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Original article

Glutathione reduces the toxicity and improves quality of life of women diagnosed with ovarian cancer treated with cisplatin: Results of a double-blind, randomised trial

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Summary

Background: Early clinical trials have suggested that glutathione (GSH) offers protection from the toxic effects of cisplatin.

Patients and methods: One hundred fifty-one patients with ovarian cancer (stage I–IV) were evaluated in a clinical trial of cisplatin (CDDP) ± glutathione (GSH). The objective was to determine whether GSH would enhance the feasibility of giving six cycles of CDDP at 100 mg/m^2 without dose reduction due to toxicity.

Results: When considering the proportion of patients receiving six courses of CDDP at any dose, GSH produced a significant advantage over control – 58% *versus* 39%, (P = 0.04). For these patients there was a significant difference between the reduction in creatinine clearance for GSH treated patients compared with control – 74% *versus* 62% (P = 0.006). Quality of life scores demonstrated that for patients receiving GSH there was a statistically significant improvement in depression, emesis, peripheral neurotoxicity, hair loss, shortness of breath and difficulty concentrating. As an indication of overall activity, these patients were statistically significantly more able to undertake housekeeping and shopping. Clinically assessed response to treatment demonstrated a trend towards a better outcome in the GSH group (73% *versus* 62%) but this was not statistically significant (P = 0.25).

Conclusions: The results demonstrate that adding GSH to CDDP allows more cycles of CDDP treatment to be administered because less toxicity is observed and the patient's quality of life is improved.

Key words: cisplatin, glutathione, platinum, toxicity

Introduction

Ovarian cancer is the fifth most common cancer in women in the United Kingdom. Each year there are 5,000 new cases and 4,000 deaths [1]. In the United States, it is the fifth leading cause of death in women and there are more deaths from ovarian than from cervical and endometrial cancers combined [2].

Cisplatin (CDDP) is one of the most effective drugs in the treatment of ovarian carcinoma [3, 4] and its well-documented efficacy has resulted in an intensive search to identify the optimal dosing regimen. Evidence suggests that both its efficacy and toxicities are dose-dependent and has prompted an interest in looking at the response to higher doses of cisplatin. This approach would appear to be justified from observing patients who achieve a complete pathological response and have a good prognosis for long-term survival, irrespective of their second-line therapy [5]. In addition, the clinical response and survival appear to be related to the relative dose-intensity of CDDP administered to the patient. The accepted conventional dose of single agent CDDP is 100 mg/m^{2}q 21 days, with the intention of administering treatment six times. However, in routine practice, despite the use of hydration and optimal anti-emetics, the toxicities of CDDP – predominantly nephro- and neurotoxicity – necessitate some dose reduction or restriction in number of cycles administered in the majority of patients. The investigations of CDDP analogues with different dose-limiting toxicities has not resolved this problem – especially for neurotoxicities, and there is therefore great interest in the development of new strategies to enhance the tolerability to CDDP whilst preserving its anti-cancer efficacy.

Glutathione (GSH) is a naturally occurring non-toxic, tripeptide (glutamyl-cysteinyl-glycine). The thio-nucleophilic region of the tripeptide is indicative of a compound which has a high affinity for heavy metals and it has been postulated that GSH may reduce the toxic effects of cisplatin.

Early clinical studies have suggested that GSH offers neuroprotection [6, 7], nephroprotection [8] and may even improve response rate [9]. However, these studies were small open trials. Thus, a large phase III double blind randomised study was designed to investigate the potential protective effect of GSH, and to determine whether GSH allows higher doses of CDDP to be administered. The dose and dose regimen of GSH was

chosen on the basis of experimental and clinical data which have shown that the pharmacokinetic half-life of intravenous GSH in man is 15 minutes [10] and greatest protection is achieved when GSH is administered in the period that ranges from 30 minutes prior to, to simultaneously with CDDP administration. It appears that a GSH : CDDP ratio of 30 : 1 allows good protection without interfering with therapeutic activity [11].

Patients and methods

A total of 151 women with ovarian cancer stage I–IV (mean age 57 years, range 21–76) received i.v. CDDP 100 mg/m^2 + GSH 3 g/m^2 (placebo controlled) every three weeks for six courses.

