Ciliated Cells of the Trachea of the Rabbit, Treated with Cis-Diamminedichloroplatinum (II) Alone, or in Combination with Ionizing Radiation

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CILIATED CELLS OF THE TRACHEA OF THE RABBIT, TREATED WITH CIS-DIAMMINEDICHLOROPLATINUM (II) ALONE, OR IN COMBINATION WITH IONIZING RADIATION

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Abstract

The ciliated epithelium of the rabbit trachea was irradiated with daily fractions of 2 Gy to an accumulated dose of 20 Gy (TD: 2, 6, 10, 16, or 20 Gy). Fifteen to forty-five minutes before start of the first irradiation (treatment day 1), 5 mg cis-DDP was given by intraperitoneal injection to each rabbit. Examination was made 1 - 10 days after each fractionation schedule, when specimens were taken for investigations.

Scanning electron microscope investigations showed a gradual development of ciliary damage from blebs on the cilia to swollen tips, broken and bent cilia and finally an epithelial lining with areas free from cilia with a surface covered with microvilli-like structures. SEM also showed cell loss, and remnants of dead cells on the surface together with detritus. By transmission electron microscope ciliary damage, cell death and cell loss of the ciliated cell layer as well as exfoliation of portions of goblet-like cells on the surface could be confirmed. The irradiated ciliated epithelium and the untreated control epithelium in each animal showed no difference in this respect. Thus no enhancement of the effects of radiation could be observed. The development of ultrastructural damage may be due to a cytotoxic effect of the drug on the ciliated epithelium. However, 19 days after the start of cis - DDP injection, a hyperplasia of the basal cell layer was observed, which indicates that the observed cytotoxicity of the drug is reversible and a normalisation occurs during the last days of observation in this study.

KEY WORDS: Cilia, tracheal epithelium, cis-diamminedichloroplatinum, irradiation, scanning electron microscopy, transmission electron microscopy.

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Introduction

Irradiation of malignant tumours is a well established method of treatment and the method usually chosen for treatment of malignant head-and - neck cancers, alone or combined with surgery. With this therapy modality of stage IV tumours, 10-20% of the cases survive for five years. Local relapse after treatment is often the cause of death. Therefore attempts have been made to improve the survival rate, with different kinds of chemotherapy before or during radiation treatment. Preliminary results show an improvement with cis - DDP containing regimens.

During radiation treatment, cis - DDP has been administered in different ways, either as a high single - dose (100 mg/m^2) every third week, an intermediate dose (30 mg/m^2) every week or in a sensitizing way (6 - 8 mg/m^2) every day before each irradiation (Creagan et al. 1981, Reimer et al. 1981, Haselow et al. 1982, Leipzig 1983, Pinedo et al. 1983, Keizer et al. 1984, Coughlin and Richmond 1985). In most of these reports a small number of patients is treated and the tumour response, as well as the toxicity of the treatment, shows great variation. The cellular and biochemical mechanisms are most likely to be different when the drug is administered in a high (100 mg/m^2), intermediate (30 mg/m^2) or low (6 - 8 mg/m^2) dose both on the tumour as well as the normal tissue.

In order to study further the possible mechanisms of interaction between cis - DDP and irradiation, an ultrastructural study has been performed on the ciliated epithelium of the rabbit's trachea. The normal ultrastructure of this tissue is well described by Hakansson and Toremalm (1965), as is the influence of fractionated irradiation alone, described earlier by Albertsson et al. (1983).

In the present study, the effects of cis - DDP and fractionated irradiation have been investigated on the ciliated epithelium of the rabbit trachea. The drug is given i.p. as a high single dose 5 mg on the first irradiation day, which corresponds to the dose (100 mg/m^2) in humans.

Materials and Methods

The effect of irradiation alone on the trachea of the rabbit has been described in an earlier paper (Albertsson et al. 1983). For the evaluation of the effect of cis - DDP relevant data have been extracted from that work.
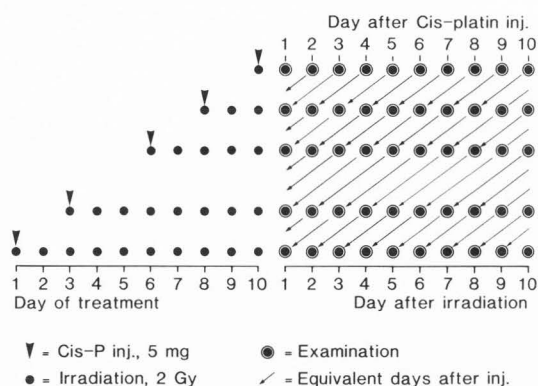
Figure 1. Treatment schedule for combined cis-DDP and irradiation. The drug was given at a dose of 5 mg, 15 – 45 minutes before the first irradiation. Total dose ranged from 5 mg cis – DDP/2 Gy to 5 mg cis – DDP/20 Gy. After completion of treatment, experiments were made daily from day 1 to day 10.

Rabbits

The material selected for study consisted of 60 full – grown rabbits weighing between 1.8 and 2.3 kg. Ten animals served as untreated control animals. Fifty rabbits received fractionated irradiation (2 Gy/F) according to the schedule shown in Fig 1.

Irradiation

Each rabbit was anesthetized by intraperitoneal injection of pentobarbital (40 mg per kg body weight) for about 15 minutes during the administration of irradiation.

Irradiation 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 trachea just beneath the larynx. Fifteen mm beyond the caudal part of the irradiated area, the absorbed dose was negligible. The absorbed dose in the trachea was controlled by thermoluminescent dosimeters. The spatial distribution between the irradiated area and control area was 40 mm.

Drug

Cis - diamminedichloroplatinum (II), (Cis-DDP, cis – platinum) (Platinol, Bristol Myers Company) was available in 20 ml flasks dissolved in normal saline at a concentration of 0.5 mg/ml.

