A Study on the Toxicity of Antineoplastic Drugs (Bleomycin, Peplomycin and *cis*-Diamminedichloroplatinum) by Simultaneous Administration (Part 1)

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(Received 27 September and accepted 2 October 1989)

Key words: semiacute toxicity, bleomycin, peplomycin,
cis-diamminedichloroplatinum

Abstract

In order to clarify the toxicity of the antineoplastic drugs, bleomycin (BLM), peplomycin (PEP) and *cis*-diamminedichloroplatinum (CDDP), which are commonly used to treat head and neck cancer by simultaneous administration, the semiacute toxicity of each of these drugs in rats was studied as an initial step. Body-weight change, general behavior, red blood cell count (RBC), white blood cell count (WBC), serum biochemistry (s-GOT, s-GPT, BUN, GLU, ALB, TP), A/G ratio, relative organ weight and histopathological features were determined. BLM and PEP given by ip administration once a week produced severe diarrhea, decreased diet consumption, emaciation, piloerection and loss of hair, enhancements of RBC and WBC, several changes in serum biochemistry, proliferation and mitosis of epithelial cells in the forestomach and occasional granular and vacuolar degeneration in the liver. CDDP administered in the same manner produced a significant decrease of WBC, several changes in serum biochemistry parameters especially BUN, an increased focal-segmental mesangial matrix in the glomeruli and vacuolar degeneration of epithelial cells of the convoluted tubule of the kidney.

Introduction

Bleomycin (BLM), a family of glycopeptide antibiotics, is an antineoplastic agent which is effective against a number of squamous cell carcinomas, such as cancers of the head and neck (including the upper jaw, tongue, lip, pharynx, larynx and other parts of the oral cavity), skin, lung, esophagus and uterus. It is also effective against lymphadenoma, gliocytoma and thyroncus.

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Peplomycin (PEP), a derivative of BLM, is also effective against a number of squamous cell carcinomas, such as cancers of the head and neck (including the upper jaw, tongue, other parts of the oral cavity, pharynx and larynx), and lung. It is also effective against prostatic cancer and lymphadenoma.

cis-Diamminedichloroplatinum (*cis*-platin, CDDP) has also been used effectively in Hodgkin's disease, lymphoma, head and neck cancer, as well as thyroid, endometrial, bladder and ovarian neoplasms. The most striking effect of CDDP is observed against testicular cancer.

In general, cancers of the head and neck are very effectively treated by irradiation and surgery, so that some have proposed that chemotherapy for cancers in this region should be done very carefully^[1]. On the other hand, a large proportion of deaths due to head and neck cancer occur through distant metastasis, such as that in cancers of the upper jaw (43%)^[2] and tongue (30%)^[1]. Therefore, it seems quite important to introduce some form of cancer chemotherapy in addition to the irradiation and surgery for the treatment of head and neck cancer.

In recent years, following the introduction of CDDP, the simultaneous use of CDDP-BLM or CDDP-PEP has been becoming common for head and neck cancer, resulting in an increased complete response rate (CR rate), which indicates that it is a sufficient therapeutic modality for head and neck cancer^[1]. KONNO et al^[2] have reported their own experiences of unsuccessfully treated cases before and after the introduction of CDDP. The failure rate before the use of CDDP was 35.3%, whereas that after CDDP decreased to 15.6%. Among these unsuccessful cases, the rate of distant metastasis before the use of CDDP was 73.3%, whereas that after CDDP was 20.0%. Thus, it was clearly demonstrated that the addition of simultaneous use of CDDP-PEP was very effective for preventing distant metastasis.

However, it is not rare for BLM and PEP to cause severe pulmonary fibrosis through pulmonitis. This has been explained by the fact that the distribution of BLM hydrolase (aminopeptidase B-like enzyme), which hydrolyzes the amide bond of beta-alanine amide to form inactive BLM-deamide, is low in the lung, skin, and their associated tumors^[3]. Thus, it is common to observe keratinization of the skin, chromatosis and alopecia among patients receiving BLM or PEP. The activity of BLM hydrolase is high in the spleen, testis, digestive tract and bone marrow^[3]. This might be the reason why BLM and PEP show low blood toxicity. As for CDDP, toxic effects on the kidney, blood and nerves (nerve octavus) are common as a dose-limiting factor^[4,5]. Moreover, toxicity in the digestive tract is seen in almost all cases^[4].

