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COMPARISON BETWEEN THE IMMEDIATE EFFECTS OF PHOTON AND ELECTRON RADIATION ON THE CILIARY ACTIVITY OF THE TRACHEA OF THE RABBIT

An in vitro investigation

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Abstract

The immediate effects of 6 MV roentgen rays and 4 MeV electron rays on the mucociliary activity of the trachea of the rabbit were studied. In spite of the same average LET for the two radiation qualities, there was a clear difference in their effects on the ciliary motility during irradiation. The increase of the mucociliary activity after an absorbed dose of 1.5 Gy roentgen rays reported earlier was confirmed by the present investigation when using 6 MV photons. Electron radiation increased the beat frequency to a corresponding degree already at an administered dose of 0.5 Gy. The differences found in the pattern of the mucociliary activity during irradiation are discussed. The results indicate that the conventional RBE values cannot be used for early physiologic effects of irradiation.

The ciliated epithelium of the trachea has proved to be an almost unique test object for investigating the early (e.g. during irradiation) and the late (i.e. hours or days after irradiation) effects of ionizing radiation. In a study of the physiologic activity of the cilia (their beat frequency), BALDETORP et coll. (6) were able to demonstrate an increased beat frequency when their preparations were exposed to radiation. This increase was usually between 20 and 25 per cent. These findings again raised the well-known question in radiation biology as to the stimulation induced by radiation.

It was assumed that this increase of the beating frequency was the result of influence on the ATP producing mitochondria of the ciliated cells. Although the underlying mechanism of this phenomenon is not fully understood, the results of low-voltage roentgen rays (50 kV) and those of gamma radiation from a cobalt-source have always been a stimulation of the beating frequency, and this has been valid for different dose rates, as well. As to the immediate reaction following irradiation, FUJIWARA et coll. (14) found a decrease of the activity in the respiratory tract by single doses up to 30 Gy, which was restituted within two hours.

No experiments have so far been conducted with photon energies higher than ^{60}Co or with high energy electrons, but since their use in clinical radiation therapy is of increasing importance, experiments are called for to gain information about their interaction with matter.

The present study has aimed at investigating the effects of 6 MV photons on the ciliary beat frequency, and to compare these effects with those of 4 MeV electrons. As in previous investigations, the focus of interest has been limited to the events during irradiation, as these studies touch on a controversial problem within radiation biology—radiative stimulation.

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Material and Methods

The trachea from 20 healthy rabbits was used, ten animals being assigned to each radiation quality. They were sacrificed by a blow on the head, after which the trachea was immediately dissected and placed into the experimental chamber, which had a temperature of 37°C and a humidity of more than 90 per cent. The specimens, which were about 30 mm in length, were placed in the manner described by HÅKANSSON & TOREMALM (20), and stretched to their original length (15–20 per cent more than in the collapsed state). They were then opened by an incision in the membranous part (length 20 mm), and left to adapt to the chamber conditions during 30 minutes. The recording of the beating cilia was made according to the method of BALDETORP et coll. (6), which permits examination during the irradiation of the ciliated epithelium. Before starting irradiation, it was first ascertained that the beat frequency was stable, i.e. that the specimen had been adapted to the conditions in the chamber. The beat frequency was then registered continuously during 30 s before the treatment.

Irradiation was performed with two different qualities: 1) Photons generated at 6 MV from a linear accelerator (Siemens Mevatron 6) with FFD = 600 mm, and the field size 100 mm × 100 mm at the focus-object distance 680 mm. In order to get an improved roentgen ray spectrum with regard to high energy photons, a Cu flattening filter was utilized in the linear accelerator. The filter had a high absorption coefficient for low energy photons up to 2 MV. Absorption was reduced at higher energies, with a minimum at 8 to 9 MV.

The dose rate was 0.07 Gy/s at the tracheal mucous membrane. The dosimetry was performed with thermoluminescent dosimeters (TLD) at the same place as the tracheal specimen in the experimental chamber. The deviation from the calculated dose rate was within 3.5 per cent. Each tracheal specimen received 10 Gy.