Experiments

The rabbits were treated with fractionated irradiation (2 Gy/F), with a total cumulative dose ranging from 2 – 20 Gy. Fifteen to forty – five minutes before the first irradiation treatment in each dose group, each animal was injected with 5 mg cis-DDP intraperitoneally according to the schedule in Fig 1. The rabbits were then laid on their back and the upper part of each trachea (20 mm) was irradiated. The animals were treated in groups of ten. After completion of irradiation, one animal was removed from the group on each of the ten consecutive days. The animal was sacrificed by a blow on the skull, in order to avoid the side effects of pharmacological intervention. The trachea was dissected out in its entire length (7 cm). Samples for scanning electron microscopy (SEM), transmission electron microscopy (TEM) and light microscopy (LM) were taken from the upper part of the trachea (irradiated area: T1) and the lower part of the trachea (control area: T2). Control investigations were also performed in the same way on untreated animals.

Preparation for SEM

The pieces for examination by SEM were not rinsed, but were placed directly in 2.5% glutaraldehyde for fixation for 12 h (in 0.15 M cacodylate buffer at pH = 7.3). They were then transferred into the same buffer for washing, and fixed in 1% osmium tetroxide in 0.15 M cacodylate buffer for 2 h.

Dehydration took place with a graded series of ethanol, after which the preparations were transferred to Freon TF 618. The specimens were later dried according to the critical point method in a Balzer's 000 Critical Point Dryer. They were finally sputter - coated with gold/palladium in a Polaron SEM - coating unit E 5000. Then they were examined in a Cambridge Stereoscan Mark II A or a Zeiss Nanolab Electron Microscope. The microscopes were operated at 20 kV.

Preparation for TEM

The samples were fixed as for the SEM preparations and postfixed in 1% osium tetroxide in cacodylate buffer (pH 7.4) for 2 h, rinsed in 0.15 M cacodylate buffer, dehydrated in ethanol and embedded in Vestopal W or Epon. Sections of 1 μm thickness were cut with an LKB Ultrotome, stained with toluidine blue and examined with a light microscope. Ultrathin sections were cut out and stained with lead citrate and uranyl acetate, or en bloc with 0.5% uranyl acetate. A Zeiss EM 10 electron microscope was used for examination.

Results

Control Material

Both the upper part of the trachea close to the larynx (T1) and the tracheal epithelium close to the carina, constituting the control area (T2) was examined. No difference in the ultrastructural pattern was observed between these two different parts. In the ten untreated rabbits, the ultrastructure consistently presented a homogeneous pattern. SEM showed gracile cilia, regularly arranged (Fig 2) with a minimum of blebs, usually on the side but occasionally on the tip of the cilia. The microvilli were intact and no mucus or particles were found covering the ciliated surface.

TEM of the normal ciliary epithelium showed a layer of basal cells resting on the basal lamina and apical cell layers consisting of ciliated cells, goblet cells and intermediate cells. In the basal cells, a large, usually lobulated nucleus was found, as well as both rough and smooth endoplasmic reticulum, a few mitochondria and sparse ribosomes (Fig 3).

The ciliated cells had a central nucleus, normally spherical. Adjacent to the nucleus, rough endoplasmic reticulum was seen, and free ribosomes were dispersed throughout the cytoplasm. In the cytoplasm Golgi complexes could also be identified. Mitochondria were scattered throughout the whole ciliated cell, but tended to accumulate at the apex. A great number of cilia with the classical 9 + 2
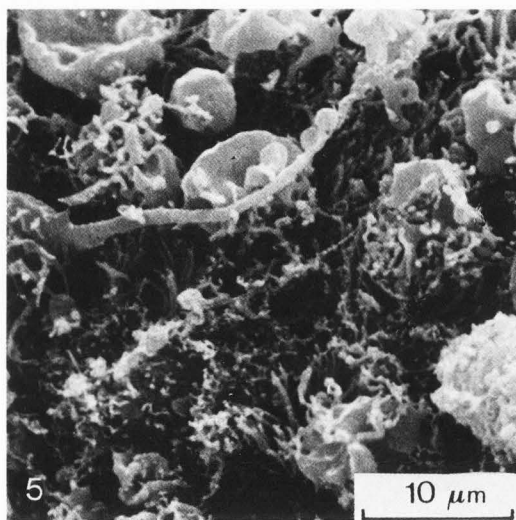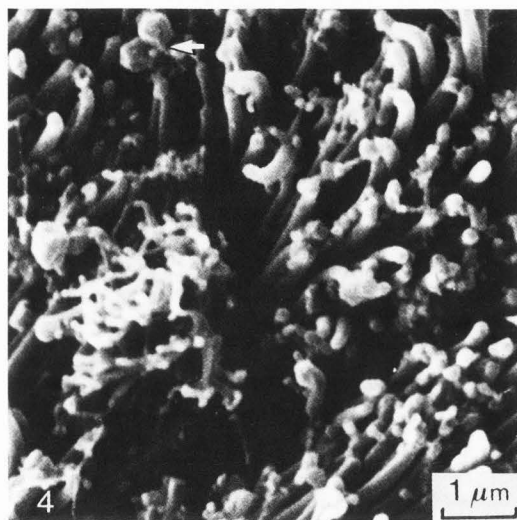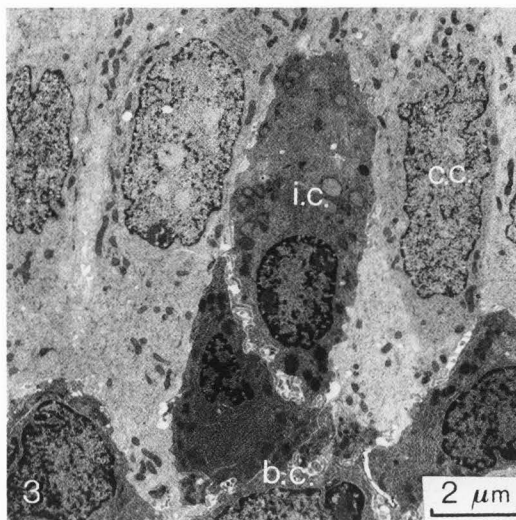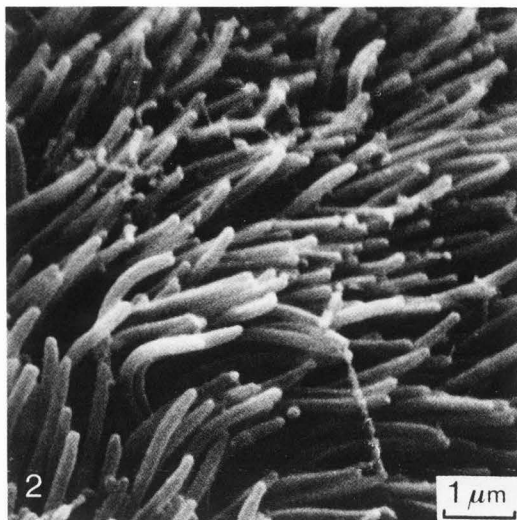
Figure 2. SEM micrograph of normal tracheal epithelium. The cilia are gracile, regularly arranged with a minimun of blebs.