It might, therefore, be reasonable to assume that a different untoward effect on oral tissues might appear through simultaneous use of CDDP-BLM or CDDP-PEP. However, evidence for any such untoward effect has so far been lacking. TSUCHIDA^[6] studied the influence of BLM on oral tissue and demonstrated that the hydroxyproline content of the lung and especially that of the parotid gland paralleled each other. Histopathological examination also revealed that cases of severe pulmonary fibrosis had accompanying severe regressive change and fibrosis

of the stroma in the parotid gland. This indicated the possibility of predicting pulmonary fibrosis through observations of changes in the salivary gland. WAKUI^[7] demonstrated that cells with rapid proliferation were easily affected by antineoplastic drugs. Thus, squamous cells in the oral cavity and digestive tract might be affected to a certain degree, resulting in stomatitis, glossitis, esophagitis, and intraoral ulcer. He pointed out that BLM was one of the antineoplastic agents that caused such untoward effects.

The present investigation was undertaken to clarify the appearance of untoward effects due to simultaneous use of CDDP-BLM or CDDP-PEP. As a first step, each of BLM, PEP and CDDP was given ip to rats, and body-weight change, general behavior, red blood cell count, white blood cell count, serum biochemistry (s-GOT, s-GPT, BUN, GLU, ALB, TP), albumin/globulin (A/G) ratio by serum protein analysis, alterations of the A/G ratio, relative organ weight, and histopathological features of the digestive tract including the oral cavity were studied.

Materials and Methods

1. Experimental animals

Wistar male SPF rats, 5 weeks old, were purchased from Sankyo Lab. Service (produced by Japan SLC Inc.), and then grown for 1 week to obtain 24 rats with normal growth. The animal room was maintained at a temperature of $23 \pm 1^\circ\text{C}$, relative humidity of $60 \pm 5\%$, and a light-dark cycle of 12 h (7 : 00-19 : 00). Oriental MF (Oriental Yeast Co.) was given *ad libitum* as a diet together with filtered tap-water. Body weight at the initiation of the experiment was 174.8 ± 8.1 g.

2. Drugs and reagents

Bleomycin (BLM, Lot No. 7678AC09) and peplomycin (PEP, Lot No. U02010S) were kindly provided by Nippon Kayaku Co., *cis*-diamminedichloroplatinum (CDDP, Lot No. BPV73A) was kindly supplied by Bristol-Myers Co. All other reagents were purchased from Wako Pure Chemicals Inc., except where indicated.

3. Experimental methods

Rats were divided into 4 groups (6 rats/group). The first group (G1) was given BLM ip at a dose of 25 mg/kg, the second group (G2) was given PEP ip at a dose of 20 mg/kg, and the third group (G3) was given CDDP ip at a dose of 2 mg/kg. All the drugs were given ip once a week for 4 weeks. The fourth group (G4) was given physiological saline solution in the same manner and treated as a control.

During the experimental period of 4 weeks, body weight was measured once a week. Four days after administration of the drugs, 0.5 ml of blood was drawn from the jugular vein and then 0.3 ml was mixed well with 0.45 mg of EDTA-Na for measurement of red blood cell count (RBC) and white blood cell count (WBC). The residual 0.2 ml was centrifuged at $12,000 \times g$ for 30 s (Eppendorf centrifuge 5414S) to obtain serum for protein analysis by cellulose acetate electrophoresis. Additional 0.5-ml samples of blood were drawn at 0, 2 and 4 weeks after the start of the experiment and the resulting serum was subjected to biochemical analysis. General behavior of rats was observed every day during the experiment.

At termination, 4 weeks after the start of the experiment, the rats were

sacrificed by decapitation and the gingiva of the mandible, labial mucosa of the mandible, lung, thymus, esophagus, stomach, duodenum, liver, spleen, kidney, adrenal, testis, ileum and rectum were extracted and then fixed with 10% neutral formalin. Decalcification was carried out for tissues containing hard material. The samples were embedded in paraffin and then thin sections cut, followed by HE staining for histopathological examination. Relative organ weights of the liver, spleen, kidney, adrenal and testis were also determined, using the following equation: (wet tissue weight)/(body weight) x 100 (%).

RBC and WBC were measured using a Celltac MEK-4300 (Nihon Kohden Co.). Serum biochemistry, including measurements of s-GOT, s-GPT, TP, BUN, ALB and GLU, was performed using a Monocard Chemistry System (Amco Inc.). Determination of serum protein was carried out using cellulose acetate electrophoresis under the conditions described in Table 1, and then the A/G ratio was

Table 1 Analytical conditions for cellulose acetate electrophoresis

Equipment for electrophoresis: Automatic constant current and potentiostat, and a bath for electrophoresis (Jookoo)
 Membrane: Separax (Jookoo), 6x1 cm.
 Buffer: Barbitol buffer (pH 8.6, $\mu = 0.06$)
 Amount of sample: Serum, 0.4–0.6 μ l/cm
 Current: 0.8 mA/cm, 60–70 min
 Staining: Ponsour 3R solution, 90 s
 Decolorization: 1% acetic acid solution
 Transparent finish: Decahydronaphthalene
 Densitometry: Programmable densitometer ADC-20SP (Kayagaki Irika)
 wavelength 500 nm

calculated from the data obtained by densitometry. The alteration ratio compared with the value of the A/G ratio at the start of the experiment was also calculated.