We wished to compare the number of patients receiving six cycles of 100 mg/m^2 CDDP in each arm, compare the toxicities of CDDP therapy in each arm of the trial and evaluate quality of life in both groups. A comparison of response was made to assess whether GSH afforded any protection from the therapeutic effects of CDDP.

The study was of a multicentre, randomised, double-blind, parallel-group design where patients were randomised to one of two treatment groups following staging and debulking surgery.

Clinical assessments plus full blood count, serum electrolytes and liver and renal function were performed at each visit. Renal function was monitored by calculating creatine clearance using the standard formula: creatine clearance (ml/min) = {[(140 − age in years) × weight in kg]/(72 × creatinine mg/dl)} × (1.73/surface area in m^2). Audiogram and neurological examination were performed at baseline, after three cycles and after six cycles. In four of the participating centres, quality of life was measured at each visit by HAD score [12] and Rotterdam Symptom Checklist [13].

Although the CDDP dose to be investigated was 100 mg/m^2 at each cycle, this could be reduced in the event of clinically significant toxicity defined as. nephrotoxicity or neurotoxicity = grade II (CTC), ototoxicity = grade III (CTC), emesis = grade IV (WHO). In the event of toxicity greater than those above, CDDP therapy was discontinued.

Also, if the white cell count was $<3 \times 10^9$/l or platelets $<100 \times 10^9$/l or neutrophils $\geq 1.5 \times 10^9$/l, treatment was delayed either for a week or until the appropriate levels were achieved.

The drug/dose regimen was as follows: 3 g/m^2 GSH was diluted in 200 ml normal saline and infused in a large vein over 20 minutes immediately before CDDP; for control patients 200 ml normal saline only was infused in a large vein over 20 minutes immediately before CDDP; 100 mg/m^2.

Table 1. Clinical details.

	CDDP (n = 77)	CDDP + GSH (n = 74)
Age (mean/range)	58 (28–76)	57 (21–76)
Performace status		
0	29	30
1	38	35
2	10	9
Grading		
1	4	3
2	22	13
3	22	34
Not specified	29	24
Stage		
I	9	13
II	9	6
III	48	48
IV	10	7
Uncertain	1	0

CDDP was administered in 250 ml normal saline over 45 minutes; hydration: 1 litre normal saline as prehydration infused in two hours before GSH and 2 litres normal saline as post-hydration over 24 hours; diuretics were only used if a patient had a diuresis <100 ml/h after CDDP administration. All patients received 5HT3 antiemetic premedication.

Randomisation and blinding

A computer-generated randomistion list was prepared for each centre using consecutive numbers.

Due to the nature of GSH, the pharmacist at each centre could not be blind to the trial treatment.

Within each centre patients were randomly but evenly assigned to one of the two groups. The pharmacist was informed of the treatment group and prepared either a saline infusion or a saline infusion containing GSH. Both were of identical appearance.

Statistical plan and evaluation

The study was a two treatment, multicentre, parallel group trial. Analysis was based on all patients who were randomised and received any study medication.

Centre effects were allowed for in the analysis of the main outcome variables because of substantial between centre differences. Tests of homogeneity of treatment effects across centres involved dichotomising the outcome variable where necessary and carrying out an exact test of homogeneity of odds ratios using the method of Zelen [14]. Tests of overall treatment effects allowing for centre effects were performed using stratified trend tests [15, 16]. Exact confidence limits for odds ratios were calculated by the method of Gart [17]. Data was analysed initially using SAS, with the use of StatXact for the tests described above. The analysis of the HAD scores was based on a weighted average of changes in HAD scores where baseline levels were available, and first treatment HAD where baselines were not available. Other tests reported are standard.

Results

One hundred fifty-two were randomised for entry into the trial. One patient withdrew consent prior to treatment thus did not provide any data and was not considered further. Seventy-seven of the remaining patients were randomised to receive CDDP alone and 74 to receive CDDP plus GSH.

The demographic features of the patients in the two treatment arms are summarised in Table 1. In no instance was there substantial imbalance between the two treatment arms. The biggest difference was found with the histological grade, with 68% of classified tumours being grade III in the glutathione arm, compared to 46% in the control arm.

Considering the proportion of patients receiving six courses at any dose, then this is achieved by 39% of the CDDP alone group and 58% of the CDDP plus GSH group, with the stratified Mantel–Haenszel test yielding a statistically significant difference (P = 0.04).

