Inhibitory Action of Statolon Against Polyoma Virus

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Rowe and collaborators (1) have shown that although cytopathic effect (CPE) on mouse embryo tissue culture, hemagglutinin inhibition (HI) antibody produced in suckling mice, and tumors produced in suckling hamsters may serve as indicators of the titer of mouse polyoma virus, the first two of these are subject to less variability than the latter in testing this virus. We have chosen tests of CPE of polyoma virus on mouse embryo tissue culture for use in examining the effects of statolon on this rodent virus, and, although in vivo work is now indicated and still needs to be done, this paper shows statolon has considerable inhibitory action against this virus. The experimental results reported here are part of a larger study of the extension of the spectrum of statolon and this will be reported in detail later.

Materials and Methods

Whole mouse embryo trypinized tissue culture in Eagle's basal medium with five per cent inactivated horse serum plus antibiotics was obtained from Microbiological Associates, Polyoma virus VR 252 was obtained from the American Type Culture Collection. Statolon lot 209-617B-242-1 was furnished by Lilly Research Laboratories. Tissue culture tubes when received were subjected to two addtional days of incubation at 37°C for a more complete monolayer to form. At this time the medium was drained off and new medium of the same kind added. Then 0.1 ml of a series of doubling dilutions of statolon made in basal medium as indicated in Table 1 was added to all tubes except virus controls. After twenty-four hours additional incubation of the tissue cultures in the presence of statolon on roller drums, all fluid was again drained off, new fluid was added, then a series of 0.1 ml doses of various decimal dilutions of polyoma virus were added using three tubes of tissue culture per virus dilution and the tubes reincubated. Statolon toxicity tubes had no virus added. Medium changes were made after intervals of 4, 4, 6, and 7 days. Tissue culture tubes were examined at the six times indicated in Table 1 for evidence of damage by virus or drugs and results are as indicated from — to 4+. Since this test included three variables, i.e., statolon dilutions, virus dilutions, and different incubation periods, tabulation of direct CPE readings appeared most feasible for indicating results.

Results

It is apparent from Table 1 that dilutions of statolon of 1-400 to 1-12,800 show substantial inhibition of polyoma virus and practically no toxicity with the exception of one or two tubes on 1-6,-400 statolon. These may have been "cross infections" from virus tubes which at this time had been opened several times. Other dilutions of statolon showing no toxicity and decreasing inhibition, but

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TABLE 1

Statolon	Virus	8 days	10 days	14 days	18 days	21 days	25 days
1-400	10^{-2}	+ + +	++ + +	+++ +++ +++	+++ +++ +++	+++ +++ +++	++++ +++ ++++
”	10^{-3}	+ + +	+ − +	++ ++ +	+++ +++ ++	+++ +++ +++	+++ +++ +++
”	10^{-4}	− − +	+ + +	+ + +++	+ + ++	+ + ++	+ + +++
”	10^{-5}	+ − −	+ + −	++ + −	++ + −	++ + −	++ + −
”	0	− − −	+ − −	+ − −	+ − −	+ − −	+ − −
1-800	10^{-2}	− − −	+ + +	++ ++ ++	+++ +++ +++	+++ +++ +++	+++ +++ +++
”	10^{-3}	− − −	+ − +	+ + +	++ ++ ++	++ ++ ++	++ ++ ++
”	10^{-4}	− − −	− − −	− − +	− − −	− − −	− − +
”	10^{-5}	− − −	− − −	− − −	− − −	− − −	− − −
”	0	− − −	− − −	− − −	− − −	− − −	− − −
1-1,600	10^{2}	− − −	+ − +	++ +++ +++	+++ +++ +++	+++ +++ ++++	++++ ++++ ++++
”	10^{-3}	− − −	− + +	− + +	++ ++ +++	+++ +++ +++	+++ +++ +++
”	10^{-4}	− − −	− − −	− − −	++ ++ +	+++ +++ ++	+++ +++ ++
”	10^{-5}	− − −	− − −	− − −	− − −	− − −	− − −
”	0	− − −	− − −	− − −	− − −	− − −	− − −
1-3,200	10^{2}	− − −	++ ++ ++	+++ +++ +++	+++ +++ +++	++++ ++++ ++++	++++ ++++ ++++
”	10^{-3}	− + −	− + +	+ ++ ++	++ +++ +++	+++ ++++ ++++	++++ ++++ ++++
”	10^{-4}	− − −	− − −	− − −	+ − +	+++ + ++	+++ ++ +++
”	10^{-5}	− − −	− − −	− − −	− − −	− − −	− − −
”	0	− − −	− − −	− − −	− − −	− − −	− − −
1-6,400	10^{2}	+ − −	++ ++ ++	+++ +++ +++	+++ +++ +++	+++ ++++ +++	++++ ++++ ++++
”	10^{-3}	− − −	+ + −	++ ++ ++	+++ +++ +++	+++ ++++ ++++	++++ ++++ ++++
”	10^{-4}	− − −	− − −	− − +	++ + +++	++ ++ ++++	+++ ++ ++++
”	10^{-5}	− − −	− − −	− − +	− + −	− − +	− − +
”	0	− − −	− − −	− + +	− + −	− ++ −	− +++ −
1,12,800	10^{2}	− ++ ++	− ++ ++	+++ +++ +++	+++ +++ +++	++++ ++++ ++++	++++ ++++ ++++
”	10^{-3}	− − −	+ + +	++ ++ ++	+++ +++ +++	++++ ++++ ++++	++++ ++++ ++++
”	10^{-4}	− − −	+ − +	++ − ++	++ ++ ++	+++ +++ +++	++++ ++++ ++++
”	10^{-5}	− − −	− − −	− − −	+ ++ +	++ +++ ++	+++ +++ +++
”	0	− − −	− − −	− − −	− − −	− − −	− − −
0	10^{2}	++ ++ ++	+++ +++ +++	++++ ++++ +++	++++ ++++ +++	++++ ++++ +++	++++ ++++ ++++
”	10^{-3}	+ + +	+++ +++ ++++	++++ ++++ ++++	++++ ++++ ++++	++++ ++++ ++++	++++ ++++ ++++
”	10^{-4}	+ + −	++ ++ ++	+++ +++ +++	++++ ++++ ++++	++++ ++++ ++++	++++ ++++ ++++
”	10^{-5}	− − −	+ − −	++++ − +	++++ ++ +++	++++ +++ +++	++++ ++++ +++
”	0	− − −	− − −	− − −	− − −	− − −	− − −

