NOTES

UNUSUAL SUSCEPTIBILITY OF ONCOGENIC VIRUSES TO AN ANTIVIRAL ANTIBIOTIC (BXM-10)

VINCENT GROUPÉ* and RICHARD DONOVICK**

Life Sciences Research Laboratories
St. Petersburg, Florida 33710, U.S.A.

(Received for publication August 22, 1977)

Preparations of BXM-10, active against a variety of viruses in tissue cultures, have been shown to inhibit oncogenic viruses *in vivo*. Such preparations have failed to affect certain transplanted tumors of other viral infections in mice. BXM-10 was produced by one of 17 unidentified actinomycete cultures provided by Dr. ROBERT W. SIMPSON (Rutgers University). All of these cultures were known to produce beers which inhibited (>1/2,000) influenza A virus (WSN) in chick embryo tissue cultures when serially diluted beers were introduced one hour post infection. Beers from these 17 cultures were prepared and tested against ROUS sarcoma virus (RSV) *in vivo*[1]. Interestingly, all but two of them were found to delay the latent period for tumor development in chickens. When these beers were extracted with *n*-butanol, 8 of 15 yielded butanol-soluble materials (BXM) which significantly ($P<0.01$) delayed tumor development by RSV. Of these, BXM-10 was the most effective and its biological characteristics are described below.

Actinomycete No. 10 was maintained on EMERSON agar slants. Preinoculum shaken cultures [50 ml/250 ml (Erlenmeyer flask)] were seeded with suspended growth from an agar slant into PGM medium (5 g peptone, 10 g dextrose, 20 g Brer Rabbit molasses in 1 liter tap water) and incubated for 96 hours at room temperature on a rotary shaker at 150 rpm. Inocula were prepared by seeding 10 ml of the pre-inoculum into each of 18 flasks containing 100 ml of PGM medium. After 72 hours of incubation, 10 ml of the inoculum was seeded into 180 flasks and incubated 4~5 days (luxuriant growth) as above. The 180 shaken cultures were pooled, filtered through Whatman #4 paper, divided into 3 aliquots, and stored in plastic containers at −10°C.

The frozen beer was thawed and serially thrice extracted with *n*-butanol (final: 20% v/v) for 30 minutes with agitation (rotary shaker) at pH 4.5. The contents were transferred to appropriate separatory globes and allowed to separate overnight. The aqueous phases were discarded and the butanol layer was aspirated and evaporated under vacuum at 45°C to dryness. The residues were reconstituted in 0.01 N Na_2CO_3 at a concentration of about 50 mg/ml. This solution was distributed in 1.0-ml aliquots among 5 ml vials which were then freeze-dried under vacuum and stoppered in dry nitrogen. The preparations were stored at −10°C.

Influenza A virus (WSN) and RSV (Bryan) were both grown in chick embryo fibroblast cultures[2] prepared in 60 mm plates by seeding 5×10^5 cells per plate. The appropriate culture nutrients containing 100 μg/ml each of penicillin and streptomycin were removed 3 days later and each culture was inoculated with approximately 100 PFU in 0.5 ml of the indicated virus. After one hour at 37°C the inoculum was removed and appropriate dilutions of BXM-10 were added to triplicate sets of cultures. Minutes later 4.5 ml of 0.5% Seakem agarose-MEM-5% FCS overlay was added. The cultures were incubated at 37°C (5% CO_2 atmosphere). Then 3 ml of appropriate medicine (neutral red v/5,000) was added to each plate and three hours later the plaques or foci were examined. Preparations of BXM-10 were found to inhibit (>50% plaque reduction) WSN and RSV in dilutions of 1/4,000 and 1/8,000, respectively.

