Scanning Microscopy

Volume 6 | Number 4 Article 13

10-2-1992

Response of the Esophageal Epithelium to Concomitant Cis-Dichlorodiammineplatinum(II) and Radiation Treatment. An Electron Microscopic Study in Rabbits

M. Albertsson
University Hospital

M. Cwikiel
University Hospital

C. H. Håkansson
University Hospital

M. Palmegren
University Hospital

Scanning Microscopy, Vol. 6, No. 4, 1992 (Pages 1023-1034)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/92$5.00+.00

RESPONSE OF THE ESOPHAGEAL EPITHELIUM TO CONCOMITANT CIS-DICHLORODIAMMINEPLATINUM(II) AND RADIATION TREATMENT. AN ELECTRON MICROSCOPIC STUDY IN RABBITS

M. Albertsson*, M. Cwikiel, C.H. Håkansson and M. Palmegren

Department of Oncology, University Hospital, S-22185 Lund, Sweden.

(Received for publication May 10, 1992, and in revised form October 2, 1992)

Abstract

The rabbit esophageal mucosa was irradiated with daily fractions of 2 Gy up to an accumulated dose of 20 Gy (total dose 2, 6, 10. 16 or 20 Gy). Fifteen to forty-five minutes before the start of each irradiation 0.3 mg Cis-dichlorodiammineplatinum (cis-DDP, cisplatinum) was given by intraperitoneal injection to each rabbit. Examinations were carried out 1-10 days after each fractionation schedule, when specimens were taken for morphological investigations.

Scanning electron microscope (SEM) examination showed a gradual development of damage with cell loss and structural disarrangement of the microridges and whorls on the surface. However, with further treatment the esophageal mucosa exposed to cis-DDP and radiation normalized faster and more complete compared to the esophageal part exposed to cis-DDP alone. The difference may depend on an accelerated proliferation in the part of the trachea that is exposed to a combined treatment.

Key words: Esophagus, mucosal epithelium, cis-dichlorodiammineplatinum(II) (cis-DDP), cisplatinum, light microscopy, scanning electron microscopy, transmission electron microscopy.

*Address for correspondence:
Maria Albertsson
Department of Oncology
University Hospital
S-221 85 Lund Sweden
Phone No.: Int + 46 (40) - 331308
Fax No.: Int + 46 (40) - 336231

Introduction

It has been a time-honoured axiom in cancer therapy that the two principal treatment modalities, radiotherapy and chemotherapy should not be given simultaneously but staggered to ensure that the side effects of treatment are tolerable. In cases of inoperable squamous cell carcinoma of the head and neck, the esophagus or the lungs, for many years, the principle treatment has been radiotherapy, which at best has yielded brief palliation and where the patient has generally died of local uncontrolled tumour. When cis-dichlorodiammineplatinum(II), (cis-DDP, cisplatinum) was introduced into clinical treatment and found to be effective against squamous cell cancer, a new weapon was added to the therapeutic arsenal. In addition, it was found to have radiosensitizing properties, to affect repair processes both of potentially lethal and sublethal radiation damage [PLRD and SLRD, respectively, (Douple and Richmond, 1979; Dritschilo *et al.*, 1979; Luk *et al.*, 1979)], and to sensitize both hypoxic and non-hypoxic cells (Richmond and Powers, 1976; Richmond *et al.*, 1977). Cis-DDP has also been shown to inhibit G1 to S phase transition (Szumiel and Nias, 1976). A number of phase II trials have been published where cis-DDP given concomitantly with fractionated radiotherapy was shown to result in improved local control and survival (Coughlin and Richmond, 1985; Al Sarraf *et al.*, 1987; Forastiere *et al.*, 1990).

In some studies, however, concomitant treatment has been shown to be associated with increased toxicity to the normal tissue surrounding the tumour, and to constitute a limiting factor. As the optimal approach to the combination of cis-DDP and radiotherapy remains to be established, we have launched a series of animal studies in the rabbit, where irradiation of the superior mediastinum is combined with concomitant cis-DDP treatment at various dosages. Acute effects in the trachea and esophagus have been investigated, and the results published (Albertsson *et al.*, 1986, 1987, 1990, 1991; Albertsson and Håkansson, 1988).

The aim of the present study was to observe the pattern of cellular damage and effects on repair and proliferation processes in the esophagus, and if possible to

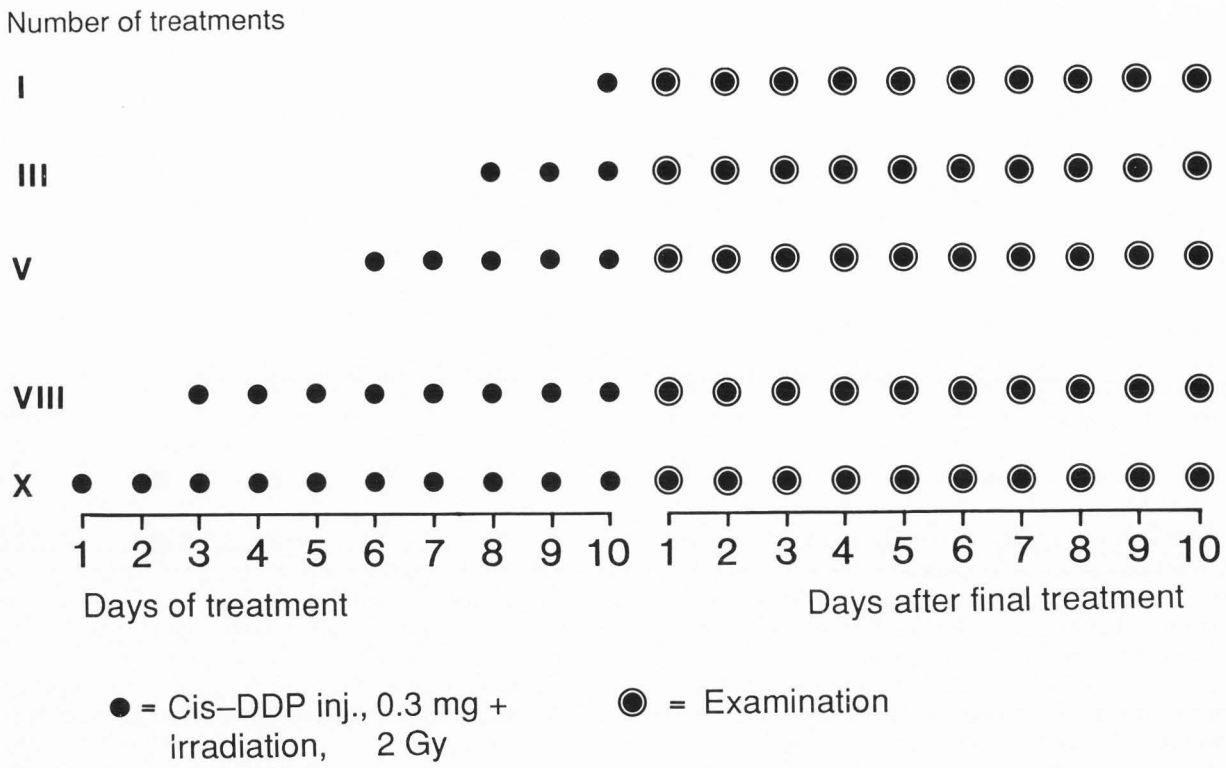


Figure 1. Schedule for combined treatment with cis-DDP and radiation. The drug was given in a dose of 0.3 mg, 15-30 minutes before each irradiation, total dosages ranged from 0.3 mg cis-DDP + 2 Gy to 3.0 mg cis-DDP + 20 Gy. Experiments were carried out from day 1 to day 10 after the completion of treatment.

elucidate the interactive mechanism(s) of action of the cis-DDP and ionizing radiation combination.

