NOTE

INFLUENCE OF A NEW ANTIBACTERIAL AGENT, BRONOPOL, UPON THE GROWTH OF CULTURED CELLS

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INTRODUCTION: In the previous paper[1] we reported that the *in vitro* antibacterial effect of bronopol (2-bromo-2-nitropropane-1,3-diol) synthesized by CROSHAW *et al.*[2] was not at all inferior to that of chlorhexidine on *Pseudomonas aeruginosa.*

The present paper deals with the inhibitory effect of both agents on the growth of cells of a culture line.

MATERIALS AND METHODS: The cultured cell line used was supplied by Nichimen Jitsugyo Company (Osaka). This line originated from human skin cells of X strain, which had been kept in EAGLE's minimal essential medium (MEM) with an addition of calf serum at 10 per cent and it reached 216 generations. The cells that had been cultured for 4 days were used for the observation. Mono-layered cells were treated with a trypsin solution and suspended in EAGLE's MEM with an addition of calf serum at 10 per cent. Five ml of the suspension were inoculated into a plastic dish (60×15 mm; Toyoshima Seisakusho Co., Tokyo) to make the final cell number of 4×10^5 per dish and incubated at 37°C in a CO_2 incubator. After the 24 hours' cultivation the culture medium was exchanged for the fresh one containing either of the drugs. Bronopol (Bronosol®; Midorijuji Co., Ltd., Osaka) was added to the medium to make a final concentration of 50, 25 and 12.5 mcg/ml and chlorhexidine digluconate (5% Hibitane® solution; Sumitomo Chemical Co., Ltd., Osaka) was added to it to produce a final concentration of 12.5 and 6.25 mcg/ml. Medium change was made using the same medium for each dish 2 and 4 days after each drug was added. To the control dishes 5 ml of EAGLE's MEM containing neither of the drugs was added. The state of cell growth was observed and cell count was made on the first, 2nd, 4th and 6th day of the drug treatment. On the occasion of the cell count cells were digested with a 0.1 per cent pronase solution.

RESULTS: As shown in Fig. 1, bronopol had a very weak damaging effect on cells and allowed them to grow at almost the same rate as in the control dishes at 12.5 mcg/ml., In contrast, in a 25 mcg/ml concentration of bronopol part of the cells fell off 24 hours after the addition. Thereafter, however, the remaining cells grew well in spite of 2 medium changes. Though not shown in Fig. 1, almost all the cells were submitted to degeneration and fell off in the medium containing 50 mcg/ml bronopol. On the other hand, chlorhexidine strongly

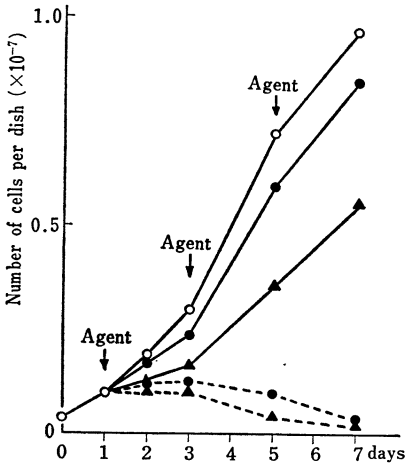

Fig. 1. Influence of bronopol and chlorhexidine on the growth of human skin cells (X-cells).
●—●, 12.5 mcg/ml of bronopol; ▲—▲, 25 mcg/ml of bronopol; ●·····●, 6.25 mcg/ml of chlorhexidine; ▲·····▲, 12.5 mcg/ml of chlorhexidine; ○—○, control. The arrows indicate the days that culture media were replaced by fresh media with agents (bronopol and chlorhexidine) of the concentrations indicated above.

inhibited the growth of cells even at a concentration of 6.25 mcg/ml and many cells degenerated and fell off. These changes were far more conspicuous at 12.5 mcg/ml of chlorhexidine.

COMMENTS: ARIMURA *et al.*[3] investigated the growth inhibitory effect of 6 antibacterial agents; bronopol, chlorhexidine gluconate, acriflavine, benzalkonium chloride, hexachlorophane and thimerosal, on cultured fibroblasts by coefficient growth and reported that bronopol and thimerosal had a weaker growth inhibitory effect. In the present observation we compared bronopol with a strong antibacterial action against *Pseudomonas aeruginosa* and chlorhexidine which is widely used and has a wide antibacterial spectrum for growth inhibitory effect on cultured cells and obtained the result that this activity was far weaker in bronopol. Recently, it is generally admitted that Gram-negative bacilli, especially *Pseudomonas aeruginosa*, are very important as pathogens causing inflammation in postoperative infections, and so the appearance of an effective disinfectant for these organisms is required for the prevention of this disease. Bronopol is regarded as an antibacterial agent which should be applied in practice because of its strong antibacterial activity against *Pseudomonas aeruginosa* and its weak growth inhibitory effect on cultured cells.

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REFFERENCES:

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