Therapeutic Antiviral Action of 5-Trifluoromethyl-2'-deoxyuridine in Herpes Simplex Keratitis

Abstract. *5-Trifluoromethyl-2'-deoxyuridine (F_3TDR) has potent therapeutic antiviral activity in herpes simplex infection of the rabbit cornea with strains of herpes virus both sensitive and resistant to 5-iodo-2'-deoxyuridine, and in corneal infection with vaccinia. 5-Trifluoromethyl uracil is not therapeutically active in herpetic keratitis.*

5-Iodo-2'-deoxyuridine (IUDR) and 5-bromo-2'-deoxyuridine have been found to have potent therapeutic antiviral activity against herpes simplex infection of the cornea (*1*, *2*). This activity, however, is selective. Iodine and bromine are similar in ionic radius to the CH_3^- group of thymidine and the 5-iodo deoxyuridine (IUDR) and 5-bromo deoxyuridine are highly effective in the therapy of herpetic keratitis (Fig. 1). 5-Chloro deoxyuridine is less similar to thymidine and is less effective, but still retains antiviral activity. 5-Fluoro deoxyuridine is unlike thymidine in that the effective atomic radius of fluorine is more like that of hydrogen and this compound is similar chemically to deoxyuridine, being an efficient inhibitor of thymidylic synthetase (*3*). The 5-fluoro deoxyuridine, therefore, is chemically different from the other halogenated pyrimidines and appears to have no therapeutic antiviral activity. Recently, a new compound in this group was synthesized, 5-trifluoromethyl-2'-deoxyuridine (trifluorothymidine or F_3TDR.) (*4*). This compound is mutagenic to, and is incorporated into, the DNA of bacteriophage $T4^5$. Trifluorothymidine is also incorporated into the DNA of human cells grown in culture, and confers upon these cells an increased radiosensitivity (*5*). Trifluorothymidine has been shown to be a potent inhibitor of the growth of transplanted tumors in mice (*6*). The nucleotide, trifluorothymidine phosphate, like 5-fluoro deoxyuridine phosphate, also inhibits the enzyme, thymidylate synthetase (*7*). It seemed of considerable interest to determine whether this substance and its free base trifluorothymine would have therapeutic antiviral activity and whether the two substances would be effective against virus resistant to 5-iodo deoxyuridine.

New Zealand albino rabbits weighing 1 to 2 kg were infected with herpes simplex virus of the McKrae strain as described previously (*2*). Three days after infection, all of the rabbits' eyes were examined for dendritic ulcers, and when it was found that all of the eyes were infected they were assigned at random to appropriate groups. Treatment with coded drops was carried out every 2 hours, and the severity of the ulcers was evaluated after 2 days of therapy. Extremely severe ulcers occupying nearly the whole cornea were graded four, and eyes that were completely healed were graded zero, the remainder being ranked between these categories.

The comparison of control and treated groups is shown in Table 1. Trifluorothymidine is clearly active in eliminating dendritic ulcers, and a comparison with previous data indicates that this agent is more potent than IUDR or cytosine arabinoside (*8*).

Virus resistant to IUDR was made by serial passage of McKrae strain virus in the presence of IUDR as already described (*9*). In vivo, IUDR has no therapeutic activity against this virus, but trifluorothymidine retains activity comparable to that against virus sensitive to IUDR (Table 1).

The free base, trifluorothymine, possessed no demonstrable therapeutic antiviral activity against the IUDR-sensitive or IUDR-resistant virus in concentrations as high as 10 mg/ml.

As with the other substituted pyrimidines which have therapeutic antiviral activity against herpes simplex, trifluorothymidine is also active against vaccinia virus (Table 1).

The free bases of all thymidine analogs (iodouracil, bromouracil and trifluorothymine) have no demonstrable therapeutic antiviral activity, although trifluorothymine can inhibit thymidlylic synthetase and under some circumstances can be incorporated into DNA. The nucleotides of all except cytosine arabinoside can be incorporated into DNA, and they are phosphorylated and must take part in the polymerase reaction. Inhibition of some degree is possible at any step in the final phosphorylation of the compounds or in their polymerization into DNA. Whether such enzyme inhibition is most important for antiviral activity, or whether incorporation into DNA is essential, remains to be clarified (*10*). Similarly, the biochemical reasons for the effectiveness of trifluorothymidine against IUDR-resistant virus requires further study.

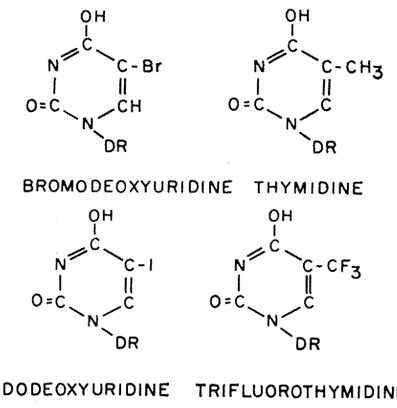

Fig. 1. The Van de Waal radius of Br^- is 1.95 Å, CH_3^- is 2.0 Å, I^- is 2.15 Å, and CF_3^- is 2.44 Å.

Table 1. Effect of trifluorothymidine (F_3TDR) on keratitis produced by the McKrae strain of herpes simplex (IUDR sensitive), by a strain of herpes virus resistant to IUDR, and by vaccinia virus. The control eyedrops contained only saline.