Therefore, cis-DDP was given daily as a sensitizer prior to each radiation treatment, at total dosages of 0.3 mg (2 Gy) to 3 mg (20 Gy); and light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) being performed 1-10 days after the completion of treatment.

Material and Methods

Animals

Sixty full grown rabbits weighing about 2.0 kg were selected for the study. Fifty received a combination of cis-DDP and radiation according to the schedule presented in Fig 1. Ten animals acted as controls.

Drug

Cis-dichlorodiammineplatinum(II) (cis-DDP, cisplatinum), (Platinol®, Bristol Myers Company), was dissolved in isotonic saline at a concentration of 0.5 mg/ml.

Radiation

Radiation was delivered by a Siemens X-ray machine operating at 160 kV X-ray, filtered by 4 mm Al, at a focus-skin distance of 50 cm, giving an absorbed dose of 2 Gy to 2 cm of the esophagus just beneath the larynx. The absorbed dose in the esophagus was controlled by thermoluminescent dosimetry. Fifteen mm beyond the caudal part of the irradiated area, the absorbed dose was < 0.05 Gy. The distance between the irradiated and non-irradiated area was 40 mm. The time interval between fractions was 24 hours.

Experiments

Each rabbit was anaesthetized by intraperitoneal (i.p.) injection of pentobarbital (40 mg per kg body weight) before the administration of irradiation.

The rabbits were exposed to fractionated irradiation (2 Gy/F), with a total cumulative dose ranging from 2-20 Gy. Fifteen to forty-five minutes before each irradiation, each animal was given 0.3 mg cis-DDP intraperitoneally according to the schedule shown in Fig. 1. The rabbits were then laid on their backs and the upper part of each esophagus (20 mm) was irradiated. The animals were treated in groups of ten. After completion of radiation, one animal was removed from the group on each of the ten consecutive days and sacrificed by a blow to the skull (in order to avoid pharmacological side effects). The esophagus was dissected out in its entire length (7-8 cm). Samples for SEM, TEM and LM were taken from the upper part of the esophagus (irradiated area: E1), and the lower part of the esophagus (E2). Control investigations were also performed in the same way on untreated animals.

Preparations for SEM

The specimens for SEM examination were not rinsed. They were fixed in 2.5% glutaraldehyde (in 0.15 M cacodylate buffer, pH of solution = 7.3) for 12

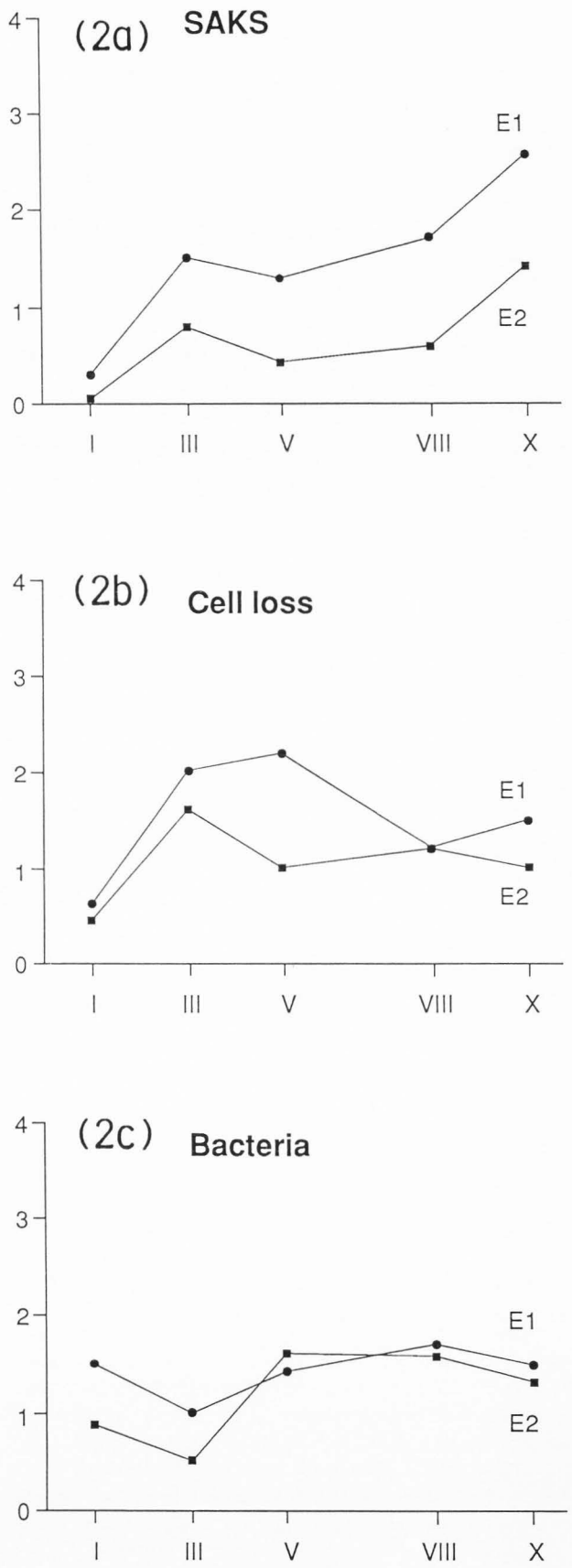


Figure 2. Scores (determined from SEM micrographs) by dosage. The range of scores was from 0 (normal) to 3 (maximum abnormality). Each point on the graphs represents all ten values for each dosage group, as assessed by three independent raters. The Roman numerals denote number of days after treatment, the dosages being: I = 0.3 mg cis-DDP + 2 Gy; III = 3X (0.3 mg cis-DDP + 2 Gy); V = 5X (0.3 mg cis-DDP + 2 Gy); VIII = 8X (0.3 mg cis-DDP + 2 Gy); X = 10X (0.3 mg cis-DDP + 2 Gy). **Fig. 2a** shows damaged microridges looking like small knobs or snakes (S.A.K.s), **Fig. 2b** shows cell loss, and **Fig. 2c** shows bacteria.