2) Electrons with an energy of 4 MeV from a linear accelerator (M.E.L. SL 75, Philips). With an electron therapy applicator mounted on the treatment head, the field size was 100 mm × 100 mm at a focus-object distance of 1 080 mm. A thin Al foil was inserted in the treatment head in order to absorb scattered electrons from the main beam. The dose rate was 0.05 Gy/s at the tracheal mucous membrane. Dose measurements were carried out with plane parallel ionization chambers. Each tracheal specimen received 5 Gy.

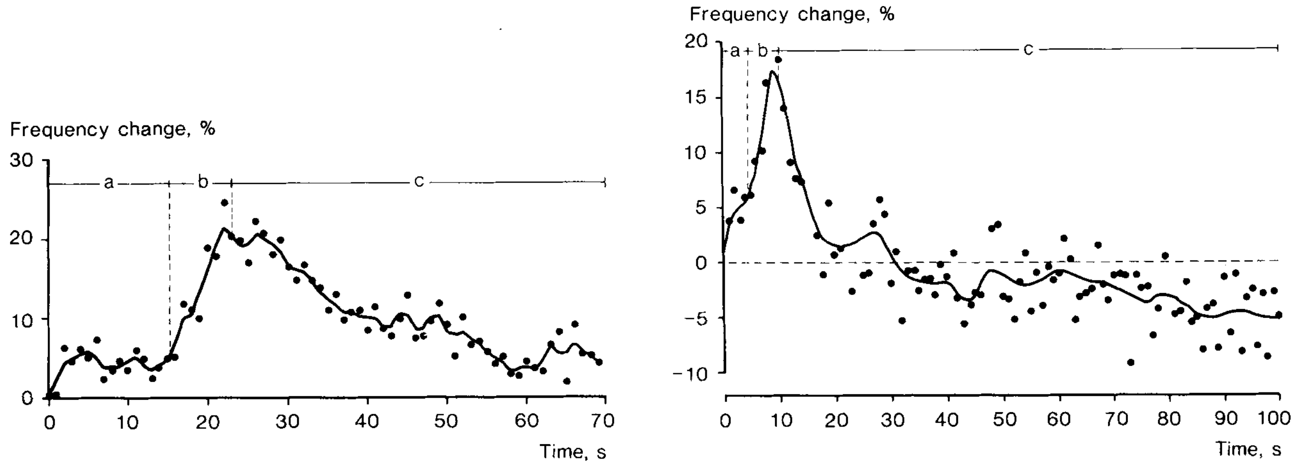
Table 1

Mean values for the increase of the mucociliary activity during irradiation with photons 6 MV (n=10)

Time after start of irradiation (s)	Accumulated dose (Gy)	Increase of mucociliary activity (%±SD)
5	0.35	4.5±2.1
10	0.70	4.2±1.7
15	1.05	5.7±3.5
20	1.40	13.7±7.2
25	1.75	20.0±2.3
30	2.10	17.8±2.6
35	2.45	13.7±2.5
40	2.80	10.4±2.1
45	3.15	9.3±1.7
50	3.50	8.9±2.4
55	3.85	6.3±2.3
60	4.20	4.4±1.5
65	4.55	6.2±2.5
70	4.90	6.1±2.2
75	5.25	5.6±1.3
80	5.60	6.9±2.9
85	5.95	7.4±3.0
90	6.30	7.3±2.4
95	6.65	6.6±2.4
100	7.00	6.1±1.4
105	7.35	5.0±2.0
110	7.70	4.5±2.9
115	8.05	5.9±2.0
120	8.40	8.6±1.5
125	8.75	6.3±1.6
130	9.10	5.5±2.3
135	9.45	5.7±2.4
140	9.80	4.7±1.6

Results

Photon radiation. During the 30 seconds before the start of irradiation, the beat frequency varied in the different specimens from 14.5 to 22.5 beats/s, when calculated second-by-second. Despite this difference in beat frequency, the frequency increase during the irradiation was nearly the same for all specimens. In the single specimen, the standard deviation (SD) was 1.0 beat/s, but for the whole material, the SD was 2.5 per cent. The mean value of these pre-radiation measurements constituted the reference value (zero-level) for the further measurements. Each specimen was thus its own control.