4. Statistical analysis

Statistical analysis of the data was performed in the following manner: Extreme values were eliminated by Smirnov's analysis at $p_0 < 0.05$. The significance of the mean was then calculated by Student's *t* test for parametric analysis and Welch's *t* test for nonparametric analysis. The significance was expressed as observed probability (p_0).

Results

1. Body weight

The influence of BLM, PEP and CDDP on body weight is shown in Fig. 1. Significant decreases of body weight in the groups given BLM (G1) and PEP (G2) were found in comparison with the control (G4) from 1 week after the start of the experiment ($p_0 < 0.05$). In the case of CDDP (G3), a trend of lower body weight was seen from 2 weeks after the start, and was clear after 4 weeks ($p_0 < 0.10$). Thus,

Fig. 1 Influence of antineoplastic drugs, bleomycin, peplomycin, and *cis*-diamminedichloroplatinum, on body weight in rats.

○: bleomycin, 25 mg/kg

□: peplomycin, 20 mg/kg

△: *cis*-diamminedichloroplatinum, 2 mg/kg

●: intact rat

B: Measurement of body weight

D: Drug administration

Statistical significance: + $P_o < 0.10$; * $P_o < 0.05$; ** $P_o < 0.01$; *** $P_o < 0.001$

the inhibition of growth in G3 was not as great as those in G1 and G2.

2. Observations of general behavior

Diarrhea was seen in all groups except for G4 (control); it was severe in G1 (BLM) and G2 (PEP), but only mild in G3 (CDDP). Decreased diet consumption was also seen in G1, G2 and G3. Furthermore, depravation and slight alopecia were also seen in G1 and G2.

3. Red blood cell count

The influence of BLM, PEP and CDDP on RBC is shown in Table 2. A trend of increased RBC with increased age was seen in G4 (control), and the increase rate over 4 weeks was 13.6%. The increase rates over 4 weeks in G1 (BLM) and G2 (PEP), 23.4% and 29.0%, respectively, were greater than that in G4. On the other hand, G3 (CDDP) showed a tendency for a lower count (significantly lower at 3 and 4 weeks in comparison with G4) with the increase rate over 4 weeks of 10.6%.

Table 2 Effects of antineoplastic drugs, bleomycin (BLM), peplomycin (PEP), and *cis*-diamminedichloroplatinum (CDDP), on red blood cell count ($\times 10^4/\text{mm}^3$)

Group	Drug	Time after initiation of experiment				
		Before	1 Week	2 Weeks	3 Weeks	4 Weeks
1	BLM	776.3 $\pm$ 21.5	944.2 $\pm$ 25.3***	944.0 $\pm$ 28.2***	891.5 $\pm$ 76.2	957.7 $\pm$ 55.3*
2	PEP	773.8 $\pm$ 37.2	930.5 $\pm$ 71.0**	893.0 $\pm$ 46.2*	896.3 $\pm$ 106.4	998.2 $\pm$ 25.9***
3	CDDP	773.2 $\pm$ 23.4	816.5 $\pm$ 17.6	835.2 $\pm$ 14.4	838.0 $\pm$ 37.7*	855.5 $\pm$ 11.3***
4	—	787.8 $\pm$ 16.6	810.4 $\pm$ 23.1	843.0 $\pm$ 17.0	890.3 $\pm$ 13.7	894.7 $\pm$ 20.5

*, $p_0 < 0.05$, **, $p_0 < 0.01$, ***, $p_0 < 0.005$

Table 3 Effects of antineoplastic drugs, bleomycin (BLM), peplomycin (PEP), and *cis*-diamminedichloroplatinum (CDDP), on white blood cell count ($\times 10^2/\text{mm}^3$)

Group	Drug	Time after initiation of experiment				
		Before	1 Week	2 Weeks	3 Weeks	4 Weeks
1	BLM	93.3 $\pm$ 16.0	92.5 $\pm$ 8.3	110.7 $\pm$ 15.1*	112.0 $\pm$ 19.6*	107.8 $\pm$ 10.0***
2	PEP	92.5 $\pm$ 15.4	91.8 $\pm$ 8.5	112.0 $\pm$ 19.5 ⁺	103.5 $\pm$ 19.6 ⁺	97.3 $\pm$ 16.2
3	CDDP	97.7 $\pm$ 8.1	92.2 $\pm$ 7.8	80.3 $\pm$ 16.9	87.3 $\pm$ 11.4	70.8 $\pm$ 6.0***
4	—	94.0 $\pm$ 11.0	100.2 $\pm$ 2.3	92.0 $\pm$ 11.0	85.8 $\pm$ 8.3	89.7 $\pm$ 4.6