---

hours. They were then transferred into the same buffer, and were later osmium-fixed in 1% osmium tetroxide in 0.15 M cacodylate buffer for two hours. After dehydration with graded series of ethanol, the preparations were transferred to Freon TF 618. The specimens were later critical point dried in Balzer 000 critical point drier. They were then sputter coated with gold plus palladium in a Polaron E 5000 coating unit, and examined in a Philips 515 SEM operating at 20 kV.

Preparation for TEM

The sample were fixed, treated with osmium tetroxide, and dehydrated in ethanol in the same manner as for SEM preparations. The sample were then embedded in Vestopal W or Epon. Ultrathin sections were cut out and stained with lead citrate and uranyl acetate, and examined in JEOL 2000X TEM.

Scoring system

The scoring system for detached microridges was based on the number as calculated from SEM (at 5000X) in an area of 17 X 11 cm; score 0: 0-50; score 1: approximately 100; score 2: approximately 200; and score 3: greater than 250. The score for cell loss was based on the number of cell flakes in an area of 17 x 11 cm on a SEM micrograph (at 100X); score 0: less than 50; score 1.5: 1-100; score 2: 101-150; score 3: greater than 150. The score for bacteria was based on the number as calculated from SEM micrographs (at 1000X) in an area of 17 X 11 cm; score 0: 0-25; Score 1: approximately 200; score 2: approximately 400; score 3: approximately 600. The scores are shown in Fig. 2.

Measurement of epithelial thickness

Epithelial thickness (in 1/100 mm) was measured with a Leitz 12.5X micrometer ocular. From each animal 8-10 sections were investigated. Owing to undulations of the epithelium, 20 measurements were made at various sites for each animal.

Statistical analysis

Statistical analysis was done with regression analysis.

Results

In 10 control animals, the esophagus was examined with LM, SEM and TEM both at the upper end at

a level just below the hypopharynx (E1, i.e., that region in the treatment group which was exposed to the combined treatment), and at its lower end (E2), the distance between E1 and E2 being at least 4 cm. In controls, no difference in ultrastructure was found between the regions E1 and E2. In the treatment group, an initial thickening of the mucosa was found in the low dose range, a thickening visible at SEM where the oedema manifested itself as a coarse pattern of smoothed out microridges (Fig. 3).

SEM of normal esophageal mucosa showed flat, polygonal epithelial cells joined by discrete cell lines, with numerous microridges arranged in striking patterns that varied from cell to cell. Occasionally microridges had become detached, forming nodular or serpentine protrusions, like small knobs or snakes on the surface (S.A.K.'s) (Figs. 4-6), though this was rare in normal cases, and in no case did the number of damaged microridges on a determined area exceed 50. Superficial cellular shedding is a normal part of the regeneration and desquamation processes, and in the present normal controls ~ 50 detached cells were seen on a determined area. After treatment with cis-DDP and fractionated radiation, superficial damage was manifest in the form of damaged microridges and increased cell loss. The preparations were rated and the scoring results are presented in Fig. 2. Damaged microridges were found in the whole series, though more prominently in that part of the esophagus exposed to the combined treatment. Detachment from superficial microridges manifested itself in its various forms, the earliest visible effect being the swelling of the microridges (Fig. 4). The swelling was occasionally so great that the microridges assumed a club-like form (Fig. 4), though usually only a small part became detached, protruding in a nodular fashion (Fig. 5). Subsequent detachment involved the greater part of the microridges which then assumed serpentine configurations (Fig. 6), often coiling around surface bacteria (Figs. 6 and 7). Each preparation was scored for cell loss, the number of detached flakes being counted for a given area, the results for E1 and E2 being shown in Fig. 2. A certain amount of cellular shedding was regularly seen as the result of normal regeneration of the esophageal epithelium. Cell loss was somewhat increased both in the area exposed to combined treatment and in the E2 region. Occasionally the detachment was seen to have occurred in chunks apparently involving more than one cell layer and resulting in superficial epithelial dehiscence (Fig. 8).

TEM of normal esophageal mucosa showed a basal columnar layer comprising approximately 10 cell layers (Albertsson *et al.*, 1990), an intermediate layer comprising 10-20 layers of polygonal cells increasingly flattened toward the lumen, and an apical layer comprising 10-20 layers of cells with their long axes parallel to the surface and with pyknotic nuclei. In the treated material, counts were made both of the total number of cells and the number of basal cells. The results showed both (the total and basal) cell counts to be somewhat (i.e., non-significantly) reduced during the study period in dosage groups 6 Gy and 10 Gy. Subsequently, in dosage groups 16 and 20 Gy, both total and basal cell counts manifested a tendency to increase, the values at 20 Gy being higher than those at 2 Gy. As no significant intragroup difference could be found during the 10-day observation period, group values are given as group means. In Fig. 9, the phenomenon is shown in the form of TEM micrographs. Both (total and basal) counts were greater in the high dosage range than in the low range, the preparations with the greatest values being the group exposed to 20 Gy. Moreover, both the basal and total counts were greater for that part of the esophagus exposed to combination treatment (E1) than in the lower esophagus treated with cis-DDP alone (E2), as shown in Fig. 10 where TEM micrographs of material from the same rabbit are compared. Bacteria were occasionally seen on the epithelial surface, but did not penetrate the superficial cell layer. Counts were made, but though they varied widely from one preparation to the other, no unequivocal relationship was found between bacterial count and either radiation or cisplatinum dosage.

Figures 3-8. Scanning electron micrographs.

Figure 3. Note the swollen surface, with microridges squeezed up together with little space between them; many microridges are damaged and have become detached like small knobs or snakes (S.A.K.s, arrows), and large quantities of bacteria have adhered to the mucosa (arrow). 16 Gy + 0.9 mg cis-DDP 6 days after completion of treatment.

Figure 4. Swollen microridges, one of which is distended like a balloon (arrow). 6 Gy + 0.9 mg cis-DDP 6 days after completion of treatment.

Figure 5. Swollen detached microridges protruding from the surface as small nodules (arrows). 6 Gy + 0.9 mg cis-DDP 7 days after completion of treatment.

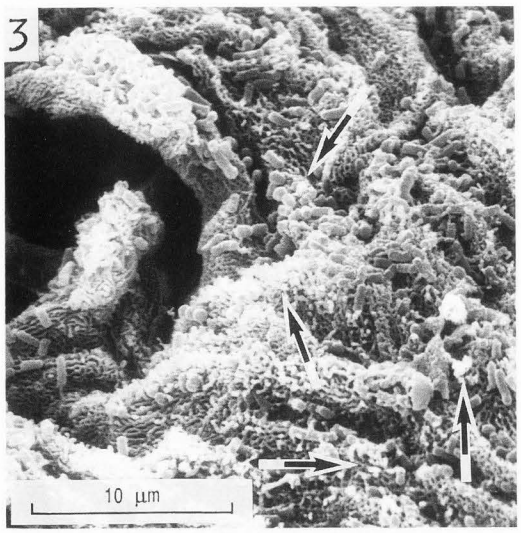
Figure 6. Serpentine detached microridges that even coil around surface bacteria (arrows). 10 Gy + 1.5 mg cis-DDP 8 days after completion of treatment.

