against human NSGCT.

In our experiments we used male and female BALB/c mice aged 6–8 weeks, obtained from Charles Rivers Laboratories. Embryo-derived tumors were produced by transplanting 8-day-old mouse embryos under the capsule of the left kidney of syngeneic female 8- to 10-week-old mice [6]. The mice were numbered individually with earmarks immediately after transplantation. The treatment was started 7 weeks after embryo transplantation. CDDP (kindly provided by the NIH drug program) was freshly dissolved in normal saline immediately before intraperitoneal injection. The mice with odd numbers were administered 18 mg CDDP/kg in 6 × 3-mg injections over a 10-day period. The evenly numbered mice received the corresponding amount of saline. The mice which were found dead in the cages and all the killed mice were autopsied. The tumors were weighed and, depending on the size of the tumor, one or two random blocks of tissue were taken for histologic examination. Primary embryo-derived tumors can be either benign or malignant. Malignant tumors contain areas of embryonal carcinoma cells (EC cells), which are not present in benign tumors [7]. Tumors over 1 g are almost exclusively histologically malignant.

We treated two series of mice. The mice of the first series ($n = 95$) and of the second series ($n = 89$) were killed three days and seven weeks after the last CDDP injection respectively. Out of the 194 mice transplanted with embryos 103 developed a tumor (56%), yielding about 25 tumors in each experimental group of which 50% were over one gram (Table 1).

After three days follow-up in the control group 13 out of 13 tumors over 1 g and in the treatment

Accepted 2 November 1982.

*Supported in part by a grant from the "J.K. de Cock Stichting", Groningen, The Netherlands, a research fellowship grant from the Netherlands Organisation for the Advancement of Pure Research (ZWO)/NATO and NIH grants CA23097 and GM29040. The *cis*-diamminedichloroplatinum was provided by the NIH drug program.

‡To whom requests for reprints should be addressed.

group 1 out of 10 tumors weighing 1 g or more were histologically malignant, containing EC cell areas. This represented a significant reduction of malignancy in the CDDP-treated group (Fisher exact test, $P < 0.001$). After seven weeks follow-up there were 11 tumors over 1 g in the control group, all of them histologically malignant. In the treatment group 7 of the 14 tumors weighing more than 1 g were malignant (Fisher exact test, $P < 0.01$). The comparison of the treatment group with a 3-day follow-up and the group with 7 weeks of follow-up disclosed significantly more malignant tumors in the latter group (Fisher exact test, $P < 0.05$). Apparently the malignant morphology of the tumors was regained due to the reappearance of EC cells (Table 1).

Among the 13 mice in the second series that died at various intervals after the last CDDP injection due to drug toxicity there were four with histologically malignant tumors. These malignant tumors together with those of three mice that were killed at the end of the experiment gave some insight about the morphogenesis of recurrent malignancy in this system. The malignant areas recurred as scattered microscopic foci of EC cells accompanied by immature tissue. As these areas increased in size they developed into circumscript nodules amidst the mature cystic tumor tissue; a distinctive pattern that was not found in untreated malignant tumors. Histologically the recurring nodules showed the same kind of somatic differentiation as found in untreated teratocarcinomas (Fig. 1).

The present study was designed to reproduce the clinical finding of mature residual metastatic tumors following chemotherapy of NSGCT in the primary embryo-derived teratocarcinoma model, a tumor system known for its resemblance to human teratocarcinomas [5, 6].

CDDP treatment had a most dramatic effect on the morphology of teratocarcinomas. All the tumors except one that were studied three days after cessation of chemotherapy had the histologic appearance of benign teratomas. The treated tumors were devoid of solid areas and were considerably more cystic than the control, untreated tumors. The CDDP-treated tumors thus showed a striking resemblance to the residual tumor masses found in metastatic foci following chemotherapy of disseminated human NSGCT of the testis. The mechanism of CDDP-induced change in the morphology from teratocarcinomas to benign teratomas could not be determined from this study.

The reappearance of malignancy in these deceivingly benign tumors illustrates the shortcomings of histologic examination of complex germ cell tumors. Thus since there are considerable similarities between human and murine teratocarcinomas, we urge caution in labelling all histologically mature tumor masses residual after chemotherapy of NSGCT as unequivocally benign. One could never be certain that a couple of EC cells have not survived the treatment and could thus serve as the basis of recurrent malignancy.

CDDP-treated embryo-derived teratocarcinomas offer a realistic model to study the biology of mature residual tumors found in patients following chemotherapy of NSGCT. This animal tumor model may prove useful in the elucidation of the mechanisms involved in the maturation process.

