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Pulmonary toxicity of the combination of bleomycin and peplomycin – an experimental study in rats

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Summary. Combination of the two drugs bleomycin (BLM) and peplomycin (PEP) may enhance their antineoplastic effects; however, it is not known as to whether this enhancement is accompanied by a concomitant increase in toxicity, especially toxic lung damage. A histologic and stereologic investigation was carried out to compare the pulmonary toxicity of BLM, PEP, and a combination of the two drugs in animals. A total of 180 female Wistar rats were randomly assigned to 4 groups: 45 animals were treated with daily i.p. injections of 4 mg/kg BLM, 45 rats received 2.5 mg/kg i.p. PEP daily, 45 animals were given i.p. injections of a combination of 2.5 mg/kg BLM and 1 mg/kg PEP, and 45 rats received 1 ml 0.9% NaCl solution. Histological examination of the lungs demonstrated varying degrees of exudative and fibrosing alveolitis in animals treated with BLM, PEP, and BLM-PEP. Stereological analysis revealed a significant thickening of the alveolar wall after 20–60 days and a significant decrease in the surface density of alveolar walls after 40–60 days in all treated groups. Both histological examination and stereological parameters indicated more pronounced inflammatory changes in the alveolar walls and a prior loss of alveolar surface after 20 and 30 days in animals receiving PEP and PEP-BLM as compared with those undergoing BLM treatment. After 40–60 days, during which time irreversible fibrotic changes prevailed, significant stereological differences between the three treated groups could not be detected. Thus, our experimental observations did not show any potentiation of the toxic pulmonary effect of BLM and PEP following their combined administration.

Introduction

Bleomycin (BLM), a mixture of different glycopeptides obtained from *Streptomyces verticillatus* [24], is clinically widely used as an antineoplastic drug [2]. Its therapeutic application, however, is limited by pronounced pulmonary toxicity, which may lead to fatal lung fibrosis [7, 13, 26].

Peplomycin (PEP), one of the new biosynthetic bleomycins [11], shows equivalent, if not higher antitumor activity in clinical and experimental studies [17]. The overall toxicity of PEP is also higher than that of BLM [12, 22], but it has been suggested that the pulmonary damage induced by the former may be less severe than that resulting from BLM treatment [9, 22, 23, 27].

Experimental studies have provided evidence that a combination of PEP and BLM may potentiate their antineoplastic effects [18, 19]; this effect would be of clinical significance only if there were no concomitant potentiation of the pulmonary toxicity. In the present study, the pulmonary damage following the administration of a combination of these two drugs was investigated in an experimental model. Quantitative stereological methods were used to obtain objective appraisals of the pulmonary changes.

Materials and methods

Experimental model. A total of 180 specific pathogen-free and Sendai virus-free female Wistar rats weighing 190±4 g (Fa. Ivanovas, Kisslegg, FRG) were randomly assigned to 4 groups. They were caged individually. In all, 45 animals were treated with daily i.p. injections of 4 mg/kg BLM (Mack, Illertissen, FRG), 45 rats received 2.5 mg/kg i.p. PEP daily (Mack, Illertissen, FRG), 45 animals were given i.p. injections of a combination of 2.5 mg/kg BLM and 1 mg/kg PEP, and 45 rats received 1 ml 0.9% NaCl solution. After 10, 20, 30, 40, 50, and 60 days, five animals randomly selected from each group were killed.

Tissue preparation. The viscera were fixed by vascular perfusion at a pressure of 110 mmHg after catheterization of the abdominal aorta [15, 16]. The pulmonary tissue was fixed in the expiratory position and then postfixed in 3% ice-cold paraformaldehyde for 2 days. One randomly selected lung per animal was embedded in toto in Paraplast. Next, 4-μm sections were stained with hematoxylin-eosin, elastica van Gieson, PAS, and Ladewig's solution for light microscopic investigations.