Regarding the proportion of patients receiving six cycles of 100 mg/m^2 CDDP, this is summarised in Table 2. Despite substantial differences between centres, the overall success rate was 23% in those receiving GSH and 15% in those who did not.

A test of homogeneity over the trial centres showed no significant deviation from a common odds ratio (exact test for homogeneity: $P = 0.57$).

Regarding the distribution of the number of full courses of CDDP received and the distribution of the number of courses received at full or reduced dose, after stratification for centre effects, there were no statistically significant differences between the treatment groups with respect to the number of full courses received (CDDP alone: mean 3.14; CDDP plus GSH: mean = 3.61; stratified trend test: $P = 0.14$) or to the total number of courses received (means 4.38 and 4.80; $P = 0.19$). Reasons for not achieving six courses are presented in Table 3.

When the actual dose of CDDP received was considered, the difference between the two groups favoured GSH (440 mg/m^2 in the GSH group, 401 mg/m^2 in the CDDP only group) but was not significant ($P = 0.13$).

Only eighty patients had disease which enabled clinical response to be determined. There was a trend to better outcome in patients treated with CDDP plus GSH, but this was not statistically significant (stratified trend test: $P = 0.25$). Complete or partial remission was observed in 73% of the 41 evaluable patients receiving CDDP plus GSH compared to 62% in the CDDP alone arm. Surgical restaging was not required by protocol but for clinical reasons this was performed in 24 patients, generating some data on 'pathological' response.

Within this group of patients the outcome was more favourable in those patients receiving GSH, and after allowing for centre effects showed a statistically significant difference (exact stratified trend test: $P = 0.014$).

Complete remission was seen in only one of 11 patients receiving CDDP alone but in six of 13 patients who also received GSH.

Table 2. Number of patients receiving six full courses of CDDP by treatment and centre.

Centre	Six full courses			
	No		Yes	
	CDDP	CDDP + GSH	CDDP	CDDP + GSH
1	23	20	0	2
2	10	12	2	0
3	4	3	0	1
4	4	3	2	2
5	9	9	6	7
6	6	3	0	2
7	2	3	1	2
8	2	1	1	1
10	5	3	0	0
Total	65	57	12	17

Table 3 Reason for not achieving six cycles.

	CDDP	GSH
General toxicity	2	1
Disease progression	4	3
Ototoxicity	4	9
Nephrotoxicity	26	11[a]
Sudden death	1	1
Allergic reaction	0	2
Myelotoxicity	0	1
Nausea and Vomiting	7	2
Patient's decision	1	0
Ineligible	0	1
Depression	1	0
Surgery	1	0

[a] $P = 0.012$.

Quality of life assessment

Quality of life data was collected at four centres.

With respect to the HAD score, the depression scores showed a clear indication of benefit in the glutathione arm. In the 72 patients with pre- and post-treatment observations (33 CDDP; 39 GSH), the mean maximum increase in depression score was 0.8 in the GSH group compared to 2.5 in the CDDP only group. The standard error of difference was 0.9.

For an additional group in whom only post-treatment observations were available, there was a mean score of 4.4 (S.D. = 4.1) in the seven patients in the GSH group, and a mean score of 8.1 (S.D. = 4.0) in the 14 patients in the CDDP only group. Combining these estimates of the treatment differences (as described in the Statistical Plan and Evaluation) gives a pooled estimate of a difference of 2.1 units with a standard error of 0.8 units, and the difference between the two groups is statistically significant ($P = 0.015$). The anxiety scores showed no indication of differences between the two groups.

Each question in the Rotterdam Symptoms Checklist was analysed separately. Forty-five of the 47 questions had the better observed mean response in the glutathione group, when the responses were scored from 1 to 4.

Eight of these differences were statistically significant at the 5% level. These comprised the questions on nausea, vomiting, tingling hands/feet, loss of hair, short of breath, difficulty concentrating, housekeeping and shopping. The overall finding is one of improved mood in the GSH group.

Toxicity

Evaluation of toxicity is summarised in Table 4.

Body weight showed no significant changes over time in the group of patients receiving cisplatin alone. However, in those receiving glutathione there was a steady gain in weight. At the last visit of each patient there was a mean weight gain of 2 kg from pre-treatment body weight in this group. This differed significantly from the zero change in the control arm ($P = 0.010$).