Figure 4. SEM micrograph of the cilia treated with 5 mg cis - DDP/2 Gy. The cilia are regularly arranged. Increased numbers of blebs are to be seen, especially on the tips of the cilia (arrow).

Figure 3. TEM micrograph of normal tracheal epithelium. A layer of basal cells resting on the basal lamina and an apical cell layer comprising ciliated cells, goblet cells and intermediate cells. b.c. = basal cells, c.c. = ciliated cells, i.c. = intermediate cells.

Figure 5. SEM micrograph of the cilia treated with 5 mg cis - DDP/10 Gy. The ciliated surface is disarranged and disintegrated.

pattern were located on the surface. The cilia were anchored to the cytoplasm by basal bodies.

The goblet cells reached the apical cell surface and microvilli were found located at the apical membrane. Both large and small numbers of mucous granules were seen, depending on the condition of the individual goblet cells. They contained heterogeneous material, were of varying size and shape, and surrounded by a membrane. The goblet cells did not contain many mitochondria but were relatively rich in rough endoplasmic reticulum and Golgi complexes. Free ribosomes were scattered throughout the cytoplasm.

Cis - DDP + Irradiation (Figs. 4 - 9)

Damage could be observed on the cilia ranging from an increased amount of blebs (Fig 4), to a cell surface with disordered and disintegrated cilia (Fig 5). Occasionally a ciliated cell was seen sloughing from the epithelium (Fig 6). The changes were scored from the SEM micrographs and graded on a scale from 0 - 3, where 0 = normal ciliated carpet, 1 = increased amount of blebs, 2 = swollen or broken tips of the cilia. 3 = disordered disintegrated cilia. Each picture was judged by shape, and surrounded by a membrane. The goblet
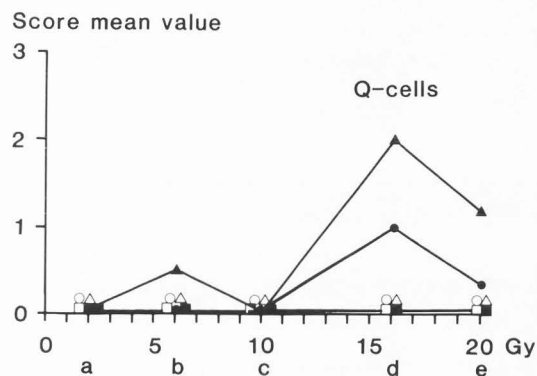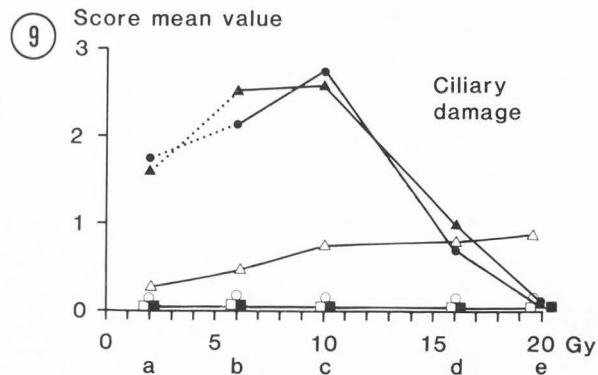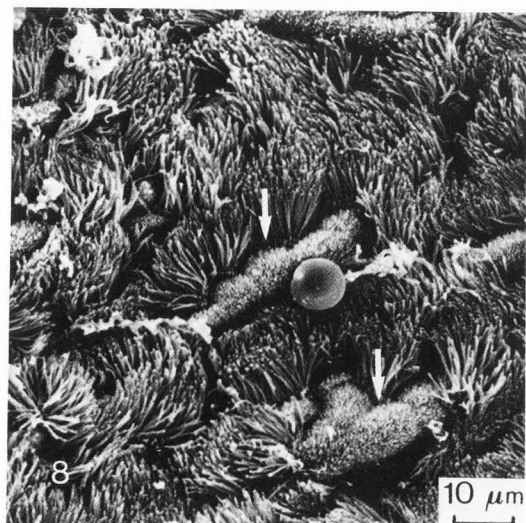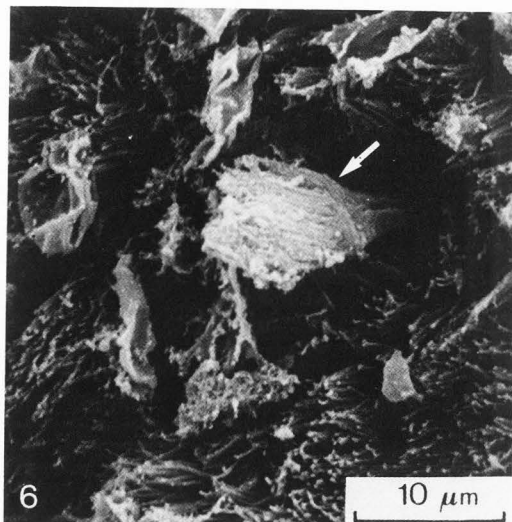
Figure 6. SEM micrograph of the cilia treated with 5 mg cis - DDP/16 Gy. An apical ciliated cell is seen sloughing from the epithelium (arrow).

Figure 7. SEM micrograph of the cilia treated with 5 mg cis - DDP/16 Gy. The figure shows a confluent cell area where the normal ciliated carpet is lost and the surface covered with microvilli-like structures.

Figure 8. SEM micrograph after treatment with 5 mg cis - DDP/16 Gy. Note areas without any cilia with a surface coated by microvilli-like structures (arrows).

