Eur J Cancer Clin Oncol, Vol. 20, No. 5, pp. 625-630, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Serum Haptoglobin and α1-Acid Glycoprotein as Indicators of the Effectiveness of *cis*-Diamminedichloroplatinum (CDDP) in Ovarian Cancer Patients—a Preliminary Report

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Abstract—*Twenty-one patients with adenocarcinoma of the ovary were given a maximum of five courses of CDDP by i.v. infusion every* 4 weeks. *With one exception, the serum levels of both haptoglobin and α1-acid glycoprotein correlate with tumour burden before therapy and at follow-up. It is suggested that a more accurate assessment of the effectiveness of CDDP therapy may be obtained by estimating these serum proteins before each infusion than by an abdominal examination.*

INTRODUCTION

LAPAROTOMY and debulking surgery are the chief methods of diagnosis and treatment of ovarian cancer. In patients who appear to have the tumour totally resected, at least one-third develop recurrent disease [1] requiring the use of post-operative chemotherapy. Recent work suggests that the metal co-ordination complex, *cis*-diamminedichloroplatinum (CDDP), when given as a single agent to patients with ovarian cancer produces tumour regression in a number of cases [2]. The assessment of the effectiveness of this chemotherapeutic agent is, however, difficult because relatively large tumour masses may not be palpable and second-look laparotomy is often required to make a more reliable estimate.

A number of substances, including glycoproteins, enzymes, hormones, and foetal and specific antigens, have been suggested as potential serum markers for patients with ovarian cancer [3–7]. With the exception of serial monitoring of human chorionic gonadotrophin and α-fetoprotein in patients with ovarian teratoma, few of these studies have been seriously concerned with longitudinal estimations of proposed 'markers' in patients with adenocarcinoma of the ovary.

The serum levels of a number of 'acute-phase' glycoproteins [8] often correlate with the extent of cancer in a variety of malignancies in man [9–12] and in tumour-bearing animals [13]. Although the non-specific nature of these glycoprotein changes precludes their use in detection of early cancers [14], it is possible that the determination of these proteins may help to assess the disease status of cancer patients, particularly during chemotherapy.

In a previous study [15] it was shown that constantly high or rising serum levels of α1-acid glycoprotein and haptoglobin during consecutive methotrexate infusions were associated with metastatic disease in breast cancer patients and falling levels of these proteins correlated with drug-induced tumour regression. This report describes sequential measurements of these glycoproteins in a group of patients with advanced ovarian cancer receiving repeated intravenous infusions of CDDP.

MATERIALS AND METHODS

Patients and treatment

Twenty-one patients of mean age 57 yr with residual FIGO stage III and IV adenocarcinoma of the ovary after laparotomy were studied. They were referred not less than 3 weeks after the operation. Twelve had palpable tumour masses of less than 10 cm and the remainder had masses greater than 10 cm. All patients received an intravenous CDDP infusion of 100 mg/m^2 over a 6-hr period. Treatment was repeated every 4 weeks to a maximum of 5 infusions. At the end of the

Accepted 31 October 1983.

drug treatment period of 16 weeks or less all patients were assessed by abdominal examination and classified into three clinical groups. Seven had a complete clinical response that was confirmed by second-look laparotomy. These patients are alive and clinically disease-free after a follow-up period of 1–2 yr. Eleven patients did not respond to drug therapy, and with the remaining three patients a partial tumour regression was suggested (i.e. 50% reduction of palpable tumour mass). Twelve of these 14 patients have subsequently died (Table 1).

Blood sampling

Sera from 46 healthy females of mean age 55 ± 15 yr were used as controls. Serial samples were collected from patients before every drug infusion and thereafter at 4- to 8-week intervals for periods up to 2 yr. Aliquots of sera were stored at −20°C. Each aliquot was thawed only once for use in this study and analysis of the serial samples of a single patient were carried out simultaneously.

Serum proteins

Haptoglobin (Hp) and α1-acid glycoprotein (AGP) were determined in duplicate by rocket immunoelectrophoresis [16]. Specific antisera and standards were obtained from Hoechst Pharmaceuticals, Middlesex, U.K. The mean ± S.D. for the control sera were 1.67 ± 0.45 and 0.76 ± 0.14 mg/ml for Hp and AGP respectively. The upper limit of the normal range was estimated as 3 S.D. above the mean serum concentration for these two proteins. Haptoglobin phenotyping was performed by gradient polyacrylamide gel electrophoresis [17]. The distribution of the three major phenotypes in the control group was Hp 2.2, 44%; Hp 2.1, 42%; Hp 1.1, 14%. Patient EW was Hp phenotype 1.1. Nine patients were 2.1 and the remaining individuals were Hp 2.2.

