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Biochemical Pharmacology, Vol. 37, No. 5, pp. 981–982, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

Time- and dose-dependent inhibition of erythrocyte glutathione peroxidase by cisplatin*

(*Received* 2 *June* 1987; *accepted* 15 *October* 1987)

Cisplatin-induced anemia is a well-known side effect [1] that develops in 9–40% of patients treated with this drug [2]. Acute hemolytic anemia has also been reported, although more rarely [3, 4]. Existence of a direct myelotoxic effect is an unsatisfactory hypothesis because leukopenia and thrombocytopenia are rare [2]. Direct toxicity of CDDP on red blood cells (RBS) has also been suggested. CDDP has already been shown to interact with glutathione (GSH) [5]. Because GSH and its related enzymatic system are a significant source of cellular detoxification [6], especially for RBC, we investigated the biochemical effects of CDDP on RBC enzymatic pathways.

A time and pharmacologically compatible dose study was thus performed *in vitro*, in triplicate using blood from three healthy volunteers (males aged 28, 35 and 38 yr). Study parameters were as follows: GSH [7], oxidized glutathione (GSSG) [7], 6-phosphogluconic dehydrogenase (EC 1.1.1.44) [8], hexokinase isomerase (EC 5.3.1.9) [8], glutathione peroxidase (EC 1.11.1.9, GSH Px) [9] and glutathione reductase (EC 1.6.4.2) [10]. Whole blood was incubated at 37° under slight agitation. Five ml blood samples were treated with four different doses (0, 1, 5 and 10 μg/ml). After appropriate intervals (0, 30 min, 1, 3, 6, 24 and 48 hr), the reaction was stopped by immersion in an ice bath (4°); this was followed by separation of the buffy coat, washing with one volume of saline solution, centrifugation at 4°, and rapid analysis of 6-phosphogluconic dehydrogenase, glucose-6-phosphate dehydrogenase, hexokinase, pyruvate kinase and phosphoglucose isomerase. An aliquot of erythrocytes (1 ml) was also quickly stored at −20° until analysis of GSH, GSSG, GHS Px and GSH reductase. Data were examined for dose and incubation time effects by two-way ANOVA analysis with repeated measurements.

As shown in Fig. 1, GSH Px was inhibited as a function of both time and the CDDP dose. A parallel inhibition occurred in GSH consumption and GSSG synthesis. For these parameters, ANOVA analysis revealed a highly significant effect, without interaction, for both the CDDP dose and the length of the incubation. In contrast, no significant modifications were observed in the other enzyme activities tested (Table 1).

* Supported by ARC, France (Association de Recherche sur le Cancer) Presented in part at the 1986 ESMO Congress (Nice, France) (European Society for Medical Oncology).

As shown by our data, CDDP exposure at concentrations compatible with existing pharmacokinetic information (1–10 μg/ml) [11–13] can significantly deplete RBC GSH Px activity. As GSH Px generates GSSG from GSH, concomitant inhibition of GSH consumption and GSSG synthesis is compatible with GSH Px depletion. These effects are strongly dose- and time-dependent. They are specific