Changes in beat frequency during irradiation. Left: 6 MV photons, 0.07 Gy/s. Right: 4 MeV· electrons, 0.05 Gy/s. Each point represents the mean value from 10 specimens expressed in per cent of individual reference values established before irradiation. The smoothed curve was calculated by a computer. a = The initial increase. b = The delayed increase. c = The decrease.

Table 2

Mean values for the increase of the mucociliary activity during irradiation with electrons 4 MeV (n=10)

Time after start of irradiation (s)	Accumulated dose (Gy)	Increase of mucociliary activty (% ± SD)
5	0.25	8.8±5.0
10	0.50	12.5±4.1
15	0.75	6.5±4.5
20	1.00	2.4±3.2
25	1.25	2.3±3.4
30	1.50	0.6±3.5
35	1.75	−1.6±1.8
40	2.00	−2.1±1.9
45	2.25	−1.1±3.1
50	2.50	−0.9±3.0
55	2.75	−2.3±2.0
60	3.00	−1.5±2.2
65	3.25	−1.5±2.4
70	3.50	−2.1±2.9
75	3.75	−3.0±3.1
80	4.00	−2.6±3.7
85	4.25	−5.0±1.9
90	4.50	−4.9±2.8
95	4.75	−4.7±2.9
100	5.00	−5.3±2.7

The mean values of the frequency change for each five-second period after the start of irradiation are given in Tables 1 and 2.

The percentual changes of the beat frequency for 6 MV photons are shown in the Figure. During the first 15 seconds after the onset of radiation the level of the beat frequency increased with 4 to 5 per cent, which was statistically significant ($p<0.0002$). This period was called the initial phase. Later, when the trachea had accumulated a dose of about 1.0 Gy, there was a rapid increase (during 5 s) of the beat frequency up to a maximum of about 20 per cent. The accumulated dose was then 1.75 Gy (the Figure and Table 1). This increased level was maintained for about 5 seconds. During the continued radiation, the frequency change decreased during the following 30 seconds to a value similar to the increase during the 'initial phase' of 15 seconds (5%) (the Figure). This increase remained constant during the whole exposure time of 143 seconds (Table 1), and was statistically significant ($p<0.0002$).

Electron radiation. During irradiation with electrons the beat frequency increased in the same way as during the photon irradiation but the initial phase lasted only 5 seconds. The maximum increase during this period, however, was the same and also statistically significant. The initial phase was followed by another increase much more rapid than for photons, but the maximum peak was somewhat lower (18%) (the Figure). This peak was reached after 10 seconds of irradiation, corresponding to an absorbed dose of 0.5 Gy, and lasted only for a few seconds. The frequency change then decreased to the zero-level after an absorbed dose of 1.5 Gy (30 s). The activity steadily decreased during the remainder of the exposure, and during the final part of treatment it was 5 per cent below the reference level (the Figure and Table 2). The total dose absorbed during this time (100 s) was 5.0 Gy.

Discussion

The discussion will first deal with some factors of importance for the interpretation of the results: Total absorbed dose, physical differences between the electrons and photons and their interaction with matter and energy consumption of the ciliae.