The serum concentrations of α1-antitrypsin, ceruloplasmin, prealbumin and albumin were also estimated by immunoelectrophoresis. These measurements, however, did not provide additional information concerning the effectiveness of CDDP therapy and are not described.

RESULTS

Before CDDP therapy

The mean serum concentrations of Hp and AGP in the 21 patients were elevated compared with the control sera (Table 2). There was an increase in both protein levels in relation to tumour burden; in the group of patients with a tumour mass less than 10 cm, 6/12 subjects had both serum protein levels in the normal range; but

Table 1. Clinical features of the patients

Patient	Age	Stage	Histology	Extent of surgery	Surgery to 1st CDDP (weeks)	No. of CDDP infusions	Clinical response	Survival (months)
Tumour size before CDDP therapy <10 cm								
HB	61	III	serous	biopsy	3	5	complete	24A
MC	53	III	serous	biopsy	4	5	complete	24A
ID	57	III	clear cell	H + O	4	4	complete	20A
EG	61	III	undiff.	O	4	5	complete	18A
GH	67	III	serous	O	3	5	complete	15A
MP	62	III	mucinous	H + O	9	5	complete	13A
KD	32	III	mucinous	biopsy	10	5	complete	25A
JJ	58	III	clear cell	biopsy	9	3	none	8D
EK	62	III	undiff.	biopsy	3	2	none	6D
BB	66	III	undiff.	O	6	3	none	10D
MS	65	IIB	undiff.	H + O	5	3	none	4D
EW	54	III	serous	O	12	3	none	15D
Tumour size before CDDP therapy >10 cm								
EF	68	III	undiff.	biopsy	4	4	partial	11D
PJ	62	III	undiff.	biopsy	4	4	partial	14A
PW	44	III	serous	biopsy	3	5	partial	12D
LE	44	III	serous	biopsy	5	5	none	13A
GE	52	III	mucinous	biopsy	5	3	none	19D
CF	68	III	serous	biopsy	6	3	none	9D
FL	59	III	mucinous	biopsy	4	3	none	4D
CV	66	IV	serous	biopsy	4	3	none	8D
AY	65	IV	undiff.	biopsy	8	3	none	4D

H = Hysterectomy, O = Oophorectomy, A = Alive, D = Dead. Clinical response was measured by abdominal examination. Complete response represents no palpable tumour that was confirmed by second-look laparotomy and a partial response indicates a 50% reduction in palpable tumour mass.

Table 2. Mean pretreatment serum glycoprotein levels and initial tumour size

Protein	Healthy individuals ($n = 46$)	Patients Tumour size <10 cm ($n = 12$)	Patients Tumour size >10 cm ($n = 9$)
Hp	1.67 ± 0.45	3.21 ± 1.15	6.14 ± 2.18
AGP	0.76 ± 0.14	1.19 ± 0.20	1.57 ± 0.35

Results are expressed in mg/ml as mean ± S.D.

serum levels above the normal range were observed in 9/9 Hp and 8/9 AGP in patients with a tumour mass greater than 10 cm.

During therapy

The mean serum levels for both proteins before every drug infusion for the 21 patients classified according to initial tumour mass and response to therapy is shown in Fig. 1. Complete or partial drug-induced tumour regression was, in general, associated with declining serum levels of Hp and AGP (Fig. 1a, c). However, in those patients who did not respond to CDDP therapy the mean serum