Nephrotoxicity was assessed applying CTC criteria and by calculating creatine clearance. Raised creatinine levels were seen in 49% of patients receiving CDDP alone compared to 39% in those also receiving GSH.

Table 4. Maximum haematological, CTC and WHO toxicity grades.

Parameter	CDDP (Grade)					GSH (Grade)				
	0	1	2	3	4	0	1	2	3	4
Anaemia	19	31	20	6	0	17	36	18	2	0
Leucopenia	27	32	16	1	0	26	33	13	1	0
Thrombo-cytopenia	74	1	0	1	0	71	2	0	0	0
Neurohearing	8	23	27	18	1	10	20	32	11	1
Nephrotoxicity	39	32	4	2	0	45	24	5	0	0
Peripheral neurotoxicity	41	34	2	0	0	45	27	2	0	0
Nausea/vomiting	2	12	20	37	6	1	8	23	39	3

There was a statistically significant difference between the groups when the reason for not achieving six courses was analysed (Table 3). In the GSH group, 11 patients compared to 26 in the control arm did not achieve six courses of treatment due to nephrotoxicity ($P = 0.012$).

A decrease of $> 25\%$ in creatinine was seen in 42% of patients on CDDP alone, compared to 23% in those receiving GSH. This difference is statistically significant ($\chi^2 = 5.2$; $P = 0.023$).

At the follow-up visit [7] after completing six treatments, there was a statistically significant difference between the reduction in clearance for GSH treated patients when compared to control – 74% *versus* 62% ($P = 0.006$).

Neurosensory toxicity was observed in 49% of the patients treated with CDDP alone compared to 39% in those who also received GSH. Although there was a consistent trend across centres towards less toxicity with GSH, the association was not statistically significant (stratified trend test: $P = 0.22$).

There was relatively little neuromotor toxicity, but this was observed in 12% of patients treated with CDDP compared to 9% in those also receiving GSH.

Some form of hearing loss was common with only 12% not experiencing any toxicity. Grade 3 or 4 toxicity was recorded for 25% of patients receiving CDDP alone compared to 17% for those also receiving GSH, but the trend towards less toxicity in the GSH arm was not statistically significant ($P = 0.38$).

Some degree of anaemia was seen in 76% of patients. There was a tendency for the degree of anaemia to be less in patients receiving GSH. In centre 1, the association was statistically significant (exact trend test: $P = 0.041$) but a stratified trend test over all centres was non-significant ($P = 0.48$).

Sixty-four percent of patients experienced leucopenia. The differences between the treatment groups were slight and non-significant (stratified trend test: $P = 0.80$).

Thrombocytopenia occurred in only four patients, two in each treatment arm.

Performance status showed no indication of between-treatment differences.

Any co-treatment aimed at reducing toxicity must also be assessed for any potential effects to reduce efficacy of the anticancer treatment. Comparing survival of patients receiving GSH *versus* control gives a hazard ration of 0.99 (0.61–1.61) $P = 0.98$ using Cox's proportional hazards analysis. The absence of any negative effect of GSH is demonstrated in Figure 1.

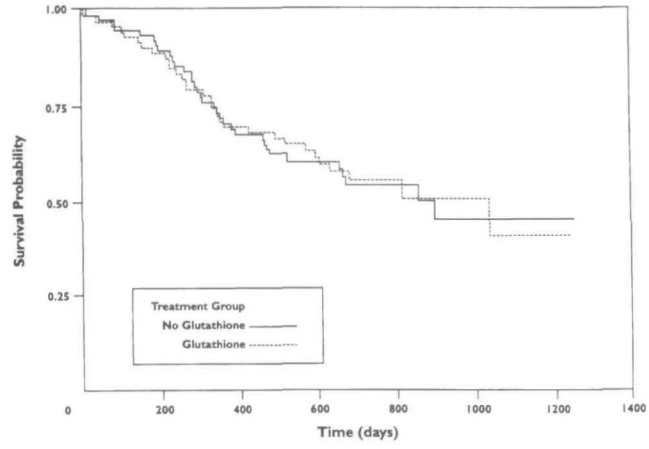

Figure 1 Survival analysis for all patients randomised.

