ANTIMICROBIAL EFFECT OF RADIATION OF IONIZED PLASMA

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Radiant energy is finding ever-widening application in modern medicine. Laser and plasma radiations are particularly promising. However, whereas much is now known about lasers, research into the physicochemical, biological, and therapeutic acion of ionized plasma on the living organism is still in its initial stage [2, 4, 5].

The aim of this investigation was to discover and substantiate the antimicrobial effect of the plasma beam.

EXPERIMENTAL METHOD

Experiments were carried out in vitro and in vivo. To study the direct effect, we used clinical strains of microorganisms; Staphylococcus aureus, Staphylococcus epidermis, Streptococcus faecalis, Escherichia coli, Proteus morganii, Proteus morabilis, Klebsiella pneumoniae, Serratia marcescens, Pseudomonas aeruginosa, and Candida albicans. The cultures were grown for 24 h at 37°C in test tubes containing nutrient broth. The grown culture was diluted 1:100 with physiological saline and poured on to the surface of Petri dishes containing blood agar, prepared by adding 5% human group A blood to nutrient agar, and allowed to stand at room temperature until dry. The response of the microorganisms was assessed on the basis of delayed growth of colonies in the zone of irradiation. The cultures of the microorganisms were irradiated by the plasma beam on the SUPR-3M helium apparatus with a current of 20 A and arc voltage of 30 V. Irradiation was given from a distance of 10 and 25 cm from the nozzle of the plasmotron for 3, 5, and 10 min. To avoid a heating effect the gas flow was drawn off by means of a vacuum pump. In the control experiments, irradiation was not carried out. In separate series of experiments, light filters made from ordinary and quartz glass, and with different transmission bands, were placed between the object and the source of radiation. In experiments in vivo on 150 albino rats weighing 100-150 g, suppurative wounds were modeled in the interscapular region, with an area of 4 cm^2, and using a clinical strain of Staph. aureus, possessing marked pathogenic properties (strain 209) by the method described in [3]. In the control series, the infected wound was not irradiated (75 experiments). In the experimental series (75 experiments) the effect of the plasma flow on bacterial dissemination in the wounds and the duration of wound healing was studied. The wounds were irradiated for 3 min from a distance of 25 cm, 24, 48, and 72 h after infection. Bacterial dissemination in the wound was calculated by the method in [1].

EXPERIMENTAL RESULTS

Plasma radiation with the above-mentioned parameters was found to have a marked bacteriostatic effect. The most sensitive strains were those of S. marcescens and of other Gram-negative bacteria of the Enterobacteriaceae family. Pathogenic strains of Gram-positive microorganisms were less sensitive to the action of plasma radiation, but their growth also was significantly inhibited when the exposure was increased. For instance, after irradiation of Staph. aureus (strain 209) for 3 min 80 colonies grew, 20 colonies after an exposure

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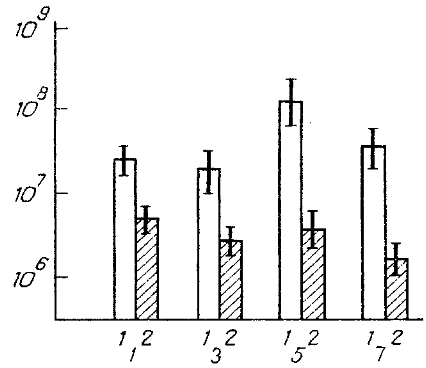

Fig. 1. Number of colonies of microorganisms in infected wound in phase I of wound healing, in control (1) and irradiated (2) groups of animals. Abscissa, days; ordinate, number of microbial cells.

of 5 min, and 5 after an exposure of 10 min, whereas in the control samples, more than 1000 colonies grew on the same area.

Thus the bacteriostatic action of plasma radiation was manifested mainly during the first 3 min. With an increase in the distance to 25 cm the bacteriostatic action decreased only a little, by between 1 and 3%. A distance of 25 cm from the nozzle of the plasmotron to the object can thus be regarded as optimal, for the temperature in the region of the light beam with this distance did not exceed 37°C, but the effect remained.

A matter of considerable importance is determination of the effective waveband. Theoretically it might be supposed that with removal of the heat-producing infrared radiation, the bacteriostatic action ought to be realized by radiation in the short-wave region of the spectrum (20-30 nm), which is close to that of the radiation of ionized helium. Delay of growth of the microorganisms was observed when a quartz glass is used. Meanwhile, when ordinary glass was used as the filter, no bacteriostatic action was observed. The use of light filters with different transmission bands showed that the bacteriostatic effect of the plasma radiation is limited to a wavelenth of 250 nm. Radiation with a longer wavelength, including visible light, has no such action.

The view expressed above, namely that the effective radiation spectrum lies in the ultraviolet region, and is limited to 250 nm, was thus confirmed.

Bacterial contamination of wounds in phase I of wound healing, which on average lasts 7 days, was studied in experiments on animals. During this period the wound was seeded with Staph. aureus, and after shedding of the scab, further contamination by the surface-vegetative microflora of the animal house was added to it. The microflora 24 h after creation of the model of an infected wound contained $4.4 \cdot 10^7$ microbial cells per gram of tissue (Fig. 1). After irradiation this level fell to $7 \cdot 10^6$. During the next two days, bacterial contamination of the wounds was reduced in both groups, to $2.6 \cdot 10^7$ and $4.4 \cdot 10^6$ microbial cells respectively. A scab formed in all the animals at this stage, and in the control rats it was rejected on the 8th-9th day, and in the treated rats on the 5th-6th day. In animals of both groups an increase in bacterial contamination of the wound was observed on the 4th-5th day after infection, and this was attributed to development of the microflora beneath the scab. However, its level in the treated group was several orders of magnitude lower. This difference persisted also on the 7th day, which was explained by shortening of phase I of wound healing in the treated animals.

Thus the light flux of ionized plasma has a marked antimicrobial effect, causing shortening of phase I of wound healing and accelerating healing of an infected wound. This action is most probably due to the ultraviolet region of the spectrum (up to 250 nm). Meanwhile, the plasma flux of helium is a component of short-wave radiation which may be responsible for a number of side effects, including its effect on the genetic apparatus of the cell. Further research both into the spectral characteristics of the plasma flux and into various aspects of its action on the living organism, is therefore essential.

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