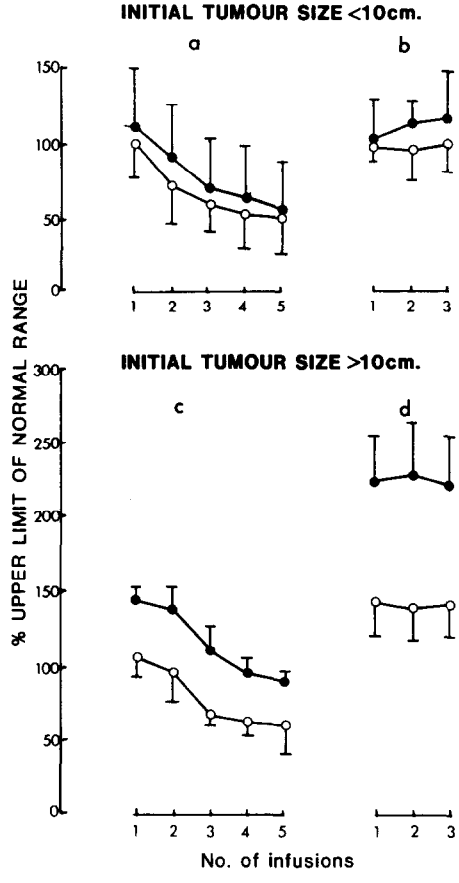

Fig. 1. Mean ± S.D. serum haptoglobin ●——● and α1-acid glycoprotein ○——○ levels before every drug infusion. The upper limit of the normal range is represented by 100%. (a) Seven patients, complete tumour regression; (b) five patients, no regression; (c) three patients, partial regression; (d) six patients, no regression.

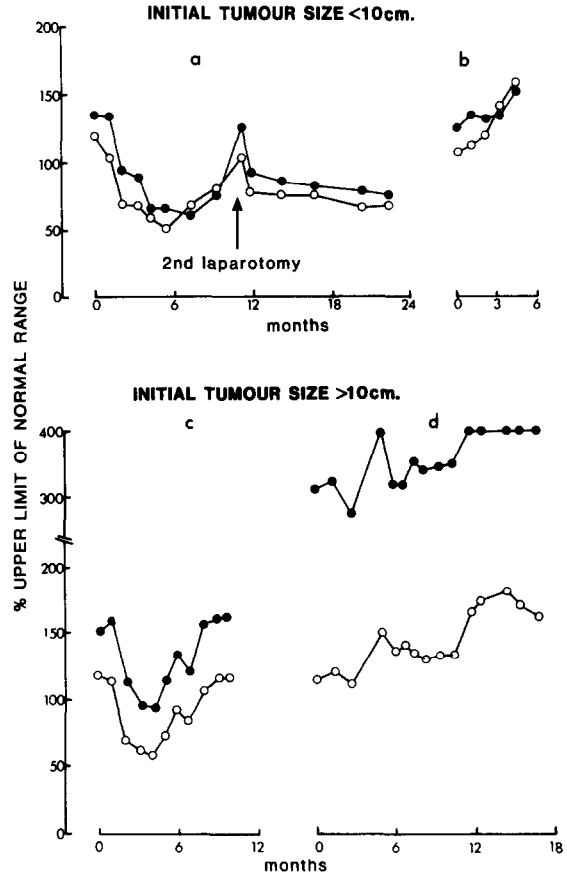

Fig. 2. Serum haptoglobin ●——● and α1-acid glycoprotein ○——○ levels in four patients. The upper limit of the normal range is represented by 100%. (a) Patient M.C., complete tumour regression; (b) patient E.K., no regression; (c) patient P.J., partial regression; (d) patient G.E., no regression.

levels for both proteins remained relatively constant, with particularly high levels of Hp occurring in patients with bulky disease (Fig. 1b, d).

At follow-up

Examples of the long-term protein profiles for four patients is shown in Fig. 2. Patient M.C. (Fig. 2a) had an initial tumour mass less than 10 cm, a complete clinical response to CDDP and no residual tumour on second-look laparotomy. She is clinically disease-free 2 yr after drug therapy and both serum proteins have remained in the normal range except for a transient increase after second-look surgery. In contrast, a progressive rise in serum Hp and AGP levels occurred in patient E.K., who was also classified in the small tumour group but did not respond to CDDP therapy (Fig. 2b).The protein profiles for two subjects with bulky disease is shown in Fig. 2 (c, d). A partial tumour regression in patient P.J. was associated with a fall in both serum protein levels during the CDDP course but was immediately followed by a continuous increase in serum levels

after therapy. The non-responding patient G.E. maintained high serum protein concentrations, which was particularly marked for haptoglobin, during drug therapy and for a follow-up period of 15 months.

One patient (Fig. 3) had a haptoglobin concentration remaining in the normal range despite tumour progression, but the serum AGP level rose steadily throughout the follow-up period.

Table 3 shows the mean and range of serum concentrations of Hp and AGP in all patients before treatment, at the end of the CDDP courses and in the last follow-up sample. Seven patients who are at present clinically disease-free had protein levels in the normal range both at the end of therapy and at follow-up. The remaining patients (except E.W.) who had tumour progression had elevated serum levels of these glycoproteins.