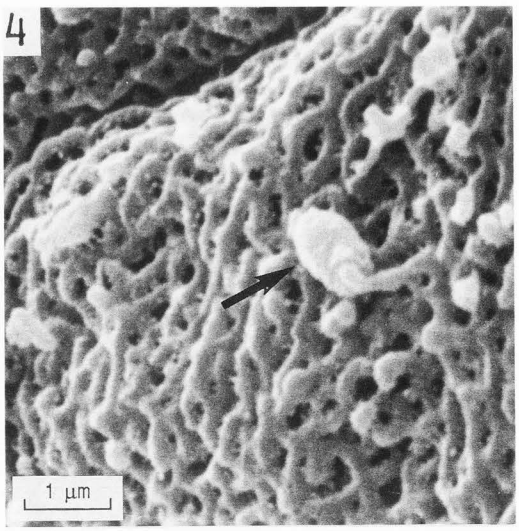
Figure 7. Long detached microridges coiled around bacteria (arrows). 10 Gy + 1.5 mg cis-DDP 8 days after completion of treatment.

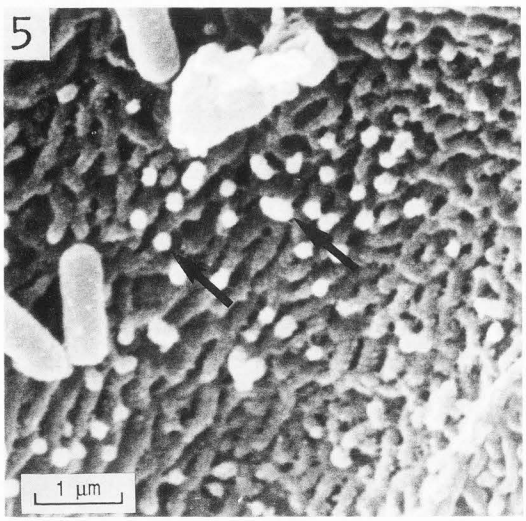
Figure 8. Cell damage and cell loss (arrow). 20 Gy + 3.0 mg cis-DDP 3 days after completion of treatment.

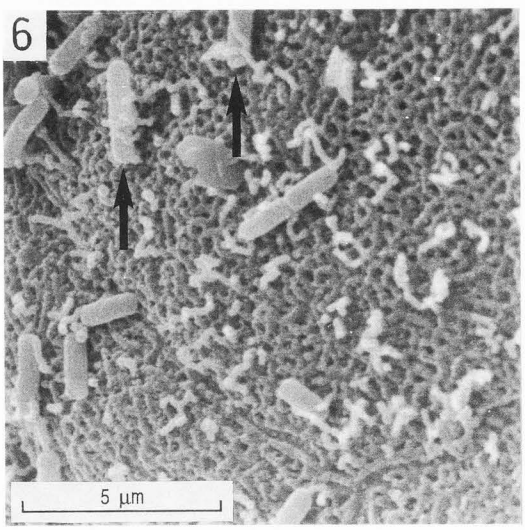
Epithelial thickness or the height of esophageal mucosa

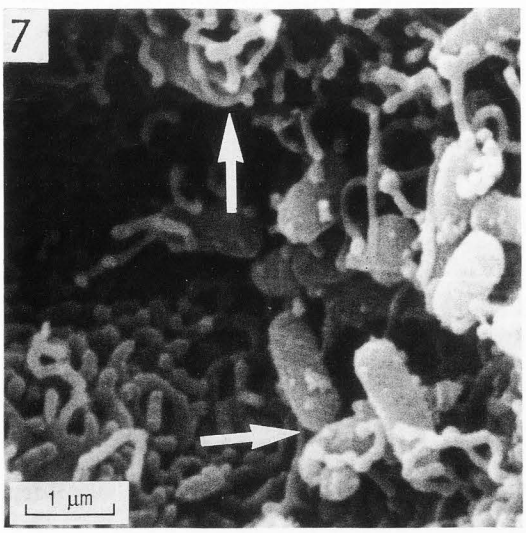
In the control and normal animals the height of the esophageal mucosa was about the same in the upper part (131 μm) as compared to the lower part (127 μm). A difference in epithelial thickness was found between E1 and E2 in the treated animals (Fig. 11). In Table 1, the measured values of the epithelial height are presented day by day. Since no significant time variation was observed in any of the groups, all values for each group are collected in one point (Fig. 12). In this figure, the