⁺: $p_0 < 0.10$, *: $p_0 < 0.05$, ***, $p_0 < 0.005$

4. White blood cell count

The influence of BLM, PEP and CDDP on WBC is shown in Table 3. Nearly the same value was found in G4 (control) during the experimental period. G1 (BLM) and G2 (PEP) showed increasing trends and the maximum values were 20.0% higher (3 weeks after the start of the experiment) and 21.1% higher (2 weeks after), respectively. However, G3 (CDDP) showed a tendency to decrease, reaching 8.7% lower at 4 weeks after the start of the experiment.

Table 4 Effects of antineoplastic drugs, bleomycin (BLM), peplomycin (PEP), and *cis*-diamminedichloroplatinum (CDDP), on blood biochemistry

Group	Drug	s-GOT KU	s-GPT KU	BUN mg/dl	GLU mg/dl	ALB g/dl	TP g/dl
Before drug administration							
1	BLM	99.0±19.5	34.5±5.0	17.8±1.9	133.3±9.0	3.48±0.36	6.86±0.08
2	PEP	84.3±21.8	33.2±8.3	17.3±0.6	132.7±8.2	3.52±0.17	6.45±0.08
3	CDDP	96.7±22.8	36.3±2.3	15.6±1.8	135.0±8.2	3.38±0.15	6.57±0.16
4	—	93.5±11.2	35.0±9.4	18.3±1.4	131.5±7.1	3.47±0.15	6.48±0.16
2 Weeks after drug administration							
1	BLM	105.2±17.0	49.2±12.9	14.2±2.0***	74.0± 6.1***	3.48±0.20*	6.67±0.35*
2	PEP	83.3±16.9*	42.0± 8.7	16.8±4.4	91.3±23.5**	3.55±0.39	6.72±0.64
3	CDDP	97.8±13.0	37.8± 4.5	18.3±1.2	122.3±11.7	3.87±0.10	7.20±0.18
4	—	114.8±20.0	38.5± 3.9	19.3±1.7	131.3±16.0	3.87±0.23	7.10±0.21
4 Weeks after drug administration							
1	BLM	96.8± 6.2***	31.0± 8.7	14.5±4.0 ⁺	93.5±22.6*	3.55±0.37*	6.52±0.36**
2	PEP	99.2±11.2***	81.0±22.4***	12.0±0.7***	88.5±19.9*	3.80±0.37	6.95±0.23
3	CDDP	80.2±11.9	27.7± 3.7	26.3±4.4***	118.2±13.9	4.07±0.26	7.20±0.25
4	—	75.2±11.8	27.5± 9.0	18.5±1.9	122.0±18.4	3.95±0.05	7.10±0.24

⁺: $p_0 < 0.10$, *: $p_0 < 0.05$, **: $p_0 < 0.01$, ***: $p_0 < 0.005$

5. Serum biochemistry

The influence of BLM, PEP and CDDP on serum biochemistry is shown in Table 4. In comparison with G4 (control), G1 (BLM) showed a significant increase of s-GOT ($p_0 < 0.005$), and significant decreases of BUN ($p_0 < 0.005$), GLU ($p_0 < 0.005$), ALB ($p_0 < 0.05$) and TP ($p_0 < 0.01$). G2 (PEP) showed significant increases of s-GOT ($p_0 < 0.005$) and s-GPT ($p_0 < 0.005$), and significant decreases of BUN ($p_0 < 0.005$) and GLU ($p_0 < 0.01$). G3 (CDDP) showed a significant increase of BUN ($p_0 < 0.005$).

6. Relative organ weight

The influence of BLM, PEP and CDDP on relative organ weight, (wet tissue weight/body weight) x 100 (%), is summarized in Table 5. Significant increases

Table 5 Effects of antineoplastic drugs, bleomycin (BLM), peplomycin (PEP), and *cis*-diamminedichloroplatinum (CDDP), on relative organ weight¹⁾

Group	Drug	Spleen	Kidney	Adrenal	Testis	Liver
1	BLM	0.215±0.017	0.360±0.017**	0.0094±0.0015***	0.692±0.053***	4.243±0.143***
2	PEP	0.227±0.025	0.415±0.025***	0.0121±0.0016***	0.755±0.063***	3.776±0.102***
3	CDDP	0.225±0.010 ⁺	0.317±0.008***	0.0084±0.0010*	0.573±0.043	3.700±0.178*
4	—	0.212±0.011	0.343±0.011	0.0074±0.0012	0.562±0.027	3.442±0.110

