[Chem. Pharm. Bull. 23(11)3038—3039(1975)]

UDC 615.33.011.5 : 615.065 : 615.076.7

Effect of Bleomycin on the Synthesis of Hexosamine-containing Substances in Cultured Fibroblasts

It was found that bleomycin was able to enhance the production of glycosaminoglycans and glycoproteins by fibroblasts isolated from carrageenin granuloma in rats.

Bleomycin, a family of glycopeptides, has been reported to have an inhibitory effect on the growth of squamous cell carcinoma of skin.[1] On the other hand, the antibiotics is known to cause some side effects such as cutaneous and pulmonary fibrosis,[2] and the measure controlling the side effects of bleomycin is desired. About the mechanism of fibrosis caused by bleomycin some works have been done from the viewpoint of collagen; Tetsuka, *et al.*[3] have reported that the antibiotics enhanced the activity of protocollagen proline hydroxylase in the process of collagen biosynthesis, and Ichihashi *et al.*[4] have demonstrated the reduction of cutaneous collagenase activity after administration of the antibiotics. On the other hand, only one report is available about the effects of bleomycin on acid glycosaminoglycans (AGAG); Ishikawa[5] showed a slight increase of cutaneous AGAG of mouse by bleomycin treatment.

In this study, AGAG-producing fibroblasts were used, which have been isolated from a rat carrageenin granuloma by one of the authors.[6] The cells were cultured on a Petri dish with a Ham's F-12 medium supplemented with 5% fetal bovine serum at 37° in a 5% CO_2 atmosphere. The cells were exposed to bleomycin sulfate (0.1 μg/ml, Nippon Kayaku Co. Ltd., Tokyo, Japan) for 8 days during their stationary phase. The total amount of AGAG produced for the culture period and the ^{3}H-glucosamine incorporation into AGAG and glycoproteins during 24 hr after the culture were determined for both the cell layer and the medium. The ^{3}H-thymidine incorporation into deoxyribonucleic acid (DNA) of the cells were examined by the cover slip method[7] using the cells in their logarithmically growing phase.

Table I. Effect of Bleomycin on Uronic Acid Content in Cultured Fibroblasts

Group	Cell layer(μg/culture)	Medium(μg/culture)	Total(μg/culture)
Control	0.96±0.08	3.02±0.13	3.97±0.15
Bleomycin (0.1 μg/ml)	1.44±0.12(150)	4.58±0.14(152)	6.02±0.19(152)

Data are shown as means±S.E. The percent of control is shown in parentheses.

Table II. Effect of Bleomycin on the ^{3}H-Glucosamine Incorporation in Cultured Fibroblasts

Group	Cell layer(cpm/culture)	Medium(cpm/culture)	Total(cpm/culture)
Control	770.8±42.6	1900.7±70.9	2671.5±91.8
Bleomycin (0.1 μg/ml)	1684.6±12.8(219)	4404.7±48.6(232)	6089.2±51.5(228)

Data are shown as means±S.E. The percent of control is in parentheses.

1) H. Suzuki, K. Nagai, H. Yamaki, N. Tanaka, and H. Umezawa, *J. Antibiotics Tokyo*, **21**, 379 (1968).
2) I.Y.R. Adamson and D.H. Bowden, *Am. J. Pathol.*, **77**, 185 (1974).
3) T. Tetsuka, S. Kawai, K. Konno, and H. Endo, *Seikagaku*, **42**, 468 (1970).
4) M. Ichihashi, H. Shinkai, M. Takei, and S. Sano, *J. Antibiotics Tokyo* **26**, 238 (1973).
5) E. Ishikawa, "Kogenbyo", ed. by Y. Otaka, Kodansha, Tokyo, 1974, p. 66.
6) S. Mutota, Y. Mitsui, Y. Koshihara, and S. Tsurufuji, *Connective Tissue*, **6**, 47 (1974); S. Murota, Y. Koshihara, and S. Tsurufuji, *Biochem. Pharmacol.*, in press.
7) K. Otubo, M. Yamada, and M. Saito, *Jap. J. Med. Sci. Biol.*, **21**, 185 (1968).

Table III. Effect of Bleomycin on the ^{3}H-Thymidine Incorporation in Cultured Fibroblasts

Group		cpm/culture
Control		4369±190
Bleomycin	0.01 μg/ml	3528±136(81)
	0.1 μg/ml	1706± 94(39)
	1.0 μg/ml	601± 17(14)

Data are shown as means±S.E. The percent of control is shown in parentheses.

The production of AGAG was markedly increased by bleomycin treatment, *i.e.* by 50% for uronic acid content in both cell layer and medium (Table I). At that time, the incorporation of ^{3}H-glucosamine into macromolecules was doubled by the treatment (Table II). However, DNA synthesis was inhibited by 60% even by rather a short exposure of 24 hr of the same concentration of bleomycin (Table III). The radioactive macromolecules produced by the fibroblasts were further analyzed with cetylpyridinium chloride. In the cell layer 64% of total radioactivity was found in AGAG fraction and the rest was in glycoprotein fraction, while in the medium 90% was in AGAG and the rest was in glycoprotein. From these results it was concluded that bleomycin was able to enhance the production of AGAG and glycoprotein as well as collagen by fibroblasts while inhibiting their proliferation.

Acknowledgement The authors express their best thanks to Nippon Kayaku Co. Ltd. for the supply of bleomycin used in the present work.

Department of Pharmacology, Tokyo Metropolitan Instiute of Gerontology, Itabashi-ku, Tokyo, 173, Japan

Katsuhiro Otsuka
Sei-itsu Murota

Department of Biochemistry, Tokyo College of Pharmacy, Shinjuku-ku, Tokyo, 160, Japan

Yo Mori

Received July 30, 1975