The results obtained with both 6 MV photons and 4 MeV electrons agree quite well with those reported by BALDETORP et coll. (5, 6) and by BALDETORP & HÅKANSSON (4), i.e. there was a stimulation of the beat frequency during the entire course of photon irradiation, or during some part of it. The appearance of this stimulation was however, quite different. During photon irradiation the maximum increase came after an absorbed dose of 1.75 Gy (25 s). The frequency had actually increased significantly (5%) during the first 15 seconds, but the great increase came suddenly, as if caused by some triggering mechanism. During electron irradiation, the course of events was much more rapid and occurred at a lower total absorbed dose. In principle, the reaction during electron irradiation took place at an absorbed dose only one-third of that observed during photon irradiation. The difference in the curves in regard to the maximum increase was, however, not significant. Another important phenomenon was that the decrease during electron irradiation came earlier than during photon irradiation, and that it furthermore dropped significantly below the zero-level.

Photons are indirectly ionizing, i.e. the Compton effect is mainly responsible for producing free radicals and ions, of which the radicals are chemically active and are known to exert a destructive influence.

Electrons are on the other hand directly ionizing and produce the same chemical reagents as photons. In clinical applications, the RBE factor is considered to be the same for both types of radiation. Determinations of RBE values are, however, based on late effects on cultivated cells or on animal tissues several days after irradiation. Such RBE factors are therefore inapplicable in the present case, when the radiation interacts with a physiologic activity during irradiation.

LET is the energy released per micron of medium (keV/μ). High LET radiation generally produces more damage to the cells than low LET radiation. RBE will usually increase with increasing LET (7). The average LET for 6 MV photons is the same as for 4 MeV electrons in regard to the calculated track average or the energy average (24). It is in spite of these values quite possible for two different types of radiation, which have the same average LET, to produce different biologic effects (21). The different kinetics for ciliar activity during photon and electron radiation, respectively, must therefore be considered together with some basic physiologic facts:

The energy of the beating cilia is provided through the hydrolysis of ATP by the dynein ATP-ase (18, 19). BROKAW & BENEDICT (12), furthermore, demonstrated a correlation between the beat frequency and the rate of hydrolysis. HOFFMAN-BERLING (22) showed a direct relationship between the ATP concentration and the beat frequency in glycerinated models of trypanosomes and grasshopper spermatozoa. This has also been confirmed by others (9, 11, 16, 23, 28).

ATP is presumed to reach the cilium by passive diffusion from the cytoplasmatic matrix, which is continuous with the ciliary matrix (10).

Investigations using electron microscopy have produced reasonable evidence that the ATP-ase activity is distributed along the length of the cilium (1, 17, 25, 29). The rate of ATP supply by a diffusion process is more than adequate (10), which means that there is a surplus of ATP available for the ciliary motion under normal conditions. If, then, the ionizing radiation is able to increase the dynein ATP-ase activity, this would result in an increased rate of the ATP hydrolysis, and, consequently, increased ciliary beat frequency. Evidence for such a radiation induced increase of the ATP-ase activity has been found in case of irradiated lymphocytes (15), but with measurements done after irradiation. As enzyme reactions, however, are very rapid, it is logical to assume that they were present during the irradiation. A stimulation from ionizing radiation has also been demonstrated by BRANDT & BALDETORP (8), who found increased fagocytozing activity of macrophages during irradiation compared with normal circumstances. The radiation must, thus, stimulate the metabolic activity in some way, but there is as yet no clear agreement on its explanation.

Another hypothesis on the stimulation effect is based on the fact that ATP is generated within the cilium, and that more ATP may be created by an increased activity of the phosphotransferase-enzyme adenylate-kinase, as an effect of the irradiation. The presence of adenylate-kinase has been demonstrated in protozoans, as well as in higher species. This enzyme has, for example, been shown to take part in the transphosphorylation on Polytoma flagella and Tetrahymena cilia (9, 13). New ATP is thus resynthesized. This reaction is capable of controlling the amount of ATP in cilia and flagella (16, 27), and therefore the concentration of ATP is not solely dependent on passive diffusion.

The effect of irradiation on the physiologic events described, and which for the two radiation qualities used is characterized by the two different curves shown in the Figure, will be considered on the basis of the following postulation: The irradiation acts on the same biochemical system in the same manner. The biochemical processes should thus actually represent the same events, but have different kinetics.