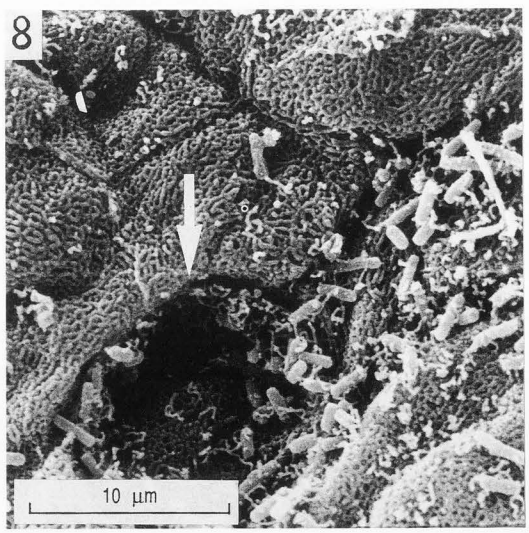

3
10 μm


4
1 μm


5
1 μm


6
5 μm


7
1 μm


8
10 μm

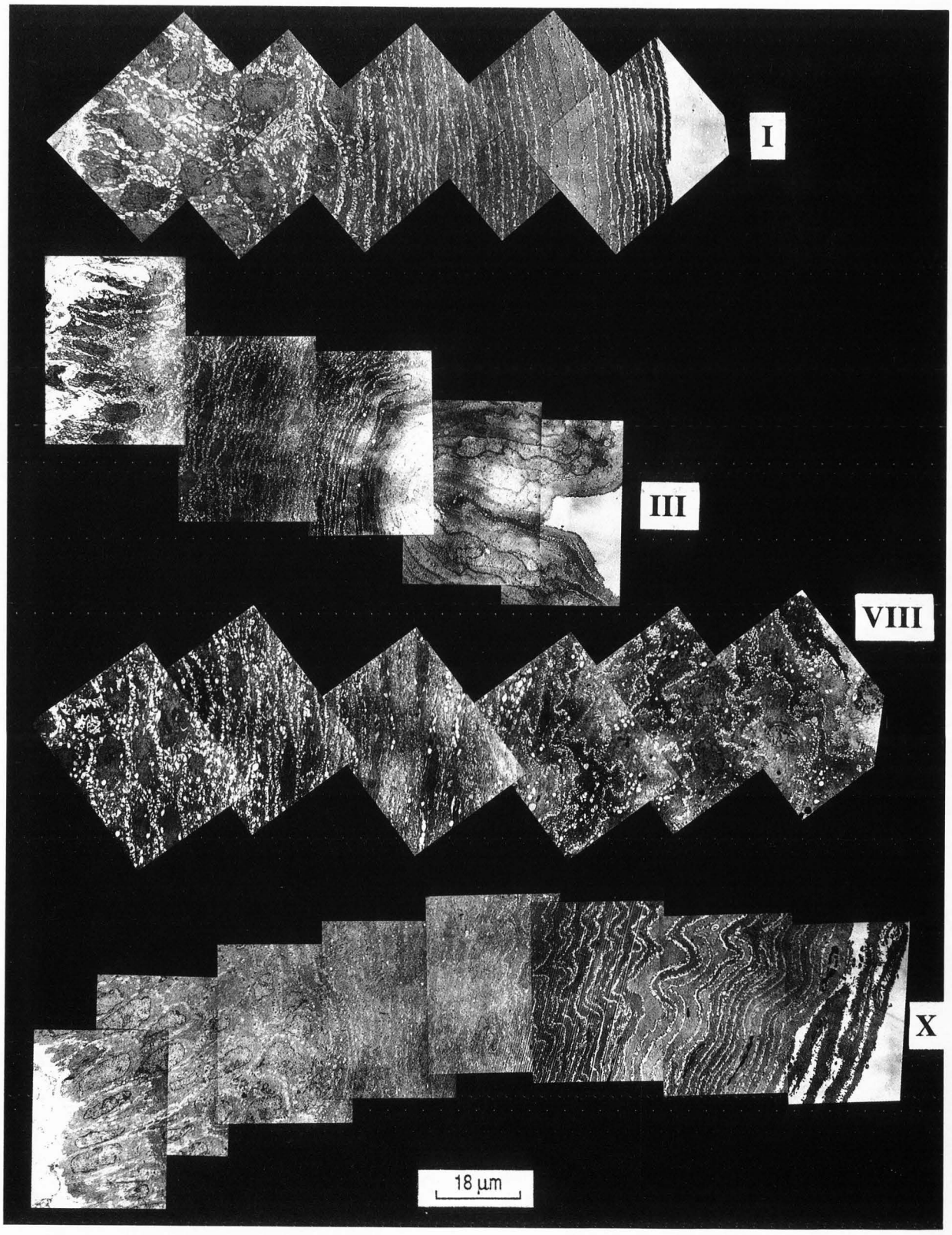


Figure 9. Comparative montage of TEM micrographs, showing the appearance of the entire cell layers. Roman numerals denote number of days after treatment, the dosages being as listed under Fig. 1. Bar = 18 μm.

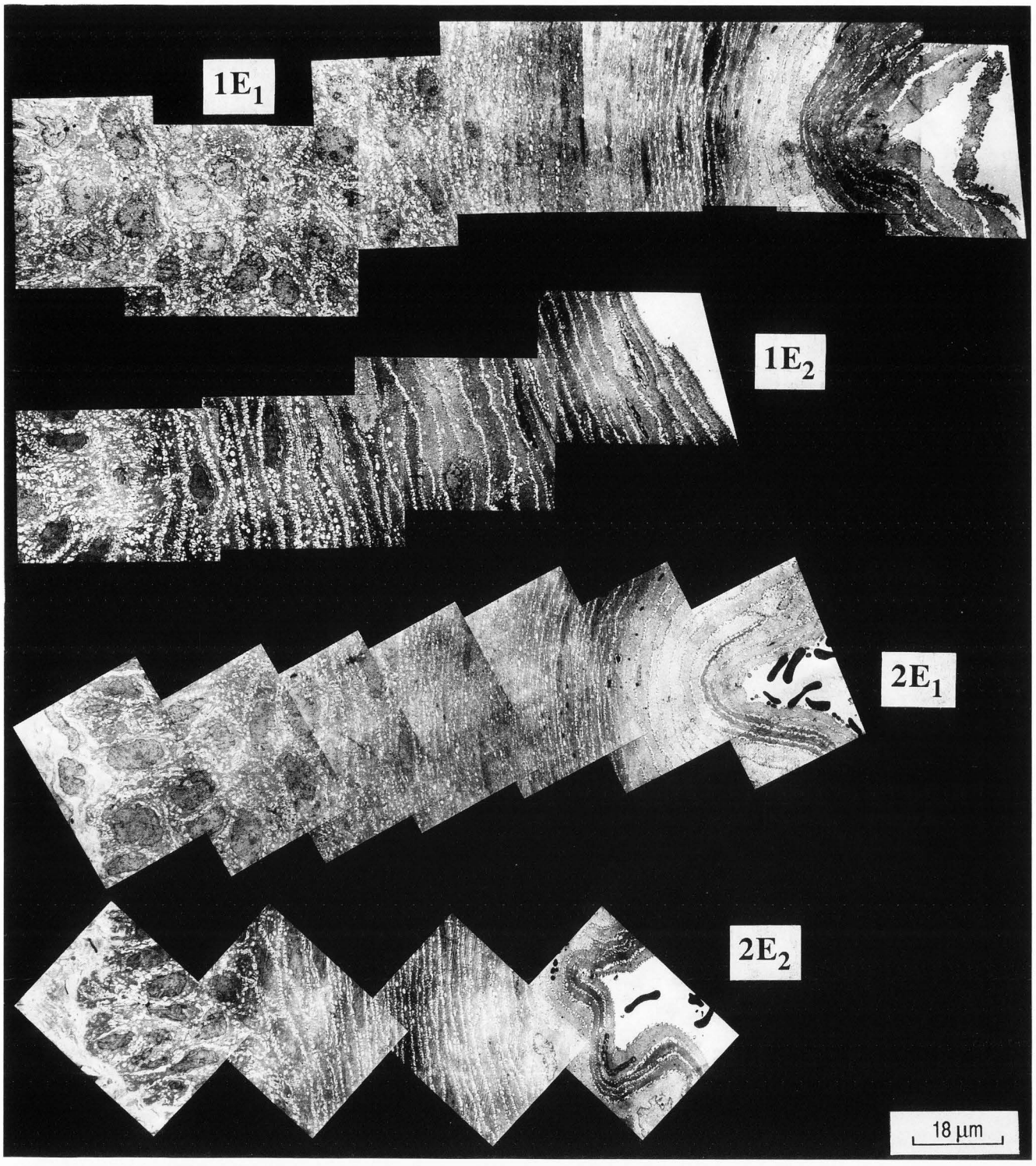


Figure 10. Montage of TEM micrographs, comparing E1 and E2 from the same animals. E1 > E2, both with regard to total cell count and proportion of basal cells. Bar = 18 μm.

