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Lung damage in the rat after irradiation and treatment with cytotoxic drugs

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Interstitial pneumonitis (IP) is one of the major complications after allogeneic bone marrow transplantation (incidence: 30 to 50%, fatality rate: 50 to 60%) (Lopes Cardozo & Hagenbeek, 1985). Cytomegalovirus is associated with 60% of the cases. When no infectious agent can be detected, it is classified as idiopathic IP (IIP). Factors related to IIP are: remission induction chemotherapy, pretransplant conditioning regimen – usually including total body irradiation and cyclophosphamide (CY) – Graft-*versus*-Host disease and post-transplant immunosuppression. The influence on the lung by these various factors, separately and in combination, is presently being investigated in a rat model. Consecutive studies have been focussed on lung injury by radiation, CY and cytosine arabinoside (Ara-C) (Collis, 1980; Haupt *et al.*, 1981). Parameters for evaluation of lung damage were lung function measurements (Travis *et al.*, 1980), animal survival (LD_{50-180}) and histopathology. Detailed histological examination will be dealt with elsewhere.

Materials and methods

Experimental animals All studies were carried out with female BN/Bi/Rij rats, 10–12 weeks of age, maintained under conventional conditions.

Irradiation and anaesthesia set-up The enflurane anaesthesia set-up and the arrangement for whole lung exposure of rats to 300 kV of X-rays (HvL: 3 mm Cu) have been described previously (Lopes Cardozo *et al.*, 1985).

Cytostatic drugs Cyclophosphamide (Cy) provided by ASTA, Weesp, The Netherlands, was dissolved in 0.9% NaCl and injected intraperitoneally. Ara-C provided by Upjohn, Ede, The Netherlands, was dissolved in 0.9% NaCl and injected intravenously (tail vein).

Lung function studies Measurements of the ventilation rate (VR) were performed with a whole body plethysmographic tube and a Fleisch type pneumomechanical flow transducer head (type: 3 × 0) connected to a pneumotachograph (Coggins *et al.*, 1981). Storage of the data was microcomputer controlled.

Experimental designs

I. *Cyclophosphamide* (A) Groups of 8–16 rats were treated with different doses of CY (range 100 mg kg^{-1} to 250 mg kg^{-1}) on day 0. To prevent death from aplasia 10^8 isologous BM cells were given on day 1 and packed blood cells on day 4 till day 7. Lung function studies were performed at one week intervals after treatment. From each group, animals were killed after 1 week, 2 weeks and 1, 3, 6 and 9 months for histopathology.

(B) Other groups of 8 animals were treated with 100 mg kg^{-1} CY on day 0, and on day 1 received bilateral thorax irradiation with single doses of X-rays at dose rates of 0.05 Gy min^{-1} low dose rate (LDR) and 0.8 Gy min^{-1} high dose rate (HDR). The dose ranges investigated were 16 to 24 Gy for the LDR and 8 to 18 Gy for the HDR. Parameters of evaluation were survival, lung function and histopathology.

II. *Ara-C* (A) A group of 20 rats received 100 mg kg^{-1} Ara-C on 7 consecutive days. Lung function studies were performed with 10 day intervals after treatment and animals were killed after 1 and 3 months.

(B) Other groups of 10–14 animals were treated with 100 mg kg^{-1} Ara-C on day −7 and received bilateral thorax irradiation with single doses of X-rays at a dose rate of 0.8 Gy min^{-1} (HDR). The dose range investigated was 13 Gy to 18 Gy. Parameters of evaluation were survival, lung function and histopathology.

Observations in experiments I and II were compared to data from experiments with groups of 10 rats receiving thorax irradiation only, with 14–24 Gy (LDR) and 8–20 Gy (HDR).

Results

I. *Cyclophosphamide*

(A) *Survival* In the 100 mg kg^{-1} treated group only 1 out of 16 rats died before day 180. When

higher doses were given, mortality before day 20 due to intestinal tract injury, bladder damage, aplasia and sepsis was observed but no mortality due to lung damage.

Lung function studies The VR did not show any difference compared to untreated rats.