1) : relative organ weight (%) = ((wet organ weight)/(body weight)) x 100

⁺: $p_0 < 0.10$, *: $p_0 < 0.05$, **: $p_0 < 0.01$, ***: $p_0 < 0.005$

($p_0 < 0.005$) in the relative organ weights of the kidney, adrenal, testis and liver were found in G1 (BLM) and G2 (PEP). The difference was quite clear for adrenal and testis (G2), and liver (G1). In G3 (CDDP), the adrenal and liver weights were increased significantly ($p_0 < 0.05$). A trend of increase in spleen weight and a significant decrease ($p_0 < 0.005$) in kidney weight were also seen in G3.

Table 6 Effects of antineoplastic drugs, bleomycin (BLM), peplomycin (PEP), and *cis*-diamminedichloroplatinum (CDDP), on A/G ratio¹⁾

Group	Drug	Time after initiation of experiment					
		Before	1 Week	2 Weeks	3 Weeks	4 Weeks	
1	BLM	A/G	1.64±0.08	1.69±0.13	1.72±0.09	1.66± 0.09	1.82± 0.10
		var.	100	102.95±8.03	104.84±8.51	103.32± 2.49*	110.71± 6.87
2	PEP	A/G	1.54±0.09	1.58±0.06	1.60±0.09	1.54± 0.04	1.70± 0.11
		var.	100	102.75±9.80	104.25±6.61	102.46±10.12 ⁺	110.63±10.05
3	CDDP	A/G	1.59±0.09	1.59±0.11	1.63±0.07	1.57± 0.03	1.84± 0.13
		var.	100	99.82±8.36	102.52±6.20	100.55± 6.75*	115.72± 8.84
4	—	A/G	1.50±0.07	1.55±0.10	1.60±0.15	1.68± 0.14	1.71± 0.07
		var.	100	103.14±5.30	106.84±8.51	112.27± 6.12	117.62± 7.46

1) : Albumin/globulin ratio

var. : The value was compared with that before the experiment.

⁺ : $p_0 < 0.10$, * : $p_0 < 0.05$

7. Albumin/globulin ratio in serum and its alteration ratio

The influence of BLM, PEP and CDDP on the A/G ratio and alteration ratio relative to the value before the start of the experiment are summarized in Table 6. G4 (control) showed a trend of increase that paralleled the increase in age, and the alteration ratio at 4 weeks after the start was 17.62%. The experimental groups G1 (BLM), G2(PEP) and G3 (CDDP) showed trends of increase, although the alteration ratios for these groups were much lower than that for G4. The alteration ratios for G1 and G3 at 3 weeks after the start of the experiment were significantly ($p_0 < 0.05$) lower than for G4.

8. Histopathological findings

The most striking findings were proliferation and mitosis of epithelial cells in the forestomach (Fig. 2), occasional granular and vacuolar degeneration, and acidophil cells in the liver (Fig. 3) due to BLM and PEP treatments. An increased

Fig. 2 PEP, 20 mg/kg, once a week, ip, 4 weeks.

Proliferation and mitosis of epithelial cells in forestomach. H. E. stain, x400.

focal-segmental mesangial matrix in the glomeruli and vacuolar degeneration of epithelial cells of the convoluted tubule of the kidney (Fig. 4) due to CDDP were also observed.

Fig. 3 PEP, 20 mg/kg, once a week, ip, 4 weeks. Occasional granular and vacuolar degeneration and acidophil cells in liver. H. E. stain, x400.

Fig. 4 CDDP, 2 mg/kg, once a week, ip, 4 weeks. Increased focal-segmental mesangial matrix in glomerulus and vacuolar degeneration of epithelial cells of convoluted tubule in kidney. H. E. stain, x400.

Discussion

1. Influences of bleomycin, peplomycin and *cis*-diamminedichloroplatinum on body weight

Irrespective of differences in the manner of administration, BLM, PEP and CDDP all cause decreases in body weight^[6,8-18].

TSUCHIDA^[6] has reported maximum rates of body weight decrease of 26.6% and 23.9% in mice, after 10 or 7 days of continuous administration of BLM (10 mg/kg per day, ip or iv) and after 7 days of continuous administration of PEP (7.14 mg/kg per day, iv), respectively. TSUBOSAKI et al.^[11] have reported that continuous administration of PEP (0.3 mg/kg per day, iv) for 30 days produced no influence on the body weight of SD-strain rats, whereas PEP (0.9 mg/kg per day, ip) produced a significant body weight decrease. In a chronic toxicity study^[12], PEP (0.15 mg/kg per day, ip) for up to 40 days did not show any influence on body weight of SD rats.

