ing experiments with mammalian CNS synaptic membranes are consistent with a close interaction of bicuculline and GABA recognition sites [16, 17]. Similar approaches however, have indicated that picrotoxinin recognition site may be relatively distinct from the GABA receptor, [14, 18]. Modifications of GABA receptor responses in the mammalian CNS showing parallel dose–response curve shifts with picrotoxinin have also been described previously, [7]. It is important to point out that although this is difficult to reconcile with a true non-competitive mode of action, these results could be explained in terms of a 'mixed' (or uncompetitive) type of antagonism. In the simplest model of such cases the antagonist combines with some species of the receptor which are themselves unable to combine with the ligand, producing kinetics with elements of both competitive and non-competitive antagonism. The present results provide some support for the concept of independent sites of action for picrotoxinin and bicuculline methiodide, consistent with the results of radioligand binding experiments. The antagonism by bicuculline methiodide showed a remarkably steep concentration dependence curve and high Hill coefficient, consistent with cooperative effects. Interestingly, radioligand binding studies have also provided data consistent with cooperative interactions between bicuculline and GABA [14, 17].

This investigation therefore provides evidence to propose a novel subtype of bicuculline-sensitive GABA receptor which is selectively activated by APS and isoguvacine, and which may represent the low affinity binding site for [^{3}H]GABA. Picrotoxinin and bicuculline methiodide inhibit this receptor's response, probably at separate sites, and in a fashion not at variance with their antagonism of other GABA receptor responses. It would therefore seem likely that the APS–isoguvacine receptor may utilise the same receptor–effector mechanism suggested for other bicuculline-sensitive GABA receptors.

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Pyrrolopyrimidine lethality in relation to ribonucleic acid synthesis in Sarcoma 180 cells *in vitro**

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Sangivamycin (SGM)† is the parent member of the pyrrolopyrimidine group of adenosine analogs [1]. This antitumor antibiotic has cytotoxic activity in a variety of experimental systems *in vitro* [2, 3] and *in vivo* [1, 4]. Following intracellular phosphorylation by adenosine kinase [5], SGM is incorporated into DNA [6, 7] and RNA [6, 7]. SGM has been shown to inhibit DNA [2] and RNA [2, 8] syntheses, *de novo* purine synthesis [9], and tRNA acylation [10]; however, it is not known whether these biochemical lesions are responsible for the lethal effects of the drug. SGM cytotoxicity is most pronounced following prolonged drug exposure [2]. Inhibition of nucleic acid synthesis by the drug occurs gradually, and RNA synthesis is inhibited to a lesser degree than DNA synthesis [2]. Recent studies have shown that SGM is preferentially incorporated into RNA, and that incorporation into poly(A)RNA correlates with cell killing by the drug [6].

Several pyrrolopyrimidine nucleoside analogs of SGM

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† Abbreviations: SGM, sangivamycin; TGM, thiosangivamycin; AXGM, sangivamycin-amidoxime; AMGM, sangivamycin-amidine; ADN, adenosine; and dCF, 2′-deoxycoformycin.

have been synthesized [11]. There are considerable differences in the abilities of these derivatives to inhibit RNA synthesis [12]. Existing data suggest that continuing RNA synthesis during drug exposure is requisite for these drugs to produce a lethal effect [2, 6]. In this communication, we report on the cytotoxicity of various pyrrolopyrimidine analogs in relation to their effects on RNA synthesis. We have also examined whether cells can be rescued from the lethal effects of SGM by equimolar concentrations of adenosine (ADN), the natural competitive analog, or by co-administration of 2′-deoxycoformycin (dCF), an adenosine deaminase inhibitor.

Sangivamycin (SGM), thiosangivamycin (TGM), sangivamycin-amidoxime (AXGM), and sangivamycin-amidine (AMGM) were provided by Dr. Robert Glazer, NCI, Bethesda, MD. Adenosine (ADN) was purchased from the Sigma Chemical Co., St. Louis, MO, and 2′-deoxycoformycin (dCF) was obtained from the Pharmaceutical Resources Branch, NCI. [5-^{3}H]Uridine (25 Ci/mmole) was purchased from the New England Nuclear Corp., Boston, MA.