Stereology. A random set of five to seven parallel, equidistant 4-μm sections of the lung were investigated histologically and stereologically. Volume and surface densities (Vv, Sv) were estimated according to

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Table 1. Mean alveolar wall thickness in the control group and in the groups treated with the antineoplastic drugs BLM and PEP alone or in combination

	Days of treatment					
	10	20	30	40	50	60
Control group	7.9±0.2	8 ±0.2	8 ±0.1	8.3±0.2	8.2±0.3	8.5±0.3
BLM	8.6±0.3	8.9±0.3	9.8±0.2	10.3±0.2	10.7±0.4	10.7±0.1
PEP	8 ±0.4	10.5±0.3	12.2±0.4	9.6±0.4	10.9±0.4	11.6±0.1
BLM+PEP	8 ±0.2	9.8±0.2	11.2±0.4	10.2±0.3	11 ±0.3	9.7±0.2

Data represent mean values ± SE expressed in micrometers

Table 2. Surface of the alveoli per volume of lung tissue in the control group and in the groups treated with the antineoplastic drugs BLM and PEP alone or in combination

	Days of treatment					
	10	20	30	40	50	60
Control group	718±21	683±21	689±12	696±13	696±18	691±12
BLM	700±20	696±17	710±14	599±12	567± 9	580±12
PEP	690±18	604±12	644±11	602±10	520±19	559± 5
BLM+PEP	664± 8	622±14	613±15	602± 9	505±17	561± 8

Data represent mean values ± SE expressed as S_V (cm^2/cm^3)

Table 3. Volume of the alveolar walls per volume of lung tissue in the control group and in the groups treated with the antineoplastic drugs BLM and PEP alone or in combination

	Days of treatment					
	10	20	30	40	50	60
Control group	0.285±0.007	0.273±0.009	0.274±0.007	0.290±0.010	0.288±0.015	0.295±0.003
BLM	0.300±0.006	0.321±0.013	0.348±0.012	0.310±0.006	0.307±0.017	0.312±0.007
PEP	0.277±0.007	0.319±0.007	0.396±0.016	0.291±0.009	0.285±0.005	0.325±0.009
BLM+PEP	0.266±0.007	0.307±0.013	0.347±0.013	0.309±0.006	0.276±0.005	0.282±0.013

Data represent mean values ± SE expressed as V_V (cm^3/cm^3)

standard methods [25]. The stereological analysis was performed at two stages of magnification: volume density was calculated using a magnification of 40 : 1 (Zeiss eyepiece with 100 points) in 15 test areas, and the volume of alveolar walls per unit volume of alveoli was determined using a magnification of 415 : 1 (Zeiss eyepiece with 100 points and 10 test lines) in 25 test areas.

The ratios of stage 2 were multiplied by the volume density of alveoli (stage 1) to estimate the V_V and the S_V of the alveolar walls. The reference volume was the total pulmonary tissue. Additionally, from the V_V and S_V of alveolar septa, we obtained the volume-to-surface ratio ($V_S = V_V/S_V$). The mean alveolar wall thickness (T) was determined using the stereological ratio $2 \times V_S$, which was considered as to yield an approximative estimate.

Statistics. The arithmetic means and standard errors were calculated in each group for each period of treatment. One-way analysis of variance was used to compare the arithmetic means of the four groups for each treatment period. Scheffe's test was applied to detect significant differences between the treated groups for each period of treatment [21]. The result was considered to be significant if $P < 0.05$.

Results

The body weights were statistically significantly decreased in all treated groups as compared with the control group after 10 days and remained low throughout the experiment. Significant differences were not found between the treated groups.

Histology

The histological examinaton revealed a normal pulmonary structure in all animals of the control group throughout the course of the experiment. In all treated groups, changes in the alveolar structure could be established that depended on the duration of treatment and corresponded to an exudative alveolitis that was maximal on day 30 and had progressed to a slightly fibrosing alveolitis with focal accentuation by days 50 and 60. Fibrotic changes seemed to be most constant and severe in the BLM group, whereas they were more variable in the PEP and BLM-PEP groups.

Stereology

The mean alveolar wall thickness (T; Table 1) was significantly increased in all treated groups from day 20 to day 60. Differences between the BLM group and the groups treated otherwise were found on days 20 and 30. T was

significantly higher in the PEP group than in the BLM group after 20 and 30 days ($P < 0.01$); after 30 days the value calculated for the BLM-PEP group was higher than that determined for the BLM group but lower than that found for the PEP group ($P < 0.05$).