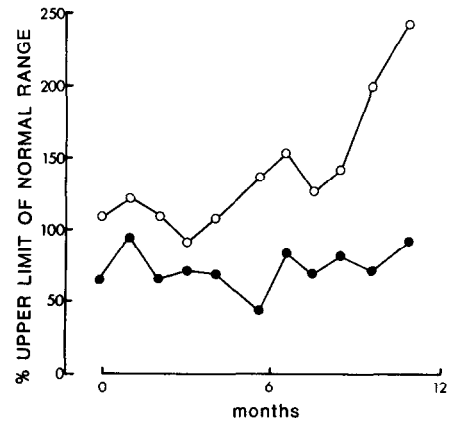

Fig. 3. Serum haptoglobin ●——● and α1-acid glycoprotein O——O levels in patient E.W., showing normal Hp levels despite tumour progression.

DISCUSSION

Only limited data are available concerning the acute-phase glycoprotein response in patients with ovarian carcinoma. The mean pre-operative serum concentration of the seromucoid fraction was significantly higher in seven patients with ovarian cancer compared with 25 cases of benign ovarian cysts [18]. This fraction is a heterogeneous mixture of serum glycoproteins, the main components of which are: alpha-1-acid glycoprotein, alpha-1-antitrypsin and haptoglobin. Marked increases in the mean pre-operative serum levels of these three proteins have been demonstrated in 15 patients with stage III and IV ovarian cancers compared with control sera [19], and haptoglobin has been suggested as an indicator of disease activity in this condition [20].

This study demonstrates that haptoglobin and α1-acid glycoprotein serum levels change in parallel and appear to reflect tumour burden before, during and after drug therapy. However, discord between these proteins can occur (patient E.W., Fig. 3), with a steady rise in the serum AGP level being accompanied by normal levels of Hp even though no drug-induced tumour response occurred and there was progressive disease after therapy. Whether a similar phenomenon occurs in other patients who carry the low-molecular-weight 1.1 phenotype of haptoglobin remains to be established.

Factors other than changes in tumour mass could be responsible for the observed alterations in protein levels in some of these patients before and during repeat courses of drug therapy. For example, transient changes in these proteins are known to occur after surgery, although in uncomplicated cases these alterations usually subside within several weeks after injury [21, 22]. However, in the present group of patients

Table 3. Mean serum glycoprotein levels after drug therapy

	Protein	Before drug therapy	End of drug therapy	Last follow-up sample
		Initial tumour size <10 cm		
Complete regression ($n=7$)	Hp	3.35(1.74–5.40)	1.57(0.53–2.30)	1.62(0.61–2.30)
	α1-AGP	1.18(0.85–1.30)	0.61(0.45–0.80)	0.72(0.65–0.80)
No regression ($n=5$)	Hp	3.04(1.93–4.10)	3.43(1.93–4.30)	4.31(2.76–6.36)
	α1-AGP	1.19(0.98–1.29)	1.17(0.86–1.44)	1.83(1.07–2.90)
		Initial tumour size >10 cm		
Partial regression ($n=3$)	Hp	4.34(4.10–4.60)	2.70(2.60–2.80)	4.73(4.68–4.76)
	α1-AGP	1.26(1.12–1.40)	0.72(0.62–0.90)	1.53(1.33–1.72)
No regression ($n=6$)	Hp	6.8(4.40–9.50)	6.7(4.60–9.10)	7.78(4.55–14.00)
	α1-AGP	1.69(1.35–2.40)	1.64(1.30–2.00)	1.80(1.43–2.04)

Results are expressed in mg/ml (ranges in parentheses). Upper limit of normal range (mean ± 3 S.D.) was 3.02 and 1.18 mg/ml for Hp and α1-AGP respectively.

stabilization of protein levels into the normal range after laparotomy is unlikely to occur since only a biopsy or debulking of the tumour mass was performed (Table 1). Moreover, in a parallel study on the kinetics of CDDP in these patients platinum could still be detected in the serum before repeat drug infusions (unpublished data). Although CDDP or its metabolites are known to be bound to serum proteins [23], the presence of platinum did not interfere, at least *in vitro*, with the immunochemical estimation of Hp or AGP.

It is suggested that monitoring the serum levels of this pair of 'inflammatory' proteins before repeat infusions may be of potential clinical value since quantitative changes in these proteins appear to reflect the effectiveness of CDDP treatment in this hidden malignancy.

Acknowledgements—The authors thank the South Glamorgan Health Authority for support and clinicians involved in the SWONS Gynaecologic Group for their interest and co-operation.

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