Sarcoma 180 cells (Foley strain, American Type Culture Collection, Rockville, MD) were grown in Earle's Medium 199 (Flow Laboratories, McLean, VA) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin [2]. Cultures were grown in monolayer in 75 cm^2 plastic tissue culture flasks containing 10 ml of supplemented medium. Cells were plated at an initial inoculum of 10^6 cells/flask (10^5 cells/ml). Medium was changed on days 2 and 4, and cells were subcultured on day 5 [2]. The proliferation kinetics of this cell system have been described in detail [13, 14].

Replicate flasks of 2-day-old log phase cells were incubated at 37° with SGM, TGM, AXGM, and AMGM at final drug concentrations of 5×10^{-6} M for 1 hr and 4 hr. At the end of the exposure period, the drug-containing medium was removed, and the cells were rinsed three times with 10 ml of Hanks' balanced salt solution. Cells were harvested by trypsinization and cloned in soft agar (0.3%) for colony formation as previously described [2]. Untreated controls were obtained at each time point. Plating efficiencies for controls were approximately 60%. The surviving viable cell fraction following drug exposure was calculated as previously described [15]. Each experiment was performed at least three times, and the reported results represent the mean ± S.E. of replicate studies at each time point.

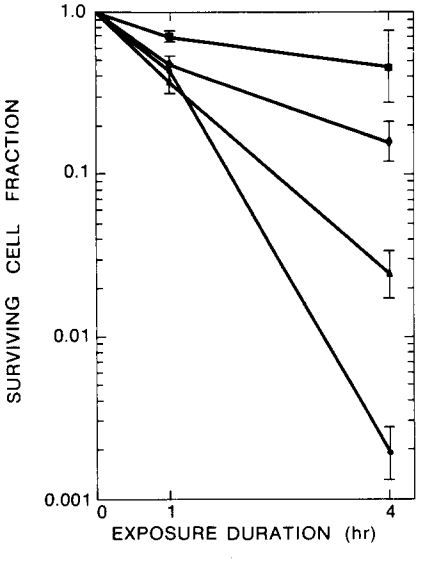

Fig. 1. Effects of 1-hr and 4-hr exposure to sangivamycin (●–●), thiosangivamycin (■–■), sangivamycin-amidoxime (▲–▲), and sangivamycin-amidine (◆–◆) at final drug concentrations of 5×10^{-6} M on clonogenic cell survival.

Replicate flasks harvested following 1-hr and 4-hr treatment with SGM, TGM, and AXGM were exposed to [5-^{3}H]uridine at a final concentration of 2 μCi/ml for 30 min prior to sample collection. Cells were rinsed three times with Hanks' balanced salt solution containing 10^{-5} M uridine at 0°, harvested by trypsinization, and counted. An aliquot containing 2×10^5 cells was washed once with 0.85% NaCl at 0°, centrifuged at 400 *g* for 15 min, and resuspended in 10 ml of 10% (w/v) trichloroacetic acid. Cell precipitates were collected on 0.45-μm Millipore filters with extensive trichloroacetic acid washing and placed in vials containing 17 ml Aquasol (New England Nuclear Corp.) for scintillation counting [2]. Radiolabeled nucleoside incorporation was expressed as a percentage of untreated control at each time point. All experiments were performed at least three times, and the reported results represent the mean ± S.E. for replicate flasks.

Cells were also exposed to ADN and/or dCF at final drug concentrations of 5×10^{-6} M, alone or in combination with SGM, for 1 hr. Replicate flasks containing only SGM were included for comparison. Following drug exposure, cells were harvested by trypsinization and cloned in soft agar for colony formation. Untreated controls were obtained at each time point. Each experiment was performed at least three times; the reported results represent the mean ± S.E. of replicate studies.

The lethal effects of the four pyrrolopyrimidine analogs examined are shown in Fig. 1. Following 1-hr drug exposure, comparable degrees of cell killing were produced by SGM, AXGM, and AMGM. The surviving cell fraction after exposure to SGM was 0.44; this is in close agreement with previous studies in this cell system [2]. In contrast, relatively few cells were killed following TGM exposure. This difference in TGM lethality was even more pronounced following prolonged drug exposure. At 4 hr, SGM was more cytotoxic than the other pyrrolopyrimidine derivatives, and the surviving cell fraction was only 0.0019; that is, SGM was approximately 240-fold, 85-fold, and 12-fold more potent than TGM, AMGM, and AXGM respectively.