Concentration of F_3TDR in eyedrops (mg/ml)	No. of rabbit eyes	Severity of ulcers*
McKrae strain of herpes simplex		
Control	12	2.00 ± 0.9
0.1	5	0.20 ± 0.3
.08	12	0.50 ± 0.1
.04	12	0.79 ± 0.1
.01	6	1.83 ± 0.2
.001	6	2.08 ± 0.3
Herpes virus resistant to IUDR		
Control	12	2.67 ± 1.3
0.1	12	0.90 ± 0.5
.08	12	1.58 ± 0.6
.04	12	1.54 ± 0.8
.01	12	2.58 ± 0.7
Vaccinia virus		
Control	6	4.00 ± 0
0.1	6	2.83 ± 0.8

* Extremely severe ulcers were graded 4.00; eyes that were completely healed were graded zero; the remainder were ranked within these categories.

Since herpes simplex keratitis is an extremely important clinical disease, the finding that trifluorothymidine is a potent antiviral agent which can be active against viruses resistant to IUDR may be of clinical value for topical administration. Because of its incorporation into DNA, however, its use as a systemic antiviral agent, except in the face of extremely hazardous virus disease, will probably be limited.

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Extracellular Polysaccharides of Algae: Effects on Life-Support Systems

Abstract. *The amount of extracellular polysaccharide produced by eight species of green and blue-green algae ranges from 174 milligrams per liter to 557 milligrams per liter. Most of the polymers are composed of four monosaccharides: a hexose, a pentose, a methyl pentose, and uronic acid. The production of excessive amounts of these photosynthetic end products will undoubtedly influence the effective recycling time of growth media in life-support systems.*

In "life-support systems" where media will be recycled for the continuous growth of algae, algal end products will undoubtedly influence the efficiency of the recycling process. Few microorganisms have been shown to be capable of breaking down their respective polysaccharides for carbon and energy (*1*). Therefore, in an efficient medium recycling process, not only will it be necessary to replace depleted minerals but some provision will have to be made to remove the accumulated extracellular by-products. Accordingly, the selection of an alga to be used in a life-support system will depend not only on its efficiency in utilizing CO_2 and producing O_2 but also on the quantity and nature of its extracellular by-products under the cultural conditions employed.

Since the early 1950's, studies have been carried out to explore the possible use of algae as a source of food for overpopulated regions of the world (*2*). In more recent years, the use of algae in life-support systems designed for long space flights has been studied (*3*). These efforts were initially designed to study mass culture, efficiency of oxygen production, suitable substrates, and so forth. One of the basic problems now concerns the production of extracellular end products of algal metabolism and their influence on the growth-medium recycling processes which would be used in life-support systems.

At least three classes of organic compounds are known to be liberated by some species of freshwater algae: organic acids (*4, 5*), nitrogenous material such as polypeptides and free amino acids (*6*), and carbohydrate polymers (*5, 7, 8*). Lewin (*7*) has presented quantitative studies on the production of extracellular polysaccharides by 18 species of green algae isolated from soil samples. The yields of the extracellular polymer ranged from 3 to 113 milligrams per liter.

In this report we describe the production and composition of extracellular polysaccharides by eight species of mucoid green and blue-green algae used for studies on life-support systems. Quantitative data are also presented for capsular and water-soluble intracellular polysaccharide production.

Table 1. Yields of polysaccharide and dry weight of cells from eight species of freshwater algae. The cultures were bacteria-free, unialgal cultures isolated from samples of fresh water and oxidation-pond water.

Algal culture	Incubation temperature (°C)	EP* (mg/liter)	CP (mg/liter)	IP (mg/liter)	TP (mg/liter)	Cells (mg/liter)
Anabaena flos-aquae	40	557	13	126	696	1379
Nostoc sp.	40	415	15	23	453	1315
Palmella mucosa	25	271	36	196	503	2133
Chlorella vulgaris	25	235	34	75	344	3203
C. ellipsoidea	25	234	15	26	275	1959
Chlamydomonas sp.	25	224	19	62	305	1391
Oocystis sp.	25	197	22	48	267	2206
Chlorella sp.	25	174	15	26	215	1959

*EP, extracellular polysaccharide; CP, capsular polysaccharide; IP, water-soluble intracellular polysaccharide; TP, total polysaccharide.

Table 2. Increase in dry weight of cells and production of extracellular polysaccharide by *Anabaena flos-aquae*.

Time (days)	Extracellular polysaccharide (mg/liter, as glucose)	Cells (mg/liter)
2	25	268
4	44	323
6	50	1128
8	77	1203
10	138	1748
12	205	2068

Bacteria-free, unialgal cultures were isolated from samples of fresh water and oxidation-pond water gathered in the vicinity of this laboratory. The algae were cultured for 12 days in modified Knop's mineral medium (*9*), *p*H 7.0, contained in sterilized 2-liter Pyrex columns (48 mm in diameter). The cultures were maintained either at 25°C or 40°C in the presence of 13.4 kilolux of continuous light intensity supplied from a bank of white, 40-watt fluorescent lamps, and were aerated with a mixture containing 5 percent of CO_2 in air.

At 2-day intervals, samples were removed for the determination of polysaccharide in the cell-free medium and for determination of the dry weight. An appropriate amount of the cell-free medium was treated with 2 volumes of absolute ethyl alcohol, mixed, and centrifuged; the precipitate dissolved in 1 ml of distilled water, and the polysaccharide was determined as the glucose equivalent by the anthrone procedure (*10*). Dry weight was determined by drying overnight at 100°C.

At the end of the growth period, the cells were removed by centrifugation. The cell-free supernatants were concentrated to one-tenth volume with a rotary evaporator at 60°C, deionized with weak ion-exchange resins, and the extracellular polysaccharides were precipitated with two volumes of absolute ethyl alcohol. The stringy precipitates were collected in tared alundum crucibles and weighed after drying. The ash content of all extracellular polysaccharides, determined by combustion at 600°C, was subtracted to give yields of organic matter. The harvested cells were killed with 2 ml of a 2:1:1 mixture (by volume) of chlorobenzene,