In the case of CDDP, 0.024 mg/kg per day, ip did not significantly influence body weight at 10 days after the start of administration in SD rats^[14]. OHTANI et al.^[16] found that CDDP at 1 mg/kg per day, iv for 10 days, 2 mg/kg per day, iv for 5 days, and 5 mg/kg per day, iv for 2 days, produced decreases in body weight of Fisher strain rats starting from 3, 2 and 2 days after the start of administration, respectively. NOMURA^[17] studied the effect of CDDP administration at doses of 12, 18 and 24 mg/kg to C57BL/6-strain mice bearing highly differentiated squamous cell carcinoma, and found that the body weight of tumor-bearing mice decreased from 1 day after the start of administration and that the lowest body weight was observed 4-6 days after the start of the experiment.

The present experimental doses, BLM 25 mg/kg ip once a week, PEP 20 mg/kg ip once a week and CDDP 2 mg/kg ip once a week, were somewhat lower than those reported previously. Therefore, the lack of increase in body weight caused by BLM and PEP and the slight increase due to CDDP were not as clear as the results reported previously. The reason for the increased body weight in G3 (CDDP) might have been the lower dose of CDDP used as well as the difference in the strain of rat.

2. General behavior

Significant diarrhea and decreased diet consumption in G1 (BLM) and G2 (PEP) were observed. However, aspects of general behavior reported previously have included depressomotor signs, a depressive state, emaciation due to decreased diet consumption, piloerection, and loss of hair^[6,8-12]. Hardly any cases of diarrhea^[6] have been reported.

In the case of CDDP, slight diarrhea and decreased diet consumption were found, but the hair was unaffected. TSUBOSAKI et al.^[14,15] have reported that CDDP caused piloerection, urinary incontinence, bloody pituita, rachioscoliosis, loose feces, melena and emaciation due to decreased diet consumption.

Aspects of general behavior reported previously were not seen in the present investigation, probably because of the relatively mild experimental conditions, as described above. However, severe diarrhea due to BLM and PEP was noteworthy.

3. Red blood cell count

RBC was increased in both G1 (BLM) and G2 (PEP) and the rates of increase were greater than in G4 (control). G3 (CDDP) also showed an increase, but this was smaller than in G4. TSUBOSAKI et al.^[11] studied RBC 30 days after administration of PEP at doses of 0.3, 0.9, 2.7, and 8.1 mg/kg per day ip, and obtained values of 770.8 ± 18.0 , 736.7 ± 19.5 , 788.8 ± 24.5 , and $586.3 \pm 34.8 \times 10^4/\text{mm}^3$ (mean $\pm$ SE), respectively, whereas the control value was $782.1 \pm 16.7 \times 10^4/\text{mm}^3$. This indicates that PEP does not have a significant influence on RBC, even when administered at doses higher than those used here.

TSUBOSAKI et al.^[14] studied RBC 35 days after administration of CDDP at doses of 2.7, 8.0, 24.0, 72.0, and 216.0 $\mu\text{g}/\text{kg}$ per day ip, and obtained values of 732 ± 19 , 697 ± 22 , 726 ± 25 , 796 ± 11 , $699 \pm 16 \times 10^4/\text{mm}^3$ (mean $\pm$ SE), respectively, whereas the control value was $730 \pm 21 \times 10^4/\text{mm}^3$. Thus, they found no significant differences.

These results indicate that BLM, PEP and CDDP have no severe toxic effect on RBC.

4. White blood cell count

Trends of WBC increase in both G1 (BLM) and G2 (PEP) with increased values of 20.0% and 21.1%, respectively, were indicated under the present experimental conditions, whereas G4 (control) showed nearly steady values through the experiment. However, G3 (CDDP) showed a trend of decrease, the rate of decrease being 8.7%.

TSUBOSAKI et al.^[11] studied WBC 30 days after administration of PEP at doses of 0.3, 0.9, 2.7 and 8.1 mg/kg per day ip, and obtained values of 90.4 ± 4.4 , 98.4 ± 5.8 , 112.8 ± 13.1 , and $123.7 \pm 13.1 \times 10^2/\text{mm}^3$ (mean $\pm$ SE), respectively, whereas the control value was $81.1 \pm 5.3 \times 10^2/\text{mm}^3$. Thus, WBC increased in relation to the increased dose of PEP. Moreover, among the white blood cells, the number of neutrophils and the neutrophil ratio increased, eosinophils and monocytes did not change, and lymphocytes decreased in relation to the increased dose of PEP.