BXM-10 was tested *in vitro* for activity against *Candida albicans, Saccharomyces cerevisiae, Escherichia coli, Pseudomonas aeruginosa, Salmonella schottmuelleri, Streptococcus pyogenes* at levels of 1, 0.1 and 0.01 mg/ml. Whatman filter paper (No. 4) strips were immersed in test solutions, carefully drained and placed on yeast dextrose agar or nutrient agar plates. One hour later, perpendicular to the filter strip, 24-hour broth cultures were surface-streaked on the agar. Bac-

* Present address: National Cancer Institute, DCCR, Blair Building, 8300 Colesville Road, Silver Spring, Maryland 20910, U.S.A.

** Present address: American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A.

terial-inoculated plates were incubated at 37°C and those with yeasts at room temperature and were examined at 20 hours. Distinct inhibition (14 mm zone at 1.0 mg/ml was observed against *S. cerevisiae* but not against any of the other selected bacteria or yeasts.

Stable, frozen standard ROUS sarcoma virus (RSV) was obtained from University Laboratories, Inc. (Highland Park, N.J.) and White Leghorn chicks 5~9 days old were used. Each chick was given 0.2 ml of viral inoculum, subcutaneously in the left wing web. Beginning with the third day after infection and daily for the duration of the test, each chick was examined for the appearance of tumors at the site of inoculation. The tumor latent period response data were analyzed by the graphic method described by BRYAN[3]. The metameter best suited to analyses of the tumor latent period responses was the reciprocal of time, in days, multiplied by 100 (100 days). Fig. 1 shows effect of 3 mg/kg of BXM-10 on latent period of chicks inoculated into the wing web with RSV diluted 10^{-2}, 10^{-4}, and 10^{-6} respectively. Daily intramuscular injections of BXM-10 were begun on the day before inoculation of RSV and continued for five days. Control and treated groups consisted of 53 chicks each. It is clear that the latent period was markedly prolonged in birds injected daily with 3 mg/kg of BXM-10 and that this inhibitory effect was not lessened when the infecting dose of RSV was greatly increased.

Dr. TIMOTHY E. O'CONNOR (National Cancer Institure, Bethesda) kindly tested BXM-10 against murine sarcoma virus (Moloney) in 3T3 cell culture and found that BXM-10 dramatically inhibited focus formation in a concentration of 0.25 μg/ml. He further reported that BXM-10 failed to inhibit 3 DNA viral polymerases (SSV-1-P; ME-DNA-P_1; ME-DNA-P_2), 2 RNA polymerases (ME-RNA-P_1; ME-RNA-P_2) and poly A polymerase (ME-Poly A-P). Dr. GEORGES H. WERNER (Rhône-Poulenc, Vitry-Sur-Seine) kindly tested BXM-10 and found that in Japanese quail (Coturnix) at 10, 3, and 1 mg/kg (i.p. or i.m.) BXM-10 did inhibit the development of tumors induced in the wing web by RSV but similar tests with transplanted sarcoma 180 and leukemia L1210 in mice were completely negative.

Stable, frozen standard RAUSCHER murine leukemia virus (RMLV) was obtained from University Laboratories, Inc. (Highland Park, N.J.) and random bred male mice weighing 15~18 g each were obtained from Southern Animal Farms. Table 1 summarizes data from a typical experiment. A large group of mice were infected i.p. with 100 ED_{50} of RMLV and then divided into subgroups of 25 mice each. Graded doses of BXM-10 were injected by the same route two hours after infection and twice daily thereafter. The mice were killed eight days after infection and their spleens removed and weighed. The spleens were then placed in PLUZNIK and SACHS fixative[4] and foci of lymphoma cells immediately appeared as white areas on a dark background on the surface of the spleen. The reduction in numbers of foci and in average weight of the

Fig. 1. Suppression of virus induced ROUS sarcoma in chicks by BXM-10

○ Control (diluent)
● BXM-10 (3 mg/kg)