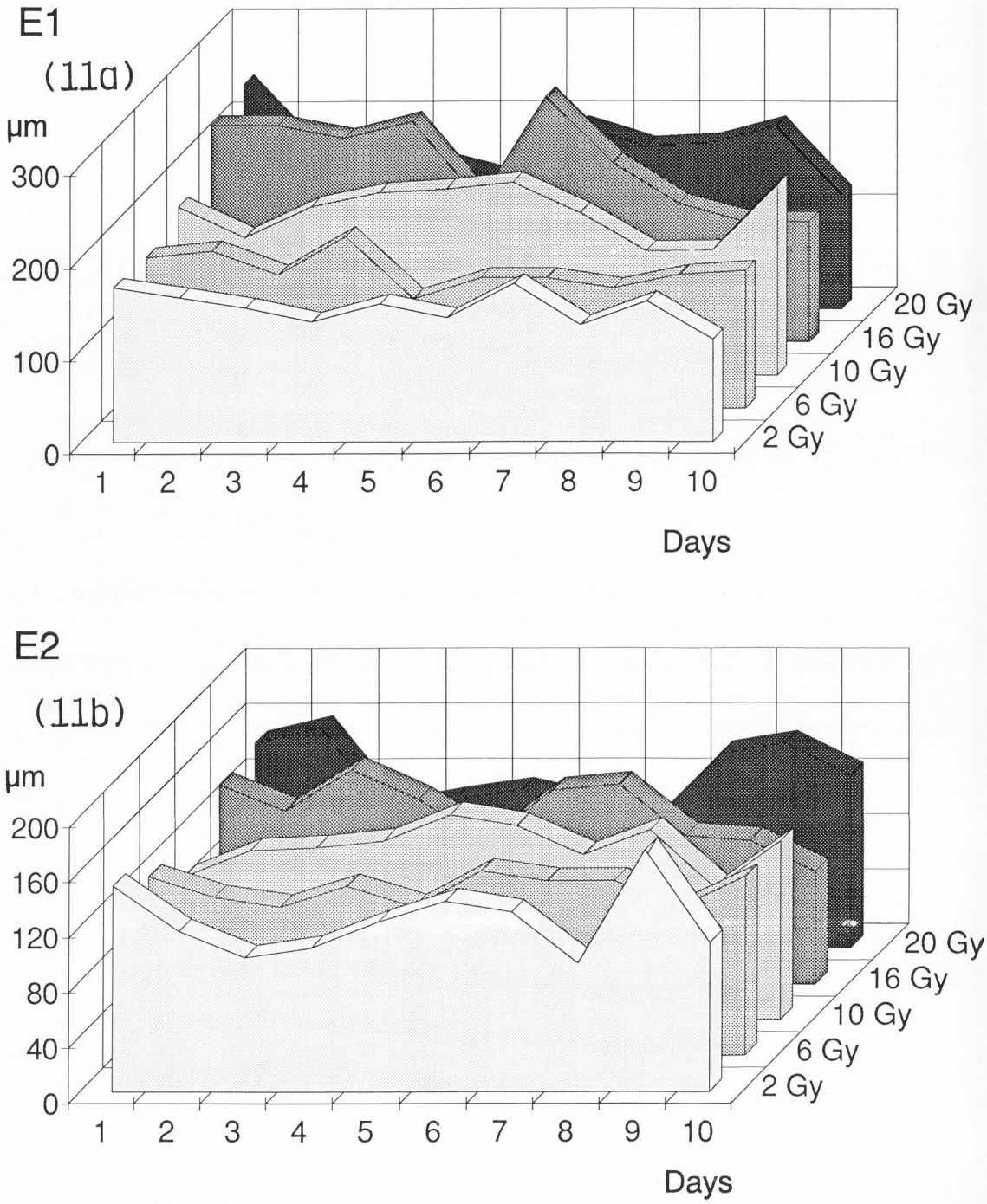


Figure 11. Three-dimensional plot of epithelial height at E1 (Figure **11a**) and E2 (Figure **11b**) as a function of dose and number of days of treatment. In Table 1 all the measured values are presented.

difference of the epithelial height in the high dose range between E1 and E2 is obvious. In E1, there is a relation between the treatment (radiation and cis-DDP) and the thickness as shown in Fig. 11: the value in group 1 being 145 μm, in group III - 150 μm and in group V - 175 μm. The highest value is found in group VIII with a value of 195 μm; in group X, it has decreased to 176 μm. In the lower part of esophagus, E2, exposed only to cis-DDP, the thickness of the mucosa is slightly (non-significantly) lower than that of the normal one (121-126 μm for all groups).

Table 1. Height of the epithelium

Gy	Day										
E1	1	2	3	4	5	6	7	8	9	10	m.v. ±S.E.
2	167	156	145	130	149	134	171	126	152	111	145 ±6
6	164	171	145	186	119	141	141	130	145	149	150 ±6
10	182	149	182	197	201	208	175	134	135	205	175 ±9
16	234	234	220	238	152	264	193	149	130	130	195 ±16
20	242	167	179	156	138	197	175	179	197	123	176 ±11
E2											
2	149	116	97	104	123	138	130	93	175	108	123 ±8
6	130	115	108	123	108	134	127	127	108	130	121 ±3
10	104	123	125	130	149	141	120	138	96	130	126 ±5
16	145	126	156	134	104	141	145	108	104	82	125 ±8
20	149	160	100	108	115	104	93	141	149	126	125 ±8

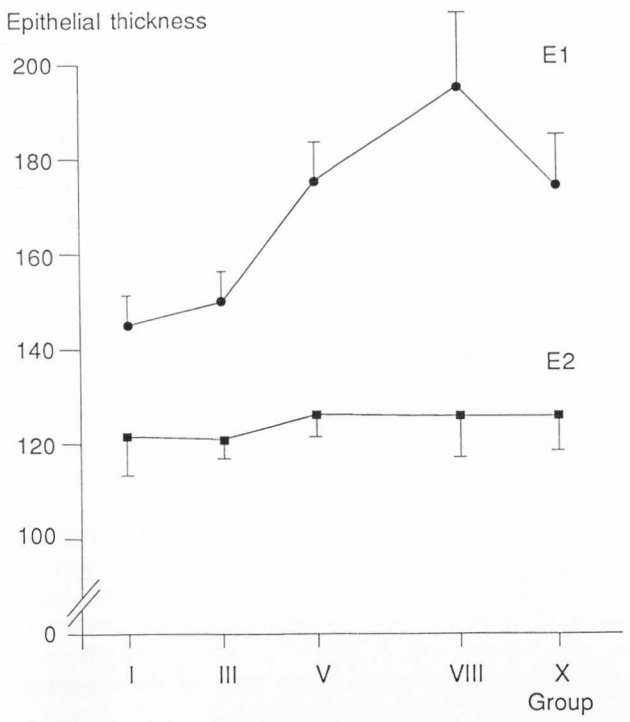


Figure 12. Epithelial height as measured for each group (I-X). Each point represents the mean value of the measurements during ten days.