TSUBOSAKI et al.^[14] studied WBC 35 days after administration of CDDP at doses of 2.7, 8.0, 24.0, 72.0 and 216.0 $\mu\text{g}/\text{kg}$ per day ip, and obtained values of 90 ± 8 , 95 ± 7 , 117 ± 8 , 85 ± 10 and $90 \pm 4 \times 10^2/\text{mm}^3$ (mean $\pm$ SE), respectively, whereas the control value was $114 \pm 10 \times 10^2/\text{mm}^3$. BODENNER et al.^[19] administered CDDP to B6D2F₁-strain mice and found a decrease of WBC and a tentative decrease in the lymphocyte/neutrophil ratio, followed by an increase.

In general, the hemotoxicities of BLM and PEP are very low, and CDDP shows weak toxicity^[20], in agreement with the data obtained in the present investigation.

5. Serum biochemistry

The present investigation revealed a significant increase of s-GOT and significant decreases of BUN, GLU, ALB and TP in G1 (BLM); significant increases of s-GOT and s-GPT, and significant decreases of BUN and GLU were found in G2 (PEP). These data indicate that hepatic dysfunction might occur due to BLM or PEP. The significant increase of BUN due to CDDP administration indicates that CDDP might cause nephropathy.

Ito et al.^[10] found increases in BUN and CRE due to PEP, indicating that PEP had strong nephrotoxicity. TSUBOSAKI et al.^[11] studied the semiacute toxicity of PEP and found that CRE, BUN and T-CHO were increased at a high dose of PEP. In a chronic toxicity study^[12], an increase of BUN was also demonstrated, indicating nephrotoxicity of PEP. In the present investigation, hepatotoxicity was found, but hardly any nephrotoxicity. The significant decrease of BUN might have resulted from reduced hepatic function. Because of the decreased diet consumption and diarrhea, a lower GLU value was obtained.

TSUBOSAKI et al.^[14] also found in a semiacute toxicity study that increases of T-CHO, BUN and CRE, and decreases of GPT and TP occurred due to CDDP, indicating nephrotoxicity. In a chronic toxicity study^[15], increases of T-CHO, BUN and CRE, and a decrease of LDH have been reported at 13 weeks after the start of administration. The present data also showed a significant increase of BUN due to CDDP, indicating possible nephrotoxicity.

6. Relative organ weight

Significant increases in the relative organ weights of the kidney, adrenal, testis and liver due to BLM and PEP were demonstrated in the present investigation. Significant increases in the relative organ weights of the adrenal and liver, and a significant decrease in the relative organ weight of the kidney due to CDDP were also found.

TSUBOSAKI et al.^[11] reported that the relative organ weights of the lung, spleen, kidney, brain, thyroid, adrenal, pancreas, testis and hypophysis were increased in accordance with the increased dose of PEP. In the present investigation, increases in relative weight for all organs were demonstrated. This might be explained by the fact that the significant decrease of body weight due to BLM and PEP occurred through loss of fat and muscle, and not a reduction of internal organ weight.

TSUBOSAKI et al.^[14] reported increased relative organ weights for the lung, liver, kidney, brain, adrenal, thyroid and testis, and decreased relative organ weights of the thymus and prostate due to CDDP at high dose.

7. Albumin/globulin ratio in serum and its alteration ratio

Trends of increase in the A/G ratios of G1 (BLM), G2 (PEP) and G3 (CDDP) were found in the present investigation. However, the increase rates in these groups were lower than that in G4 (control).

ITO et al.^[10] carried out a semiacute toxicity study of PEP and reported A/G ratios of 1.42 and 1.68 at PEP doses of 130 and 169 mg/kg ip, respectively, at the 40th day. These values were lower than that in the control group, 1.71 ± 0.23 . TSUBOSAKI et al.^[11] also reported a lower A/G ratio, 2.06-2.16, in the experimental group given PEP than in the control, 2.20 ± 0.07 , in a semiacute toxicity study of PEP.

TSUBOSAKI et al.^[14] reported that the A/G ratio was decreased by CDDP to 1.98-2.07, whereas the control value was 2.08 ± 0.03 . OHTANI et al.^[16] reported A/G ratios of 0.89 ± 0.04 for CDDP 1 mg/kg x 10, 0.89 ± 0.12 for CDDP 2 mg/kg x 5, and 0.71 ± 0.10 for CDDP 5 mg/kg x 2, whereas the control value was 0.93 ± 0.03 . Thus, it is reasonable to consider that the A/G ratio is decreased by CDDP.

In general, the A/G ratio in rats has been reported to be 1.43 for animals 9-13

weeks old^[21]. The present rats were 7-11 weeks old during the experimental period. However, the present data are somewhat higher than those reported previously, but the data lie within a reasonable range.