not recorded in this table, were 1-25,600, 1-102,400, and 1-204,800. It is apparent that inhibitory action of statolon against the virus in maximal at a drug dilution of 1-800 and this become gradually less as the concentration of statolon decreases. It is also apparent that at any given concentration of drug, the weaker virus dilutions are more easily inhibited than the stronger dilutions. Comparison of results of drug action against 10^{-2} and 10^{-5} dilutions of virus shows a maximum of 1000 fold as much virus may be inhibited under different conditions, and also the degree of inhibition noted at a shorter period of incubation may decrease with further incubation generally involving changes of media.

Discussion

Kleinschmidt and Probst (2) have partially characterized statolon as a macromolecular polyanionic polysaccharide composed of galacturonic acid, galactose, galactosamine, glucose, arabinose, xylose, and rhamnose. They did not crystallize this compound. With the exception of the report of Johnson and Baker (3) who reported this drug, known first as 8450, as effective against Rous sarcoma virus, most viruses within the drug's spectrum of activity are small neurotropic viruses viz. Semliki Forest, MM, polio, and Russian Spring Summer virus. Exceptions have been several small viruses involved to some extent in the cause of some common colds, i.e., the HGP (of Andrewes), JH or 2060, and Coe (Coxsackie A21). In some instances of evidence of activity, tissue culture alone has been used; in others, animals alone have been used; and in some cases activity has been shown in both tissue culture and animals. In all cases, pretreatmet of tissue culture or animals with statolon (later followed by virus) has shown the greatest extent of antiviral action. In a very few instances a very slight degree of effective post-infection chemotherapy can be shown. In most, if not all tissue culture experiments, statolon can be removed from once treated tissue before virus is added, and full anti-viral chemoprophylaxis results. The mechanism of antiviral action certainly is not contact, but rather prevention or blocking (for a time) of virus penetration into otherwise susceptible cells. Location of the precise site and time of action has not been determined. There is nearly always considerable margin of safety between toxic and effective antiviral doses of statolon in both tissue culture and mouse experiments. Whether statolon may have any practicality in treating human infections is not known at present. It seems any possibility of trial usage would depend on infections where direct application of drug is possible.

Summary

Statolon has been found to have demonstrable inhibitory action against polyoma virus as grown and tested on whole mouse embryo tissue culture. Considerable leeway is evident between toxic dose of this drug and effective virus inhibitory doses. Polyoma virus is the second tumor-producing virus to be included in the spectrum of activity of statolon.

Literature Cited

1. Rowe, W. P., J. W. Hartley, J. D. Estes and R. J. Huebner. 1959. Studies of Mouse Agent. Antibiot. and Chemo. **12**:298-309
2. Kleinschmidt, W. J., and G. W. Probst. 1962. The Nature of Statolon, an Antiviral Agent.
3. Johnson, I. S., and L. A. Baker. 1958. The Utility of Rous Sarcoma Virus for Detection of Antiviral Activity. Antibiot. and Chem. **8**:113-121.