Discussion

In this study where cis-DDP was given daily as a sensitizer prior to radiation treatment, damage to the surface epithelium was manifest in the form of detached or damaged microridges and increased cell loss. Epithelial damage would appear to be a gradual process, where the first event is the detachment of the microridge at one end, and its protrusion as a nodule from the surface (Fig. 5). At this stage, it often appears to be swollen and edematous, sometimes assuming a club-like or balloon-like form (Fig. 4). Subsequently, the damaged microridge becomes more and more detached, the swelling disappears, and it assumes an extended serpentine form (Fig. 6), being eventually shed either alone or together with the ejection of the whole cell into the lumen. Our findings suggest damage to the esophageal epithelium to be greater when cis-DDP is given concomitantly with radiation treatment than when either cis-DDP or radiation is given alone (Albertsson *et al.*, 1986). The damage is also greater at E1 (i.e., that part of the esophagus exposed to combined treatment), though damage also occurs at E2 (i.e., the lower esophagus exposed to cis-DDP alone). The damage would also appear to increase with increasing dose.

Damage to microridges and cell loss are probably of minor clinical importance, as the affected cells are already on the point of being shed into the lumen and replaced by new cells as part of the natural regeneration process of the epithelium. Maturing cells from the basal layer migrate successively up toward the lumen where they are finally shed into the lumen. TEM findings manifested a tendency of the number of total cells and

basal cells to be higher in the high dose range in that part of the esophagus exposed to combined treatment (i.e., E1). Of the various possible explanations of the increased epithelial thickness in the part of the esophagus exposed to the combined treatment, the three most likely alternatives are as follows:

1. Post-radiation edema. Radiation treatment has long been known to cause cellular and interstitial edema. This has been shown to be dose-dependent in the dosage range 2-20 Gy (Albertsson *et al.*, 1983). In the present study, epithelial thickness increased successively through four dose levels. The course is not unlike that to be expected after radiation therapy, where oedema appears early but subsequently subsides. Even so small a dose as 2 Gy yielded an increase in epithelial thickness of 10% as compared with the E2. In light microscopy observation, the cells have an edematous appearance, an impression verifiable both in SEM and TEM at doses of 10-20 Gy + cis-DDP.

2. Reduced cell loss. With steady state proliferation and cell loss, there is no increase in epithelial thickness (Leblond *et al.*, 1964). Cell kinetic studies of radiation effects have shown a primary mitotic delay to be followed by compensatory repopulation (Denekamp, 1982). Judging by light microscopy and TEM, there is a cell layer that appears to manifest arrested development. This is most apparent in Fig. 10 where there is an intermediate layer of coalesced cells with indefinable limits. The entire epithelium, from the lumen to the basal cell layer, consists of about 40 cells. This is consistent with the prevailing radiobiological view. An irradiation effect that first causes damage to basal cells which are the most sensitive, results in a mitotic delay, followed by hyperplasia that exerts pressure upon the overlying cell layer; the morphological effect being an increase in epithelial thickness. Otherwise the currently accepted explanation of increased basal proliferation is increased cell loss that in some manners signals the basal cells to increase their proliferative activity. In the present cases, the effect may be interpreted as the result of ionizing radiation. However, the physiological course would seem to be complex. The turn-over rate in the rabbit oesophagus is not known, and the nearest approximation might be the rat where the figures are 8.8 days for the upper esophagus and 10.6 for the lower esophagus (Bertalanffy, 1960).

3. Increased proliferation. Both light microscopy and TEM showed the number of basal cells to be increased in the E1 preparations, particularly at the end of the series at doses of 16 and 20 Gy + cis-DDP, which in itself would result in an increase in epithelial thickness if the desquamatory process remained unchanged. The reason for the increase in epithelial thickness in the E1 groups, and the differences between the groups, is probably a combination of the three alternatives outlined above. Although it is difficult to say with any certainty when one alternative is the predominant explanation, it is possible to make educated guesses: in groups 2 and 6 Gy + cis-DDP, the edema explanation is probably the predominant one, whereas in groups 16 and 20 Gy + cis-DDP, so much time has elapsed since the first day's radiation that the proliferation effect have been induced. The increased epithelial thickness in E1 in the high dose range is a noteworthy finding of manifest clinical import, in view of the time-honoured axiom that cytostatic and radiation treatment must be staggered in order to avoid unacceptable normal tissue toxicity (Steel, 1988).

In clinical work, however, where esophageal and head and neck cancer are concerned, the development has been toward more intensive pretreatment for cure with concomitant chemotherapy and radiotherapy, which in a number of studies has been found to result in improved local control and survival (Coughlin and Richmond, 1985; Al Sarraf *et al.*, 1987; Forastiere *et al.*, 1990). That such treatment can be given with tolerable levels of normal tissue toxicity is probably due to accelerated proliferation in response to damage induced in the otherwise dose-limiting normal tissue included in the field exposed to radiation. Accelerated proliferation in response to induced cellular damage is a well known phenomenon previously shown to occur both in skin (Denekamp, 1982), the intestine (Withers, 1971), and the trachea (Albertsson and Håkansson, 1988), but which would appear to be of considerably greater clinical significance than formerly thought. Although considerable attention has hitherto been focused on the problem of hypoxia, as this has been considered to be an important cause of treatment failure, radiosensitizers such as nitro-imidazoles and their derivatives have been found to be without effect in most clinical studies. Cis-DDP, on the other hand, is capable of affecting reoxygenation, repair, repopulation and redistribution, which renders it particularly interesting for use in combination with fractionated ionizing radiation. Whether treatment with concomitant cis-DDP and fractionated radiation can improve outcome, remains to be established in randomized studies, however.

Conclusions

The combination of cis-DDP and radiation induces damage to the esophageal epithelium, which is manifest within a few days after treatment in the form of damaged microridges and increased cell loss. As measured with these variables, the damage is dose-dependent and somewhat greater in that part of the esophagus which is exposed to the combined treatment. However, TEM investigations show the proportion of active proliferating basal cells to increase within the high dose range, and to be more prominent in that part of the esophagus exposed to the combined treatment. This might depend on an accelerated proliferation in the part of the esophagus exposed to the combined treatment.

Acknowledgements

This work was supported by grants from the Medical Faculty, Lund University, from Lund's Hospital

Research Foundations and from the B. Kamprad Foundation. The microscopic examinations were performed at the Electron Microscopy Unit, Faculty of Medicine, Lund University.

References

Albertsson M, Håkansson C-H. (1988). Changes in the tracheal ciliated cells in rabbits treated by cis-diamminedichloroplatinum(II) as studied by electron microscopy. Scanning Microscopy **2**, 2173-2179.

Albertsson M, Håkansson C-H, von Mecklenburg C. (1983). Scanning electron microscopy and recording of the physiological activity of tracheal ciliated cells treated with fractionated irradiation. Scanning Electron Microsc. **1983**; IV: 2019-2026.

Albertsson M, Håkansson C-H, Mercke C. (1986). Electron microscopy of the ciliated cells of the trachea of the rabbit treated with cis-dichlorodiammine-platinum (cis-DDP) alone or in combination with ionizing radiation. Scanning Electron Microsc. **1986**; III: 913-924.