Figure 9. Score mean value expressed for each dose. Each point represents all ten values from each dose group as judged by three people. 0 = normal, 3 = greatest abnormality (a-e = 1-10 days after treatment).
Control animals ☐ T1 (trachea beneath larynx)
■ T2 (trachea adjacent to tracheal bifurcation)

Fractionated irradiation: 2, 6, 10, 16 or 20 Gy
△ T'1 (irradiated area)   ○ T' 2 (control area)

5 mg cis - DDP/Fractionated irradiation 2, 6, 10, 16 or 20 Gy
▲ T''1 (irradiated area)   ● T''2 (control area)

cilia	Q-cells
0 = normal ciliary carpet	0 = no Q-cells
1 = increased amount of blebs	1 = a few Q-cells
2 = swollen, broken ciliary tips	2 = many Q-cells
3 = disordered, disintegrated cilia	
	3 = most of the surface covered with Q-cells

three persons. Fig 9 presents the results from these judgements. For each dose group all days of examination are gathered in one point. It is observed that the ciliary damage is pronounced already after cis - DDP 5 mg + 2 Gy, and maximal after 5 mg cis - DDP and 10 Gy, where the score mean value is about 2.5 for the whole dose group. In the higher dose range it is difficult to score the damage effect on the cilia, since on large areas nearly all cilia are lost. This is a phenomenon not observed in the normal trachea. In the SEM loss of cilia can be observed as confluent cell areas, where the normal ciliary carpet is lost (Figs. 7,8). These areas are defined as Q - cells (Quondam - cells, quondam is Latin for former).

The main distinction between goblet cells and Q - cells in SEM micrographs is that goblet cells are single protruding cells on the SEM surface, while Q - cells is the description of confluent areas with only remnants of cilia. However, the surface is covered with microvilli - like structures. The number of Q - cells on SEM micrographs was scored and graded on a scale from 0 - 3, where 0 represents no Q - cells, 1 = a few Q - cells, 2 = many Q - cells, 3 = most of the surface covered with Q - cells. In Fig 9 the result of this evaluation is presented, where each point represents the score for each dose group (note that the time is inevitably included as a variable). The appearance of Q - cells is late in the treatment schedule (Fig 1). From TEM micrographs (Fig 10) it also appears likely that Q-cells are remnants of earlier cilia covered cells and thus represents a late stage of ciliated cell destruction.

P - cells

Figs. 11 - 13 show what is defined as P - cells (protruding cells or pseudogoblet cells) which are cells with a surface partially covered with microvilli. In the normal untreated tracheal epithelium they represent goblet cells. The reason for this different terminology is that P - cells observable after treatment with cis - DDP and irradiation have an ultrastructural appearance somewhat different from the normal, mainly observed on TEM micrographs (Fig. 13). They are filled with a large number of heterogeneous granules surrounded by a membrane. On the surface microvilli - like structures of varying length are seen and occasionally remnants of basal bodies adjacent to the microvilli - like structures. In some P - cells, a nucleus was found as well as rough endoplasmic reticulum, Golgi complexes and ribosomes. The P - cells emptied their contents on the surface by extrusion of small granules or larger membrane - covered parts of the cell, defined as
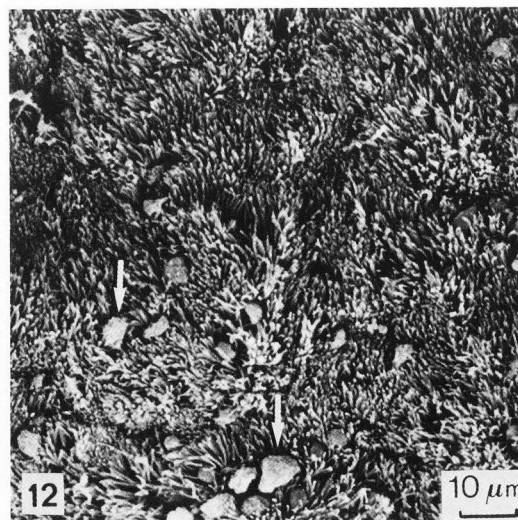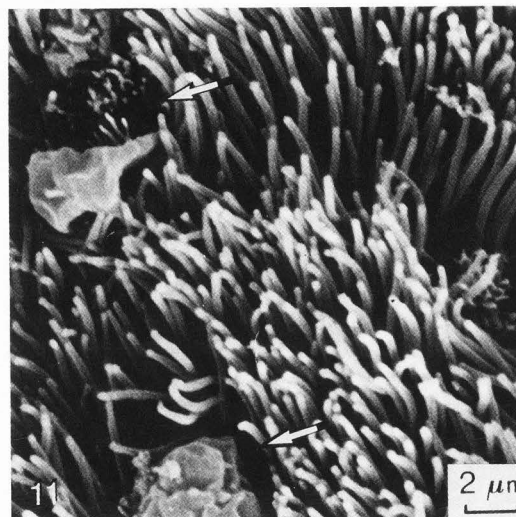

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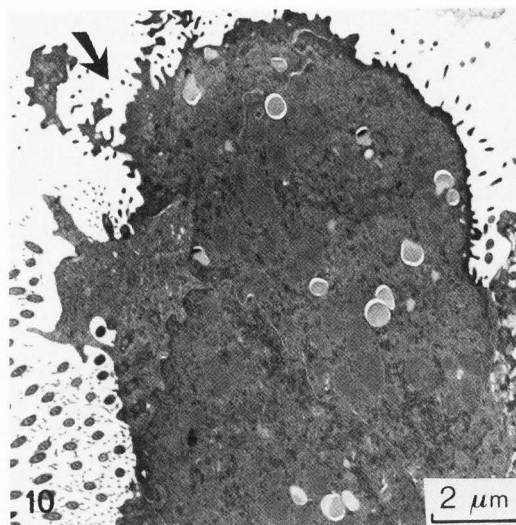

10 µm

Figure 10. TEM micrograph illustrating an apical cell with microvilli - coated surface extruding on the epithelium (arrow).

Figure 11. SEM micrograph illustrating cells partially coated with microvilli protruding on the surface after treatment with 5 mg cis - DDP/16 Gy (arrows).