The shape of the curves motivates a discussion on the course of events during three main phases: 1) The initial increase (a in the Figure), 2) the delayed increase (b), 3) the decrease (c).

1) *The initial increase*. The small but significant increase in the beat frequency, is a true phenomenon previously demonstrated (2, 4) for different photon radiation qualities and different dose rates. Little attention, however, has been paid to this response. Data from these earlier experiments were used in the present study to define the duration of the inital phase, and showed that it lasted 25 seconds when 50 kV roentgen-rays with a dose rate of 0.05 Gy/s were administered, which means following an absorbed dose of 1.25 Gy. Its duration was 65 seconds when ^{60}Co radiation was administered with a dose rate of 0.017 Gy/s (absorbed dose 1.11 Gy). In the present investigation, its duration was 15 seconds for photons (absorbed dose 1.05 Gy) and 5 seconds for electrons (absorbed dose 0.25 Gy). The increase up to 5 per cent in all experiments may indicate an intervention in the same biochemical system. This system is likely to be an action on the overflow of ATP in the cilium itself, as mentioned earlier (10). This explanation is, however, only a hypothesis, and requires further investigation for confirmation. The duration of this initial increase is dependent on the dose rate, but there is a striking difference between photons and electrons which cannot be referred wholely to the dose rate. Electrons obviously appear to have a three-fold greater efficiency, which is difficult to explain, as it does not agree with the general conception of RBE.

2) *The delayed increase*. Photons and electrons showed differing reactions in this phase. Electrons had a much faster effect, and the peak was reached at a much lower absorbed dose (0.50 Gy) than for photons (1.75 Gy). The biochemical action is not fully understood, but since the amplitude for electrons as well as for photons was about the same, it is assumed that the 'amount' of target material is the same in both cases. This question perhaps touches on the 'enzyme-release theory' of BACQ & ALEXANDER (3). It may also be that an activation of the dynein is triggered by the irradiation. Electrons were also more efficient in this phase, and exerted their effect at a third of the dose required by photons. This 'radio-physiologic efficiency' (RPE) may be due to the direct ionization action of electrons.

3) *The decrease*. After a maximum value at the end of phase 2, the beat frequency was already reduced during the irradiation for both photons and electrons. There was, however, even here a difference between the two radiation qualities: The decrease was much faster for electrons, and the ciliary activity became lower than normal after about 1.5 Gy. For photons, the activity was increased during the whole course of irradiation (10 Gy).

If the 'target material' is assumed to be the same at the peak of increase, even here the electrons are much more effective than the photons. It is impossible to give any figure of relative efficiency, and it is not known if the action is on the enzyme kinetics, on the resynthesizing of ATP, or on the passive diffusion of ATP. It is quite probable that all of these factors are involved.

OGI (26) further found that after irradiation with 28 Gy electron energy, 'the cilia movement decreases without any recovery' (please note that this is not consistent with the figure on page 822 of his article). This statement is very interesting when compared with the results reported by FUJIWARA et coll. (14). After irradiation with 70 Gy of photons these authors found a 30 per cent reduction of the ciliary activity, lasting for at least three hours without any recovery, while OGI reported a reduction of about 90 per cent during the same time. This indicates a three-fold greater efficiency of electrons if the decrease of the ciliary motility and the administered radiation dose required to eliminate recovery of the activity are taken into consideration.

In summary, these experiments together with the studies reported by OGI (26) and FUJIWARA et coll. (14), have shown that there is a significant difference between the effects produced by high and low energy photons and electrons, and that the conventionally accepted RBE factor cannot be applied to events during irradiation. A new concept is therefore proposed, called 'the relative physiologic efficiency'. This concept is as yet only considered valid for the present investigation, which showed the factor: electrons/photons = 3/1. This factor can only be definitely established by further experimental investigations.

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