Albertsson M, Håkansson C-H, Mercke C. (1987). Effects of cis-dichlorodiammineplatinum (cis-DDP) alone and in combination with ionizing radiation on the oesophageal mucosa. A scanning and transmission electron microscopic study. Scanning Microscopy **1**, 1861-1869.

Albertsson M. Håkansson C-H, Palmegren M. (1990). Changes in the esophageal epithelium in rabbits treated by 5 mg cis-dichlorodiammineplatinum as studied by electron microscopy. Scanning Microscopy **4**, 143-149.

Albertsson M, Håkansson C-H, Palmegren M. (1991). The response of the tracheal epithelium to concomitant Cis-diamminedichloroplatinum(II) and radiation. An electron microscopic study in rabbits. Scanning Microscopy **5**, 573-583.

Al Sarraf M, Pajak TF, Marcial VA (1987). Concurrent radiotherapy and chemotherapy with cisplatin in inoperable squamous cell carcinoma of the head and neck. Cancer **59**, 259-265.

Bertalanffy FD (1960). Mitotic rates and renewal times of the digestive tract epithelia in the rat. Acta Anat. (Basel) **40**, 130-148.

Coughlin CT, Richmond RC (1985). Platinum based combined modality approach for locally advanced head and neck carcinoma. Int. J. Radiat. Oncol. Biol. Phys. **22**, 915-919.

Denekamp J. (1982). Cell kinetics and Cancer Therapy. C.C. Thomas, Springfield, Illinois, 162 pp.

Douple EB, Richmond RC. (1979). Radiosensitization of hypoxic tumor cells by cis- and trans-dichlorodiammineplatinum(II). Int. J. Radiat. Oncol. Biol. Phys. **5**, 1369-1372.

Dritschilo A, Piro AJ, Kelman AD. (1979). The effect of cis-platinum on the repair of radiation damage in plateau phase chinese hamster (V-79) cells. Int. J. Radiat. Oncol. Biol. Phys. **5**: 1345-1349.

Forastiere A, Orringer M, Perez-Tanayo C Urba S, Husted S, Takasugi B, Zaharak M. (1990). Concurrent chemotherapy and radiation followed by transhiatal esophagectomy for local-regional cancer of the esophagus. J. Clin. Oncol. **8**, 119-127.

Leblond CP, Greulich R, Marques-Periera JP (1964). Relationship of cell formation and cell migration in the stratified squamous epithelia. In: Advances in Biology of Skin, Edward Adlard and Son Ltd., Dorking, U.K., chapter 5, pages 39-67.

Luk KH, Ross GY, Phillips TL, Goldstein LS. (1979). The interaction of radiation and cis-diamminedichloroplatinum(II) in intestinal crypt cells. Int. J. Radiat. Oncol. Biol. Phys. **5**, 1417-1420.

Richmond RC, Powers EL (1976). Radiation sensitization of bacterial spores by cis- dichlorodiammine-platinum(II). Radiat. Res. **68**, 251-257.

Richmond RC, Zimbrick JD, Hykes DL. (1977). Radiation-induced DNA damage and lethality in *E. coli* as modified by an antitumor agent cis-dichlorodiammine-platinum(II). Radiat. Res. **71**, 447-460.

Steel GG. (1988). The search for therapeutic gain in the combination of radiotherapy and chemotherapy. Radiother. Oncol. **11**, 31-53.

Szumiel I, Nias AHW. (1976). The effect of combined treatment with a platinum complex and ionizing radiation on chinese hamster ovary cells *in vitro*. Br. J. Cancer **33**, 450-458.

Withers HR (1971). Regeneration of intestinal mucosa after irradiation. Cancer **28**, 75-81.

Discussion with Reviewers

L.G. Friberg: The fractionated irradiation schedule (2 Gy/F) is the conventional one in the clinic. A conclusion after a total dose of at least 40 Gy instead of your 20 Gy may have been of more clinical interest. Why did you choose a dose of 0.3 mg of cis-DDP i.p.? A dose given i.p. gives a quite lower concentration in blood.
Authors: In all our studies for the past 10 years we have kept to an accumulated irradiation dose of 20 Gy, as our aim has always been to investigate the early effects of treatment. As to the cis-DDP dosages of 0.3 mg i.p., we have tried to measure the tissue content both with the particle induced X-ray emission (PIXE) method and with the atom absorption technique, but without success. However, findings in animal studies suggest that absorption after i.p. injection is very rapid [see e.g., Pretorius *et al.* (1981) Cancer Treat. Rep. **65**(11-12), 1055-1062, Yakihidi Iwamoto *et al.* (1984) Cancer Treat. Rep. **68**(11), 1367-1373]. According to these authors the absorption of cis-DDP from an i.p. injection in animal systems takes place very rapidly.

L.G. Friberg: The recorded thickening of the epithelial layers may be explained by an increased blood flow resulting in a kind of stimulation both of the number of basal layer cells and the activity on the surface which you have shown in Fig. 3 in E1 after treatment.

Authors: The hypothesis is very interesting, one that may well be true. However, we have carried out no blood flow measurements so far.

L.G. Friberg: The aim of the study included study of the repair. Is the return to a normal thickness after day 7-8 seen in Fig 11 in E1 and E2 a sign of repair? A SEM micrograph of the normal esophageal surface should be interest, before treatment and after repair.
Authors: We agree that the comparison suggested might be valuable, and in our earlier papers we have shown micrographs of normal tissue [see e.g., Albertsson *et al.*, (1987), text reference]. In the present paper, however, for reasons of space we felt it necessary to limit the figures to those selected, though in interpreting the micrograph we have considered the phenomenon to be a sign of repair.

Z. Somosy: Some literature data suggest that the apoptosis plays role in the radiation induced cell death [Story *et al.* (1992) Int. J. Radiat. Biol. **61**: 243; Schrek (1955) Radiology **65**: 912; Yamada and Ohyama (1988) Int. J. Radiat. Biol. **53**: 65; Walters (1992) Cancer Res. **52**: 883] and it is known that the cis-DDP has similar effect of cells [Barry *et al* (1990) Biochem. Pharmacol. **40**: 2353]. Did you detect cytological or ultrastructural signs of apoptosis?
Authors: In earlier publications, where cis-DDP was administered as a high dose of 5 mg at day one of a fractionated radiation schedule, apoptosis was found in ultrastructural investigations of both the trachea and esophagus. However, no signs of apoptosis were found in this investigation.

Z. Somosy: Did you find any morphological changes of cell junctional complex after irradiation and combined treatments?
Authors: No.

D.P. Penney: Has there been any attempts to shield the lungs, which are radiation dose limiting?
Authors: The radiation field size was 3 x 3 cm and placed over the proximal central trachea, which means that the radiation dose to the lungs was negligible. Moreover, in this experiment, only the early effects were investigated.

D.P. Penney: Was the radiation given at the same time each day? Were there ten consecutive daily treatments, or were weekends (i.e., Saturdays and Sundays) omitted? If the latter, then there may have been some PLD or SLD repair.
Authors: The radiation was given at the same time each day with ten consecutive daily treatments.