Figure 12. SEM micrograph of P-cells. Large numbers of protruding cells are seen on the surface (arrows).
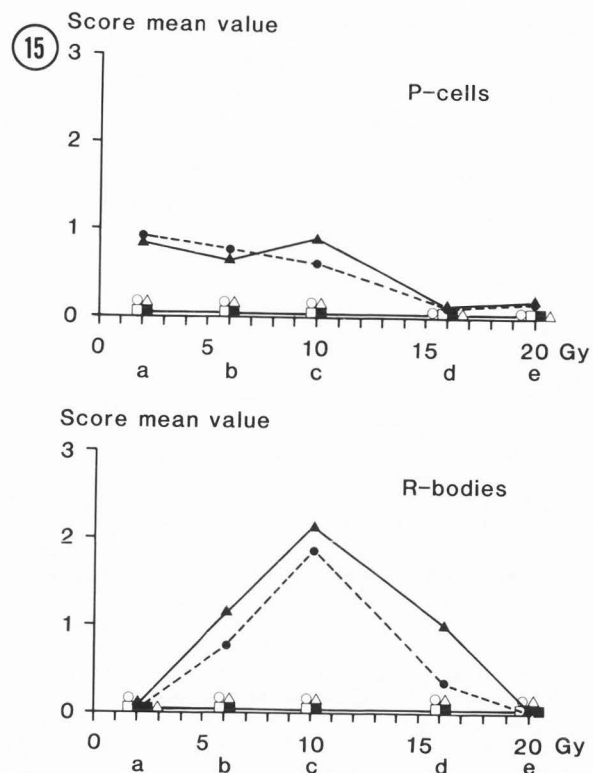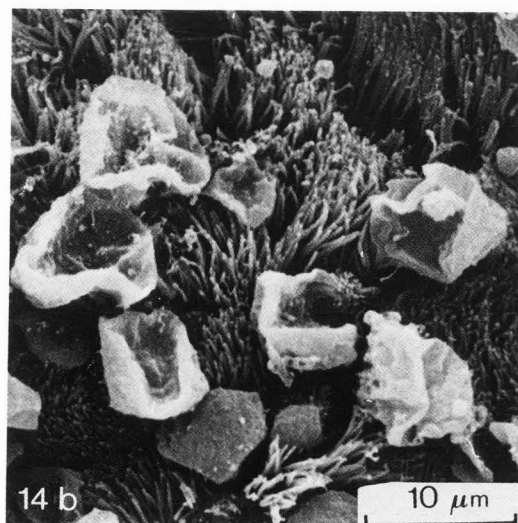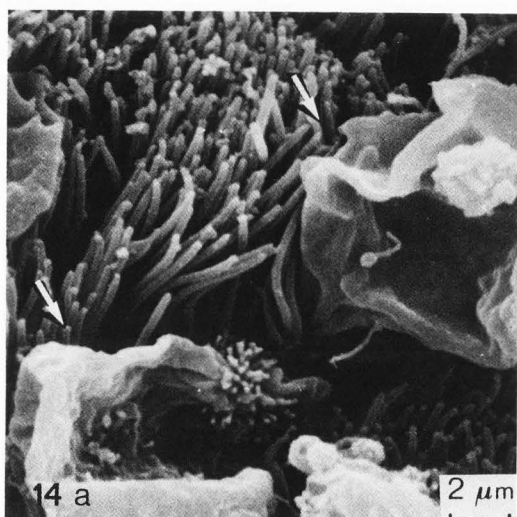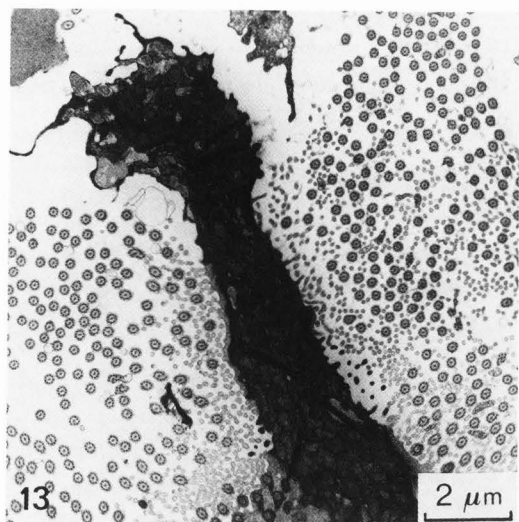
Figure 13. TEM micrograph after treatment with 5 mg cis – DDP/10 Gy. A goblet – like cell exfoliating on the ciliated surface.

Figures 14 a and b. SEM micrographs after treatment with 5 mg cis – DDP/10 Gy. Remnant bodies on the ciliated surface. The R – bodies are partially covered with microvilli and vary in size and shape (arrows).

Figure 15. Score mean value expressed for each dose. Each point represents the value from each dose group as judged by three persons. 0 = normal, 3 = greatest abnormality (a–e = 1–10 days after treatment).
Control animals: □ T1 (trachea beneath larynx)
■ T2 (trachea adjacent to tracheal bifurcation)

Fractionated irradiation: △ T'1 (irradiated area)
○ T'2 (control area)
5 mg cis – DDP/Fractionated irradiation:
▲ T''1 (irradiated area)   ● T''2 (control area)

P – cells   R–bodies
0 = no P – cells   0 = no R–bodies
1 = a few P – cells   1 = a few R–bodies
2 = more P – cells than normal 2 = many R–bodies
3 = many more P – cells than normal
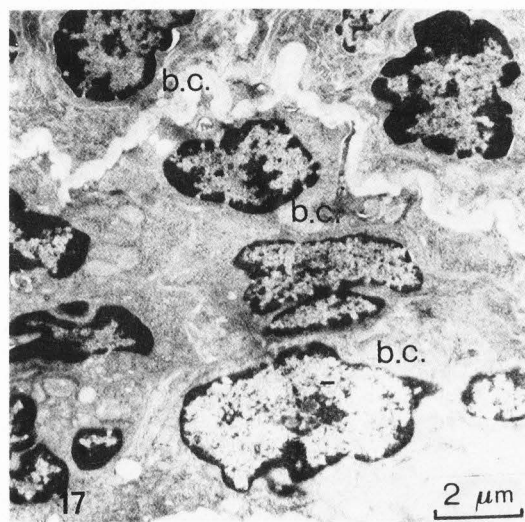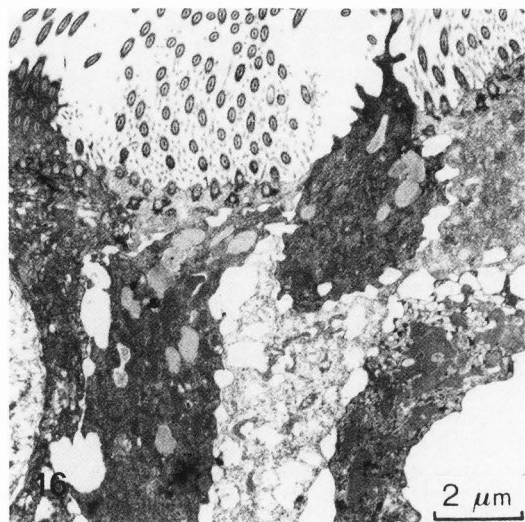   3 = the surface covered with R–bodies