Table 1. CDDP treatment (18 mg/kg) of primary embryo-derived teratocarcinomas in female BALB/c mice: weights and histologic classification

Follow-up	No. of transplated mice	Treatment	No. of tumors < 1 g	Tumors > 1 g No.	Weight (mg) Median	(Range)	Histology Benign	Malignant
3 days	95	CDDP	15	10	1659	(1050–6283)[ac]	9	1[eg]
		control	14*	13	3202	(1093–8177)[ad]	0	13[e]
7 weeks	89	CDDP	13	14	3338	(1153–15487)[bc]	7	7[fg]
		control	13	11	11587	(1736–19634)[bd]	0	11[f]

*The only histologically malignant tumor weighing less than 1 g was in this group.
Mann–Whitney U test: a and c, $P < 0.05$; b and d, $P < 0.001$. Fisher exact test: e, $P < 0.001$; f, $P < 0.01$; g, $P < 0.05$.

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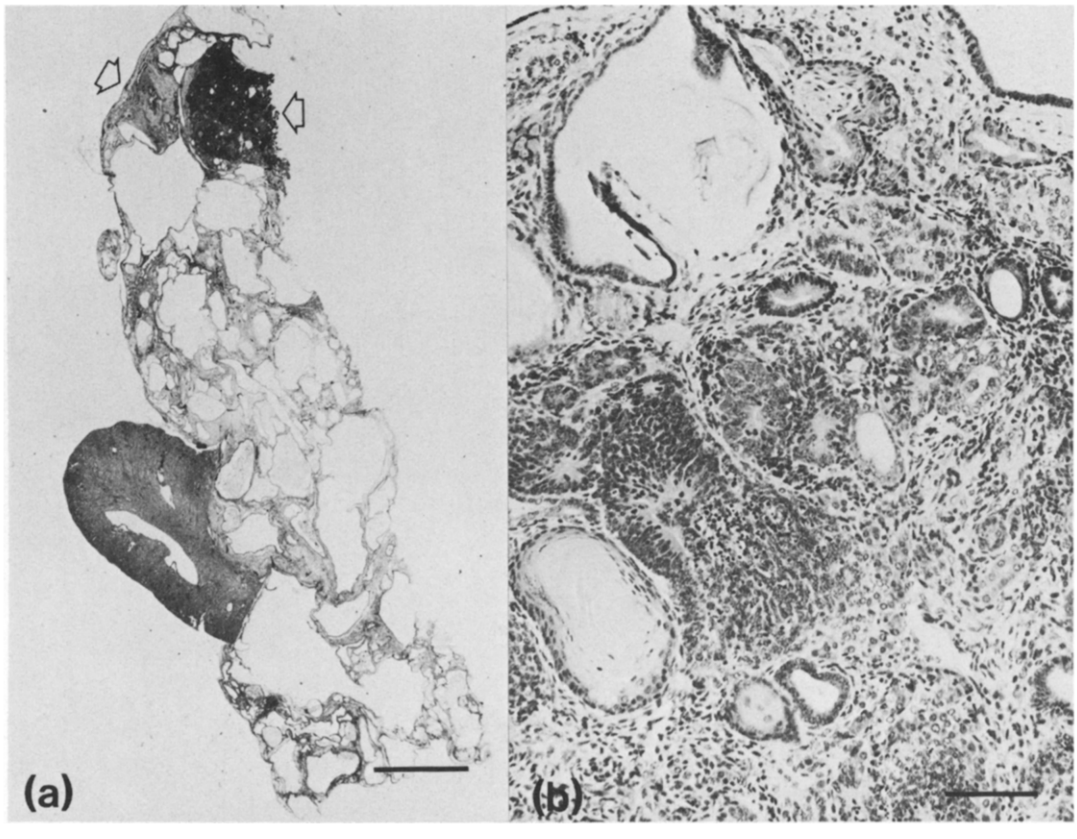

Fig. 1. *(A) A primary embryo-derived tumor transplanted under the kidney capsule* 7 weeks *after completion of CDDP treatment* (18 mg/kg *over* 10 days). *Recurrence of areas containing EC cells as two discrete nodules amidst mature cystic tissue (arrows). (B) Higher power of one of the recurrent malignant nodules: EC cells, immature teratoma and mature cysts lined with a variety of epithelial tissues* [*bar equals* 2 mm *in (A) and* 0.1 mm *in (B)*].

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