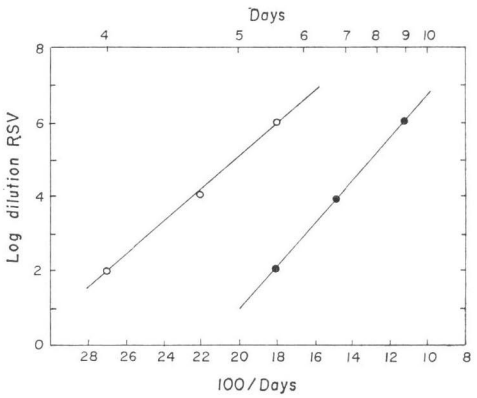

Table 1. Suppression of virus induced RAUSCHER leukemia in mice by BXM-10

BXM-10*	Average No. of foci per spleen**	Percent reduction of foci	Average spleen weight** (mg)
24 mg/kg	6.1	79	202
12 mg/kg	13.8	52	221
6 mg/kg	20.0	31	234
3 mg/kg	27.4	6	212
Virus control	29.0	—	267
BXM-10 control (24 mg/kg)	0	—	171
Diluent control	0	—	198

* Two injections per day for 7 days, beginning 1 hour post infection (i.p.).

** Twenty-five spleens per group. Mice sacrificed 8 days post infection 100 ED_{50} RMLV (i.p.).

spleens in treated and control groups were compared. Appropriate control groups of equal numbers (25) were included and the results are shown in Table 1. It will be seen that BXM-10 clearly inhibited both the development of foci in the spleen and the average spleen weight.

Dr. CLAIR G. ENGLE (Ciba-Geigy, Summit, N. J.), kindly conducted extensive and sophisticated tests on BXM-10 both *in vitro* and *in vivo*. Antiviral activity ($>1/2,000$) in tissue cultures was demonstrated against Mengo, vaccinia, and herpes simplex viruses in decreasing order. BXM-10 failed to inhibit polio virus II, Coxackie A-21, Rhinovirus 1A and vesicular stomatitis viruses *in vitro*. Extensive *in vivo* tests with BXM-10 using mice infected with herpes simplex, Mengo, influenza A and B, and Sindbis viruses failed to demonstrate inhibitory activity equal to the known antiviral agents used as reference drugs in these studies. For example, BXM-10 was effective in reducing the number of localized vaccinia lesions ($P<0.01$) in the tail of mice but not as effectively as Marboran, the reference drug.

Marked suppression of virus-induced tumor development by a broad spectrum antiviral antibiotic that fails to affect other susceptible viruses *in vivo* emphasizes fundamental differences in pathogenesis. Further, cytotoxic agents are known to inhibit oncogenic viruses including RSV[1] and RMLV[5], but antiviral antibiotic BXM-10 failed to affect transplanted sarcoma 180 and leukemia 1210 again emphasizing fundamental differences in pathogenesis. These data also suggest that candidate preparations with unsuspected anti-oncogenic virus activity would be overlooked in anticancer tests that utilize only transplanted tumors.

Acknowledgement

We thank EDWARD L. VAUGHAN for assistance in these studies.

References

1) PIENTA, R. J.; E. H. BERNSTEIN & V. GROUPÉ: Experiences with virus-induced ROUS sarcoma as a model in experimental therapy. Cancer Chemotherapy Reports, No. 31, pp. 25~39, 1963
2) VOGT, P. K.: Focus assay of ROUS sarcoma virus. *In*: Fundamental Techniques in Virology. *Eds.* K. HABEL & N. P. SALZMAN, Academic Press, New York, pp. 198~211, 1969
3) BRYAN, W. R.: Interpretation of host response in quantitative studies on animal viruses. Ann. N. Y. Acad. Sci. 69: 698~728, 1957
4) PLUZNIK, D. H. & L. SACHS: Quantitation of a murine leukemia virus with a spleen colony assay. J. Natl. Cancer Inst. 33: 535~546, 1964
5) CHIRIGOS, M. A.; F. J. RAUSCHER, I. A. KAMEL, G. R. FANNING & A. GOLDIN: Studies with the murine leukemogenic RAUSCHER virus. II. Chemotherapy of virus-induced lymphoid leukemia. Cancer Res. 23: 1646~1650, 1963