Figure 16. TEM micrograph after treatment with 5 mg cis - DDP/10 Gy. The picture reveals cell death and cell destruction affecting the ciliary cell layer.

Figure 17. TEM micrograph after treatment with 5 mg cis - DDP/20 Gy. Note the hyperplasia of the basal epithelial cell layer. b.c. = basal cells.

R - bodies (Figs. 14a and b). The number of P - cells was scored and graded on a scale from 0 - 3, where 0 = no P - cells, 1 = a few P - cells, 2 = more P - cells than normal, 3 = many more P - cells than normal. The number of P - cells is presented in Fig. 15, and the number of P - cells is increased mainly after 5 mg cis - DDP + 2,6 or 10 Gy, i.e., early in the treatment schedule (Fig 1).

R - bodies

R - bodies, or "Remnant cells" (Figs. 14a and b) are not observed in the normal tracheal epithelium. They vary in size and have the shape of emptied cells with a shell - like appearance. In some cases they have areas on the surface covered with microvilli. They appear in large numbers after a dose of 5 mg cis - DDP + 10 Gy. It is at this point in the treatment schedule that TEM shows extensive cell death and cell destruction affecting the apical ciliated cell layer (Fig. 16), with formation of P - cells extruding from the surface. The R - bodies observed on the surface most probably are remnants of dead cells. The amout of R - bodies was scored from SEM - micrographs and graded on a scale from 0 - 3, where 0 = no R - bodies, 1 = a few R - bodies, 2 = many R - bodies, 3 = the surface covered with R - bodies. The score mean value for each dose group is presented in Fig. 15. R - bodies are observed to a lesser extent in the groups 5 mg cis - DDP + 2 and 6 Gy, most pronounced after 5 mg cis - DDP + 10 Gy and have almost disappeared after 5 mg cis - DDP + 20 Gy.

Desquamation products

This concept is preserved for the appearance of fibrin - like threads, mucus and detritus on the surface. These phenomena appear to run parallel to R - bodies and are also pronounced when the ciliated carpet is most damaged.

Cis - DDP treatment

In the lower part of the trachea, where no absorbed radiation dose could be measured (T2), the ultrastructure of the tracheal epithelium showed a similar pattern of damage to that in the upper irradiated area (Figs. 9 and 15).

The results described above support the hypothesis that when 5 mg cis - DDP is given as i.p. treatment on day 1 (Fig. 1), the drug exerts a cytotoxic effect on the tracheal epithelium. The ciliated carpet undergoes a process of destruction with blebs and broken cilia, until there is a complete loss of cilia, leaving areas of Q - cells. Also the ciliated cell layer dies: the ciliated cells seem to transform to P - cells which are exfoliated on the surface and seen as R - bodies. At the end of the observation period in the treatment schedule, day 10, after 5 mg cis - DDP + 20 Gy, i.e., 19 days after injection of the drug, a hyperplasia of the basal cell layer is observed in TEM (Fig. 17). In order to clarify further the assumption that the effects on the tracheal epithelium observed here depend mainly on the cytotoxic effects of the drug and that the irradiation effects in this schedule are negligible, the investigations were carried out to estimate the degree of ciliary damage, Q - cells, P - cells and R - bodies taken into consideration only days after cis - DDP treatment according to the schedule (Fig. 1). An estimation of these effects where time after injection of cis - DDP and the score mean value are considered is presented in Figs. 18 and 19. From these figures it is possible to observe that the ciliary damage is an early phenomenon which gradually declines from day 11, which is when Q - cells appear. The appearance of P - cells and R - bodies is an early phenomenon, and reaches a maximum on days 3 - 9. P - cells and R - bodies are not observed after the first 14 days. Moreover, score mean values in the irradiated and control part run parallel and are of the same size, which further indicates that the effects observed depend mainly on the cytotoxicity of the drug, and that the irradiation in itself hardly exerts any of the observed effects.
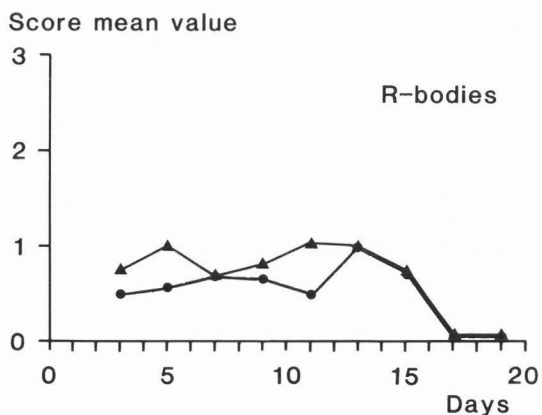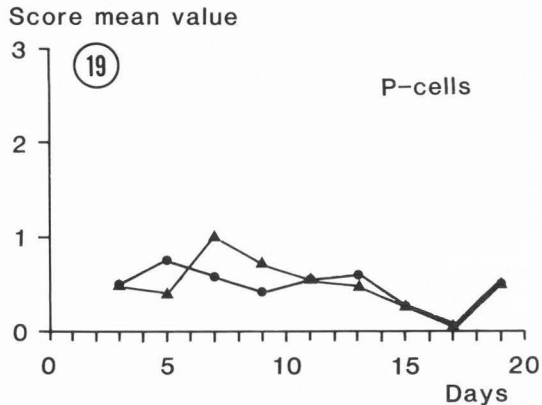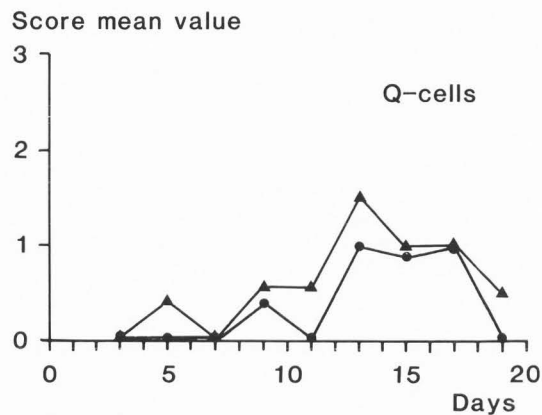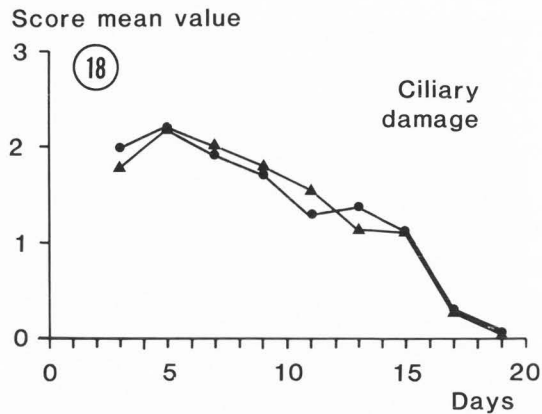
Score mean value