The surface density of alveolar walls (S_V; Table 2) was different in the treated groups from day 20 to day 60. The PEP group and the BLM-PEP group showed significantly lower S_V values than did the BLM group ($P < 0.01$). The volume density of alveolar walls (V_V; Table 3) was increased in all treated groups as compared with the control group. After 30 days, the values found for the PEP group were significantly higher than those calculated for either the BLM group or the BLM-PEP group.

Discussion

Müller and co-workers [18, 19] have detected a synergistic, if not potentiated, effect on the antineoplastic activity of BLM and PEP in mouse lymphoma cells in vitro and in vivo. Such an increase in activity could be of significant clinical importance if it were not curtailed by a concomitant rise in pulmonary toxicity.

PEP has been used clinically in Japan since 1981, and clinical studies have shown that the pulmonary toxicity of PEP is lower than that of BLM despite the higher general toxicity of the former drug [17]. Experimental studies on pulmonary tissue have yielded contradictory results: Ginsburg et al. [12] and Raisfeld [20] found equivalent overall and pulmonary toxicity for PEP, whereas Sikic et al. [22] and Yoshida et al. [27] reported observing low pulmonary toxicity for PEP despite its high lethality. The assessment of pulmonary toxicity in these studies was based on lung-hydroxyproline levels, which reflect the pulmonary collagen content, but not necessarily the extent of pulmonary fibrosis (see [3]) or functional impairment. It should be emphasized that several factors contributing to lung damage such as inflammatory response, alveolar collapse, collapse induration, epithelial metaplasia, and subsequent loss of respiratory surface can be detected only by histology and quantitatively assessed by stereological parameters.

In the present study, daily treatment with either 4 mg/kg BLM or 2.5 mg/kg PEP was compared with a combination of 2.5 mg/kg BLM and 1 mg/kg PEP daily, taking into consideration the difference in the toxicity of these two drugs (PEP being approx. 1.5 times more toxic than BLM). Preliminary experiments (unpublished data) had revealed that these doses were approximately biologically equivalent, with lower doses causing no significant lung damage and higher doses resulting in high mortality due to general toxicity and wasting of the animals.

The pathogenetic events and histologic changes involved in BLM-induced lung damage, which may be characterized as a toxic exudative and fibrosing alveolitis, are well known from various observations in humans [5] and animal experiments ([3, 4, 6] reviewed in [5]). The histological examination revealed pulmonary damage of this type in all treated groups. There seemed to be minor variations in the severity and time of appearance of these features among the three treated groups, with the PEP group exhibiting the most variable pattern. The histological changes observed in the BLM-PEP group did not seem to exceed those noted in the PEP group. The stereological analysis disclosed significant effects of the treatment from day 10 to day 60, and the parameters were consistent with the changes observed histologically.

In the early stage (10–30 days), the increase in the volume density (V_V) and thickness of alveolar walls ($2 \times V_S$) was related to edema and infiltration of inflammatory cells, mainly alveolar macrophages and a few neutrophilic granulocytes [1, 7, 10, 14]. In this stage, some differences were observed between the treated groups, indicating that PEP and, to a lesser extent, BLM-PEP induced a more pronounced inflammatory reaction and an earlier collapse of alveoli than did BLM. These findings are compatible with additive rather than with significant synergistic effects of the drugs. In contrast to fibrosis, both the inflammatory reaction and the alveolar collapse must be considered to be reversible, at least to a certain extent, and may be influenced by prophylactic measures.

After 40–60 days, during which period irreversible fibrotic changes prevailed, we did not find any significant quantitative differences between the PEP-BLM group and the groups treated with BLM or PEP alone. Our stereological results are in agreement with the morphometric results of Chandler et al. [8], who describe an increase in the numerical density of inflammatory cells followed by an increase in that of fibroblasts after 40 days of BLM treatment. These findings correspond well to the changes observed during the early and late stages of the present experiment.

The histological and stereological data presented demonstrate that combined antitumor therapy with BLM and PEP is no more pulmonotoxic in terms of fibrotic changes than is monotherapy with PEP or BLM, although the early exudative alveolitis elicited by the combination is more severe than that produced by treatment with BLM alone but less severe than that resulting from PEP treatment alone. Thus, our experimental results indicate that the clinical application of the combined antineoplastic therapy (BLM and PEP), which promises an enhanced antineoplastic effect, may not be precluded by a potentiating effect on the development of pulmonary fibrosis.

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