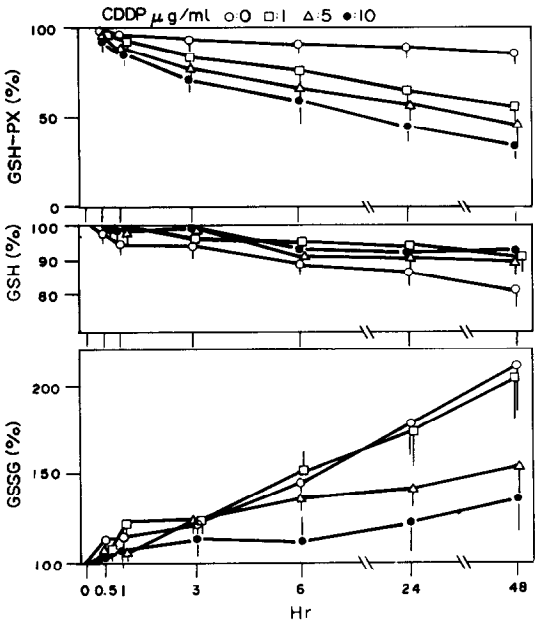

Fig. 1. Effect of incubation time and CDDP dose on GSH Px activity, GSH consumption and GSSG synthesis. Results are expressed as the percentage of values at t = 0. Two-way ANOVA analysis with repeated measurements gave the following F values for the effect of incubation time (F^6_{42}), dose (F^2_{42}) and interaction (F^{12}_{42}). For GSH Px activity: $F^6_{42} = 12.6$ ($P < 0.001$), $F^2_{42} = 10.7$ ($P < 0.001$), $F^{12}_{42} = 0.39$ NS. For GSH consumption: $F^6_{42} = 43.1$ ($P < 0.001$), $F^2_{42} = 7.79$ ($P < 0.01$), $F^{12}_{42} = 0.99$ NS. For GSSG production: $F^6_{42} = 3.67$ ($P < 0.01$), $F^2_{42} = 6.4$ ($P < 0.01$), $F^{12}_{42} = 0.89$ NS.

Table 1. RBC enzymatic activities under CDDP exposure (international units/g hemoglobin)

CDDP incubation conditions* (μg/ml)		RBC Enzymatic activities (mean, SD) G6PD	6PGD	HX	PK	GPI	GR
0 (control)	t = 0	14.0 (0.80)	8.20 (0.35)	1.13 (0.16)	12.26 (1.36)	53.50 (4.54)	8.06 (1.90)
	t = 48 hr	14.6 (1.02)	8.03 (1.10)	0.82 (0.15)	12.36 (2.23)	57.10 (7.43)	10.10 (1.51)
1	t = 0	13.5 (0.06	8.17 (0.40)	1.02 (0.16)	11.77 (0.98)	53.06 (3.72)	7.63 (1.78)
	t = 48 hr	14.5 (1.14)	7.63 (0.40)	0.68 (0.14)	11.83 (2.54)	54.76 (6.57)	9.66 (1.0)
5	t = 0	13.5 (1.45)	8.07 (0.15)	1.07 (0.33)	13.66 (4.12)	55.10 (1.57)	8.13 (0.70)
	t = 48 hr	14.2 (0.98)	7.77 (0.38)	0.78 (0.28)	11.13 (2.42)	57.23 (6.04)	10.06 (1.18)
10	t = 0	12.3 (0.30)	8.40 (0.71)	0.99 (0.26)	12.50 (2.36)	56.10 (3.84)	8.16 (0.45)
	t = 48 hr	14.9 (0.95)	7.83 (0.23)	0.70 (0.24)	11.23 (1.12)	56.16 (5.99)	10.03 (1.55)
Statistical results†		NS	NS	NS	NS	NS	NS

* Only extreme values (t = 0, t = 48 hr) have been shown to keep the table clear.
† Regression analysis for t = 0 and t = 48 hr with 0, 1, 5, 10 μg/ml.
G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconic dehydrogenase; HX, hexokinase; PK, pyruvate kinase; GPI, glucophosphoisomerase; GR, glutathione reductase.

for GSH Px, GSH and GSSG and not related to a global alteration of cell metabolism, since the other key RBC enzymes were not affected by CDDP. GSH Px inhibition might be caused by alteration of the interaction between the enzyme and its substrate GSH, because CDDP is known to bind GSH *in vitro* [15], or by a direct reaction of protein thiols with the drug. Such enzymatic inhibition could help explain the pathogenesis of CDDP-induced anemia and warrants more thorough exploration *in vivo*. Our finding that CDDP affects GSH Px activity may also have other implications equally worthy of further investigations:

Since GSH Px is a free radical detoxifying enzyme, CDDP-inhibition of this enzyme activity could account for this drug's radiosensitizing effect [14].

Additional insight might be gained concerning the neurotoxicity of CDDP, as CDDP-induced GSH inhibition may promote lipid peroxidation, especially in the nerve region. Examination of neural tissue after treatment with CDDP would be an appropriate means of determining the validity of this hypothesis.

Special attention may have to be paid to the chronology of administration of CDDP/doxorubicin combinations, because GSH Px is the main detoxification source for doxorubicin in the myocardial cell [15].

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