Score mean value

Score mean value

Score mean value

Discussion

Earlier experiments with fractionated irradiation (2-20 Gy) on the ciliated epithelium of the rabbit trachea, (Albertsson et al. 1983), described a moderate degree of ultrastructural change, mainly as an increased amount of blebs on the cilia seen by SEM. TEM investigations of that material showed signs of increased intracellular metabolic activity. The ultrastructural observations made in this study where cis - DDP and radiation are combined, follow quite a different pattern. Firstly, there appears to be no major difference between the ultrastructural pattern in the upper part of the trachea (T''1), where the animal had been treated with fractionated irradiation, and that in the lower part of the trachea (T''2) which served as the animal's own control and where the absorbed radiation dose was negligible. Thus there is no enhancement of radiation effects in this normal tissue with cis - DDP administered in a cytotoxic way.

A moderate degree of enhancement of radiation effect by cis - DDP has been reported earlier in some normal tissues (skin, intestine) in vivo, (Burholt et al. 1979, Douple et al. 1979, Luk et al. 1979, Overgaard and Kahn 1981, Bartelink et al. 1983, Baker et al. 1984, von der Maase 1984a and b, Leliveld et al. 1985). However, the results are sometimes conflicting and in a recently published article regarding dose and time effects of cis - DDP and radiation on mouse duodenal crypts Dewit et al. (1985) suggest that cis - DDP and radiation act by independent cellular mechanisms. The data from the present investigation support that hypothesis.

Cis - DDP given day 1 of a fractionated irradiation schedule exerts a cytotoxic effect on the tracheal epithelium. The ciliated carpet becomes damaged. This is seen on the cilia as blebs, swollen ciliary tips, broken and bent cilia. This damage is most pronounced the first 14 days after drug injection. Thereafter, confluent areas of cells can be observed in the SEM with only remnants of cilia with a microvilli - coated surface (Q - cells). These appear to have a maximum at day 11 - 17 after drug injection and are believed to represent areas where the cilia are lost. This assumption is supported by TEM investigations (Fig. 10). The cytotoxic effect of the drug results in cell death and loss of the apical ciliated cell layer. Ciliated cells transform to P - cells and are exfoliated on the surface as R - bodies. P - cells and R - bodies are early phenomena, appearing mainly during the first 14 days after drug injection and are of transitory character, diminishing with increasing time. The appearance of P - cells raises the question of the origin of goblet cells, which is not known. They may be produced from undifferentiated basal cells or differentiate from intermediate cells, or possibly some of the ciliated cells undergo a functional conversion to goblet cells. This latter

hypothesis is supported by the ultrastructural appearance of the P - cells, which classifies them as goblet - like cells. Moreover the effects of the drug seem to be reversible. The last days of observation, day 15 - 19 after drug injection, the ultrastructure normalizes. SEM shows a normal regular pattern of the ciliated carpet and TEM shows hyperplasia of the basal cell layer. This may represent a repopulation with a new ciliated cell layer, differentiated from the basal cell layer. Thus even though cis - DDP in itself exerts greater ultrastructural damage of the rabbit tracheal epithelium than fractionated irradiation, these effects seem to be reversible and the combination of cis - DDP and irradiation does not enhance the ultrastructural damage revealed by cis - DDP alone. This agrees with the clinical experience that when fractionated irradiation is combined with simultaneous cis - DDP - containing chemotherapy every third week, there appears to be no immediate increased toxicity from irradiation, (Taylor et al. 1985). Further studies are planned with cis - DDP administered in a low sensitizing dose (0.5 mg) before each irradiation treatment in order to see whether any effects enhancing those of the radiation are observed in that case, or if the mechanisms of action is different when the drug is administered in this way.

In the present study cis - DDP was given i.p. 15 - 45 minutes before irradiation. In 90% of the animals about 30 minutes passed between drug injection and irradiation but some variation in this time interval could for practical reasons not be avoided. However according to the drug kinetic studies by Iwamoto et al. (1984) in animal systems the absorption of cis - DDP from and i.p. injection takes place very rapidly and plasma peak concentration is reached within 15 minutes.