(B) *Survival* A change in mortality pattern was observed compared to rats receiving lung irradiation without CY pretreatment. Rats treated with CY before lung irradiation died acutely (before day 14) or early (± day 60) in the groups receiving higher dosages, and delayed (after day 180) in the lower dose groups. In the rats that received irradiation only, no acute mortality was observed, but in addition to early and delayed mortality also intermediate mortality (around day 150) was seen. Acute mortality was due to sepsis and aplasia and not to lung damage. Early mortality was due to pleural fluid accumulation and vascular damage in the lungs. Intermediate mortality was caused by pneumonitis and early fibrosis. With regards to the delayed mortality histopathology is not yet available. Figure 1 shows a Weibull analysis of the mortality on day 180 with exclusion of the acute mortality plotted against the absorbed doses. After CY pretreatment, the LD_{50-180} for the LDR group is 19.95 Gy (±0.002 Gy) and the LD_{50-180} for the HDR groups is 14.44 Gy (±0.029 Gy). The LD_{50-180} for rats receiving only thorax irradiation are 22.07 Gy (LDR) and 13.78 Gy (HDR). CY treatment prior to LDR thoracic irradiation shifts the mortality curve to the left with a statistically significant difference of 2.12 Gy. For the HDR groups the curve shifts 0.66 Gy to the right.

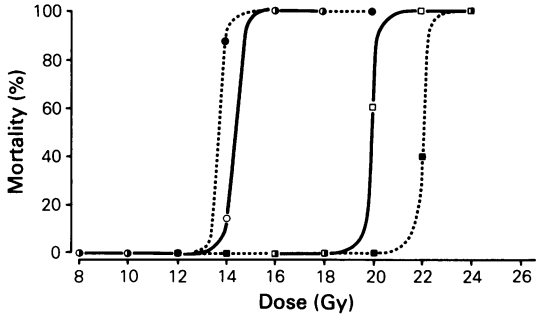

Figure 1 Mortality of rats at 180 days after treatment with thoracic irradiation alone or in combination with high-dose cyclophosphamide. (○) CY (100 mg kg^{-1}) + thoracic irradiation (0.8 Gy min^{-1}); (□) CY (100 mg kg^{-1}) + thoracic irradiation (0.05 Gy min^{-1}); (●) thoracic irradiation (0.8 Gy min^{-1}) (■) thoracic irradiation (0.05 Gy min^{-1}).

Lung function studies An increase in VR with a maximum before death was observed in the groups irradiated with the highest doses. No changes in VR were seen in the lower dose groups. In the groups in between a temporary increase around day 60 was seen (data not shown).

II. *Ara-C*

(A) *Survival* Until now (day 623) no mortality was observed in the Ara-C treated rats.

Lung function studies The lung function did not show any difference compared to untreated rats.

(B) *Survival* Preliminary data show that the mortality pattern (early, intermediate and delayed) of Ara-C pretreated rats is the same as in rats which are irradiated only. Figure 2 shows a Weibull analysis of the mortality on day 147 (day 180 has not yet reached) plotted against the absorbed doses compared to rats receiving only HDR thoracic irradiation. The LD_{50-147} is 15.57 (±0.018) for the Ara-C pretreated groups and 15.03 (±0.0017) for the groups treated with irradiation alone. There is a shift of 0.54 Gy of the curve to the right after Ara-C pretreatment.

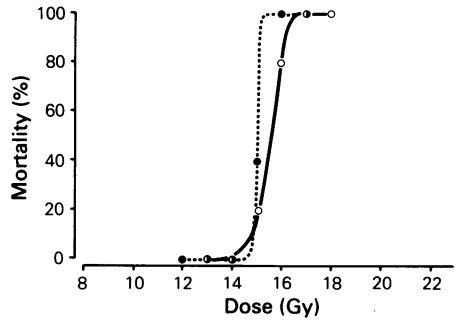

Figure 2 Mortality of rats at 147 days after treatment with HDR thoracic irradiation alone or in combination with Ara-C (100 mg kg^{-1}) (●) thoracic irradiation (0.8 Gy min^{-1}); (○) Ara-C (100 mg kg^{-1}) + thoracic irradiation (0.8 Gy min^{-1}).