Discussion

In this study we have demonstrated that the addition of GSH to CDDP allows six cycles of therapy to be given to more patients than giving CDDP alone, 58% *versus* 39% ($P = 0.04$). Dose reductions were still however required and if you consider only those that achieved 100 mg/m^2 CDDP × 6, although there was a trend in favour of adding GSH: 15% (12/77) in the control group and 23% (17/74) in the GSH group, the difference was not statistically significant.

It is well recognised that clinical response assessment in ovarian cancer is difficult and often impossible to assess. Reponse to therapy was a secondary variable but was only evaluable in 80 patients. The trend was again in favour of GSH (73% of 41 evaluable patients had complete or partial remission with GSH compared to 62% in the control group).

Another interesting feature was that patients in the GSH group had a steady weight gain of an average 2 kg over the trial period. This weight gain was statistically significant ($P = 0.010$).

Perhaps the most significant overall finding in this double-blind study is that patients themselves reported a clear improvement in the quality of their lives if they received GSH during CDDP treatment.

The quality of life scores demonstrate that in terms of depression, nausea, vomiting, tingling of hands/feet, shortness of breath, difficulty concentration, housekeep-

ing and shopping there was a statistically significant difference in favour of GSH.

Further work is required to define the optimal use of GSH in reducing the toxicities of platinum containing drugs, but the conclusion is that glutathione allows more cycles of cisplatin treatment to be administered because less toxicity is observed and that the quality of life is improved during treatment.

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References

1. Cancer Research Campaign Factsheets 1.3 & 3.3, 1990 and 1989 respectively.
2. Thigpen JT, Hoskins WJ. Cancer of the Ovary. Princeton: Bristol-Myers Squibb 1992.
3. Ozols RF, Young RC. Chemotherapy of ovarian cancer. Semin Oncol 1991; 18. 222–32.
4. Young RC, Knapp RC, Perez LA. Cancer of the ovary. In De Vita VT, Mellman S, Rosenberg SA (eds): Cancer: Principles and Practice of Oncology. Philadelphia: Lippincott Publishers 1984.
5. Lippman SM, Alberts DS, Slymen DK et al. Second-look laparotomy in epithelial ovarian carcinoma. Prognostic factors associated with survival duration. Cancer 1988, 61: 2571–7
6. Hamers FPT, Brakkee JH, Cavalletti E et al. Reduced glutathione protects against cisplatin-induced neurotoxicity in rats. Cancer Res 1993; 53: 544–9.
7. Cavalletti G, Minoia C, Schieppati M et al. Protective effects of glutathione on cisplatin neurotoxicity in rats. Exp Neurol (in press).
8. Di Re F, Bohm S, Oriana S et al. Efficacy and safety of high-dose cisplatin in the treatment of bulky advanced epithelial ovarian cancer. Cancer Chemother Pharmacol 1990; 25: 355–60.
9. Locatelli MC, D'Antona A, Vinci M et al. Cisplatinum (CDDP) + cyclophosphamide (CPA) + reduced glutathione (GSH) in advanced epithelial ovarian carcinoma. Sixth International Symposium on 'Platinum and other metal co-ordination compounds in cancer chemotherapy', San Diego, 23–26 January 1991.
10. Aebi S, Asserata R, Lauterburg BH. High-dose intravenous glutathione in man Pharmacokinetics and effects on cystein(e) in plasma and urine. Eur J Invest 1991; 21: 103–10.
11. Zunino F, Pratesi G, Micheloni A et al. Protective effect of reduced glutathione against cisplatin-induced renal and systemic toxicity and its influence on the therapeutic activity of the anti-tumour drug. Chem Biol Intern 1989; 70. 89–101.
12. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983; 67: 361.
13. De Haes JCJM, van Ostrom MA, Welvaart K. The effect of radical and conserving surgery on the quality of life of early breast cancer patients. Eur J Surg Oncol 1986; 12: 337.
14. Zelen M. The analysis of several 2 × 2 contingency tables. Biometrika 1971; 58. 129–37.
15. Cochran WG. Some methods for strengthening the common χ^2 tests. Biometrics 1954; 10: 417–454.
16. Armitage P. Test for linear trend in proportions and frequencies Biometrics 1955; 11· 375–86.
17. Gart J. Point and interval estimation of the common odds ration in the combination of 2 × 2 tables with fixed marginals. Biometrika 1970; 57. 417–75

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