All the animals in this study treated with irradiation, also received pentobarbital (40 mg/kg bodyweight). It is known that this drug can alter the physiology in such a way that changes in radiation response may occur. Radioprotection has been observed in tumours, (Milne et al. 1973, Denekamp et al. 1979, Sheldon 1979, Peacock et al. 1980, Rockwell and Loomis 1980), and in normal tissues, (Alper and Hornsey 1968, Riches et al. 1973, Keizer and van Putten 1976, Down et al. 1983). Also both in normal and tumour tissue either radiosensitization or no effect from anaesthesia has been seen (Denekamp and Fowler 1966, Kallman et al. 1972, Hendry 1978, Denekamp et al. 1979). Different mechanisms have been proposed to explain the changed radiation response. The protection has often been ascribed to physiological alterations leading to an oxygen deficit (Zanelli et al. 1975, Kallman et al. 1972, Rockwell and Loomis 1980). Investigations with multicellular spheroids have shown hypoxic radiosensitization and oxic radioprotection, which was suggested to depend on a suppression of respiration in the oxic cells and a consequent improvement in oxygen supply to previously hypoxic cells (Yau et al. 1980).

A possible effect of the anesthesia to be considered in the interpretation of the results described here, cannot be excluded. Therefore separate analyses have been performed both on 30 control animals and on all control preparations in the lower part of the trachea (T'2) in animals treated with fractionated irradiation. All of these investigations showed normal ultrastructure and no influence of the anesthesia could be seen 24 - 240 h after completion of irradiation (L. Henningsohn, personal communication).

Conclusion

Cis - DDP exerts a cytotoxic effect on the ciliated epithelium on the rabbit's trachea. The ciliary damage is observed a few days after injection and shows a gradual development from blebs on the cilia to swollen ciliary tips and broken cilia. Finally the cilia are lost and large areas of the surface are covered with microvilli - like structures, i.e., Q - cells. A few days after injection of cis - DDP cell death and cell loss is observed from the ciliated cell layer, with formation of P - cells, which are exfoliated on the surface as R - bodies. The addition of fractionated irradiation to the ciliary epithelium does not enhance these effects.

Acknowledgements

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Discussion with Reviewers

J. Reitan: The cis - DDP was given 15-45 minutes before the irradiation. What was the reason for the wide variation of timing and was there any systematic variation in such a way that, e.g., long intervals predominated in certain fractionation groups?

Authors: In 90% of the animals about 30 minutes passed before drug injection and irradiation but

some variation in this time interval could for practical reasons not be avoided. However, according to drug kinetic studies from Yukilide et al. (1984) in animal systems the absorption of cis-DDP from an i.p. injection takes place very rapidly and plasma peak concertration is reached within 15 minutes.

J. Reitan: The upper panel in Fig. 9 shows a parallel degree of ciliary damage in irradiated and control epithelium. Why is there a dose variation in the unirradiated control areas instead of a constant degree of damage after cis - DDP? The same question may be raised regarding the lower panel, and also Fig. 15.

Authors: In radiotherapy one always has to work with two parameters: dose and time in fractionated irradiation and sometimes with three as in this paper. We are fully aware of this and therefore show the effect both in relation to Gy (Figs. 9, 15) and time (Figs. 18 and 19). With the combination of these results we think that it is possible to interpret the observations made in this paper. The figures are now completed with the results of ultrastructural changes of irradiation alone which has been extracted from an earlier paper (Albertsson et al., 1983). Fractionated irradiation alone results in an increased amount of blebs on the cilia but no broken cilia or loss of cilia. No P - cells, Q - cells or R-bodies are seen. These phenomena are believed to represent the cytotoxic effect of cis - DDP with cell death and cell loss of the ciliated cell layer and is seen both in the irradiated part and control part in the animals treated with cis - DDP. The variation in dose in Figs. 9 and 15 is believed to reflect the normal turn over rate of the ciliary epithelium which is about 3 weeks. This is supported by the findings in Figs. 18 and 19 where ciliary damage, Q - cells, P - cells and R - bodies are plotted as a function of time after 5 mg cis - DDP.

K.E. Carr: Were any statistical analyses carried out on the data used for the graphs?
Authors: No.

K.E. Carr: What are the main differences between P and Q - cells?
Authors: They were scored from SEM micrographs where they have quite a different appearance. P-cells are single protruding cells not covered by cilia although sometimes remnants of cilia are present. Q - cells are confluent cell areas, with a surface covered by microvilli-like structures, regularly arranged on the surface. Q - cells could represent a late stage of ciliary cell destruction or early stage of regeneration. They appear late in the treatment schedule and further studies are planned with examination 15 days after completion of irradiation instead of ten days, in order to be able to further distinguish between P and Q - cells by more detailed TEM examination.

B.A. Afzelius: The message of the paper is that a combined treatment with the drug cis - DDP and X-irradiation will cause a transient loss of the cilia and you speculate that the loss is due to the effect of the drug alone. There is a way to examine this namely to use the drug alone (on rabbits that have not been X-irradiated). Why has this not been examined?

Authors: In this paper it has been observed that in the part of the trachea which has not been exposed to fractionated irradiation, a reaction for cis - DDP is noted. Further investigation of the tracheal epithelium in rabbits exposed only to cis - DDP is a logical consequence. We have started with these experiments and the current paper is part of a major research program.

B.A. Afzelius: The doses of the drug are given as a quantity per square meter. (e.g., 30 mg/m^2). Why m^2?

Authors: In the clinic concerned with chemotherapy treatment, the dosage of the drug is always mg/m^2. In this paper we have referred to clinical investigations where the dose of cis - DDP is in mg/m^2. This has been converted to a corresponding dose for rabbits.

B.A. Afzelius: You give evidence for the drug cis-DDP to cause a loss of cilia from the ciliated cells. This parallels the findings by Sato et al. (Exp. Mol. Pathol. 43 13, 1985) who found that another anticancer drug, bleomycin, causes damage to the ciliated airways in that there were a larger number of abnormal ciliary axonemes. The orientation of the cilia remained the same. Did you examine the ciliary axonemes or the orientation of the cilia?

Authors: We are aware of the work considering bleomycin but have not yet had time to examine the axonemes or the orientation of the cilia. This will be done at a later date.

B.A. Afzelius: One way to differentiate between goblet cells and the cells that have been ciliated but have lost their cilia (and which are called Q-cells by you) is to examine whether the cell surface has glycocalyceal bodies (see Afzelius, Ultrastruct. Pathol. 7, 1, 1984). Did you look for the glycocalyceal bodies?

Authors: Unfortunately, we have overlooked this. We are grateful for the comment.