Lung function Figure 3 shows the mean VR of the different groups plotted against time. Starting at around day 35 an increase in VR was seen in the 15, 16, 17 and 18 Gy groups. Some of the 15 and 16 Gy group and all rats of the 17 and 18 Gy group died around day 60, with breathing frequencies up to 300 before death. Around day 80 the survivors of the 15 and 16 Gy group returned to normal and slightly raised breathing frequencies respectively. The 13 and 14 Gy group showed neither enhancement of the VR nor mortality. A second period of increase in breathing frequency was

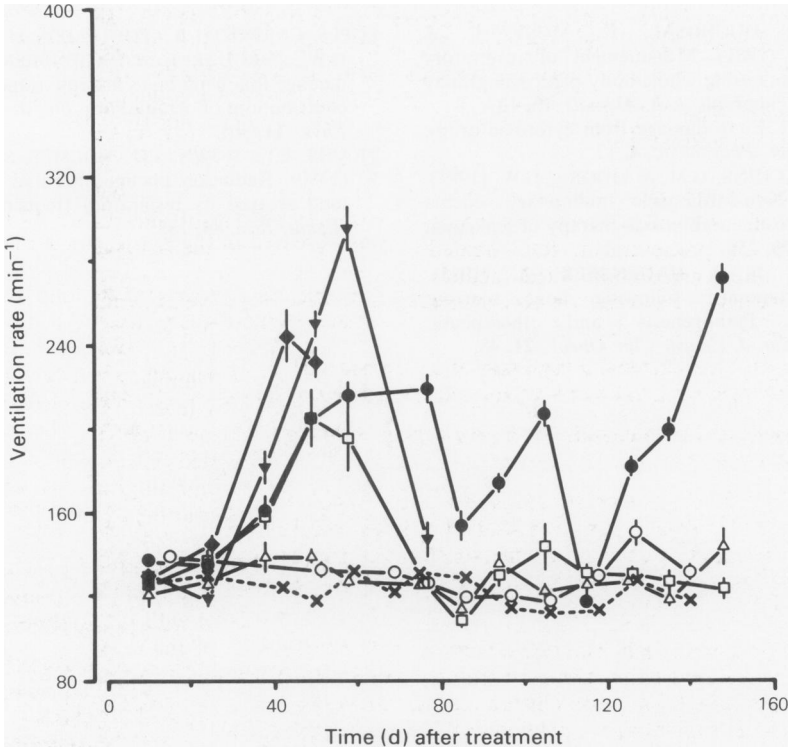

Figure 3 Ventilation rate after treatment with Ara-C (100 mg kg^{-1}) and thoracic irradiation (0.8 Gy min^{-1}). (○), 13 Gy; (△), 14 Gy; (□), 15 Gy; (●), 16 Gy, (▼), 17 Gy; (◆), 18 Gy; (X) Control (0 Gy).

observed starting after day 118 in the 16 Gy group, corresponding with the period of intermediate mortality. In the groups which received only lung irradiation a similar response was observed at an irradiation dose of 1 Gy less (data not shown).

Discussion

From mortality pattern and lung function studies it may be concluded that two types of radiation lung damage in the BN rat are seen before day 180: early and intermediate. The recovery observed between these periods makes it likely that two different targets are involved.

As far as evaluable, CY alone did not induce significant pulmonary toxicity, but an evident increase of radiation lung damage by CY in the LDR groups is observed while a slight protection seems to occur in the HDR groups. A possible explanation for this observation is that CY decreases the repair capacity of the lung. This effect exceeds the protecting effect seen in the HDR groups, and is possibly also responsible for the disappearance of the intermediate mortality around day 150.

The slight protection against radiation lung damage by Ara-C is expected to be more pronounced at 180 days, when the intermediate period of death will have passed. Compared to rats irradiated only, the LD_{50-180} will probably decrease by ± 1 Gy, in line with the observation in the lung function studies.

The lung is a complicated organ with different target tissues for chemo-radiotherapy. Several variables e.g. the radiation dose, the dose rate, the drug dose and the time interval will initiate a different effect. Further experiments and histopathological evaluation are at present in progress to unravel their interaction.

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