8. Histopathological findings

In the present investigation, proliferation and mitosis of epithelial cells in the forestomach, occasional granular and vacuolar degeneration and acidophil cells in the liver were found following treatment with BLM and PEP.

TANAKA et al.^[9] found a moderately thickened capsule in rat liver following treatment with BLM oil suspension 59 mg/kg, ip, 14 days after treatment in an acute toxicity study. Tsubosaki et al.^[11] studied the semiacute toxicity of PEP in rats and demonstrated lung congestion (85-100%), perihepatitis (65-75%), perisplenitis (15-35%), anemia of the kidney (10-20%), hydropic change in the pancreas (5-20%), atrophy of the thymus (15-20%) and pleural effusion (35-65%) in a group given PEP at 24.3 mg/kg. The appearance rates of these abnormalities decreased as the dose was reduced, and no specific abnormal finding was observed at a dose of 0.3 mg/kg. Tsubosaki et al.^[12], in a chronic toxicity study of PEP 2.4 mg/kg after 90 ip doses, demonstrated proliferation of bile ducts in the liver, hemosiderin deposition in the spleen, flattening, necrosis and degeneration of the tubular epithelium in the renal cortex, and edema, fibrosis and focal cell infiltration of the renal interstitium. Because of the limited period employed, 4 administrations in 28 days, not many such abnormal findings were obtained in the present investigation. However, proliferation and mitosis of epithelial cells in the forestomach were noteworthy.

ITO et al.^[13], in an acute toxicity study of CDDP, demonstrated the occurrence of degeneration and desquamation of the tubular epithelium, dilatation of the distal tubules, regenerative change in the proximal tubules, hyaline degeneration in the proximal tubular epithelium of the kidney, regenerative change in the mucosal epithelium, necrotic debris in regenerative foci of the small intestine, atrophy of the white pulp in the spleen, marked hypoplasia of the bone marrow and a decrease of spermatogenesis in the testis. Tsubosaki et al.^[14], in a semiacute toxicity study of CDDP, demonstrated the occurrence of dilated tubules in the outer renal cortex and flattened tubules in the corticomedullary junction, appearance of basophilic tubular epithelium and degeneration of the proximal tubules, edema and fibrosis in the renal interstitium, alteration and atrophy of the mucosa in the small intestine, hypoplasia of the bone marrow, atrophy of the testis and atrophy of the malpighian corpuscles in the spleen.

In the present investigation, an increased focal-segmental mesangial matrix in the glomeruli and vacuolar degeneration of the epithelial cells in the convoluted tubule of the kidney were observed.

9. Toxicity of simultaneous administration

In this investigation, only a single drug was administered in order to obtain basic data and for comparison with results reported previously. In the next step, we intend to investigate the simultaneous administration of BLM-CDDP and PEP-CDDP to find whether any additional untoward effect can be detected.

Conclusions

Antineoplastic drugs used widely for treatment of head and neck cancer, bleomycin (BLM), peplomycin (PEP) and *cis*-diamminedichloroplatinum (CDDP), were administered ip once a week at doses of 25, 20 and 2 mg/kg, respectively, for 4 weeks. Body-weight change, general behavior, red blood cell count (RBC), white blood cell count (WBC), serum biochemistry (s-GOT, s-GPT, BUN, GLU, ALB and TP), A/G ratio by electrophoresis and the alteration ratio, relative organ weight, and histopathology were examined to investigate the toxicity of these drugs. The following results were obtained:

1. In the groups given BLM and PEP, decreased diet consumption, significant reduction of body weight, severe diarrhea, and emaciation were observed. In the group given CDDP, a slightly inhibited body-weight increase and loose feces were seen.
2. In the groups given BLM and PEP, piloerection and loss of hair were observed.
3. In the groups given BLM and PEP, both RBC and WBC were increased. In the case of CDDP, an inhibition of increase in RBC and a significant decrease of WBC were observed.
4. Serum biochemistry revealed a significant increase of s-GOT and significant decreases of BUN, GLU, ALB and TP in the group given BLM, significant increases of s-GOT and s-GPT and significant decreases of BUN and GLU in the group given PEP, and a significant increase of BUN in the group given CDDP.
5. In the groups given BLM and PEP, relative organ weights of the kidney, adrenal, testis and liver were significantly increased, but in the group given CDDP, those of the adrenal and liver were significantly increased and that of the kidney was significantly decreased.
6. A/G ratios in all groups were increased, but were lower than in the control.
7. Proliferation and mitosis of epithelial cells in the forestomach and occasional granular and vacuolar degeneration and acidophil cells in the liver were seen in the groups given BLM and PEP. An increased focal-segmental mesangial matrix in the glomeruli and vacuolar degeneration of the epithelial cells of the convoluted tubule of the kidney were observed in the group given CDDP.

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