While the relative cytotoxicity was SGM > AXGM > TGM, the order was reversed for potency in RNA synthesis inhibition (Fig. 2). At 1 hr following SGM exposure, total RNA synthesis was inhibited by only 24%, whereas TGM had inhibited RNA synthesis by 73% at 1 hr. AXGM was of intermediate potency and inhibited RNA synthesis by 57%. At 4-hr drug exposure, RNA synthesis was inhibited by a magnitude similar to that seen at 1 hr and the same order of relative potency was maintained (not shown).

ADN had no effect on cell survival; results following 1-hr exposure are shown in Fig. 3. When ADN was given in combination with SGM, the natural competitive analog did not interfere with cell killing by SGM. The cytotoxic effects of cordycepin, xylosyladenine, and adenine arabinoside are potentiated by dCF [16]; however, SGM is resistant to the action of adenosine deaminase [17], and dCF would not be expected to directly enhance cell killing by SGM. Rather, dCF might be expected to indirectly antagonize SGM cytotoxicity by producing sustained levels of ADN which would compete with SGM for incorporation into RNA. No cell killing was produced by dCF alone, and dCF did not interfere with the cytotoxic effects of SGM. However, when cells were exposed to nontoxic equimolar concentrations of dCF plus ADN in combination with SGM, significant protection was obtained against the cytotoxic effects of SGM (Fig. 3).

Previous studies in Sarcoma 180 cells *in vitro* have dem-

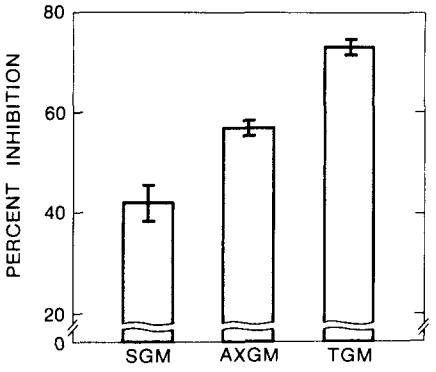

Fig. 2. Effects of 1-hr exposure to sangivamycin (SGM), sangivamycin-amidoxime (AXGM), and thiosangivamycin (TGM) at final drug concentrations of 5×10^{-6} M on [5-^{3}H]uridine incorporation.

onstrated that drug exposure duration is a major determinant of SGM lethality [2]. This has been confirmed in a human colon carcinoma cell line [3]. Inhibition of RNA synthesis by SGM occurs gradually, even in the continued presence of the drug [2]. During continuous drug exposure, approximately 10-fold more SGM is incorporated into RNA than DNA, and incorporation into poly(A)RNA exhibits the same time-dependency as the effects of SGM on cell viability [6]. If fraudulent incorporation into RNA is responsible for the lethal effects of pyrrolopyrimidines, those analogs that are more potent inhibitors of RNA synthesis would also be expected to be less toxic since RNA synthesis inhibition would self-limit drug incorporation. The present studies are consistent with this hypothesis.

TGM has been shown to be approximately fifty times more potent than SGM in inhibiting RNA synthesis in L1210 cells [18] and Ehrlich ascites cells [12]; AXGM and AMGM were similar in potency of SGM, whereas ADN had no effect on RNA synthesis [12]. However, these studies were not attempts to correlate the biochemical effect with drug lethality. Our studies show that TGM is also a more potent RNA synthesis inhibitor than SGM in Sarcoma 180 cells. We have demonstrated further that there is an inverse correlation between the effectiveness of various pyrrolopyrimidines for inhibiting RNA synthesis and log kill produced by these drugs ($r = -0.99$).

In these studies, neither ADN nor dCF antagonized SGM lethality when given in equimolar concentrations; however, it is not likely that significant levels of ADN were maintained under either of these conditions due to the activity of adenosine deaminase. This is suggested by the substantial protection against SGM cytotoxicity afforded by the ADN–dCF combination. This protection presumably relates to interference with SGM incorporation into RNA in the presence of sustained levels of ADN produced by co-administration of dCF. More conclusive studies aimed at quantitating SGM incorporation into RNA are required.

Initial Phase I evaluation of SGM employed intermittent bolus administration according to a variety of schedules [19]. In view of more recent information on time-dependent drug lethality, clinical studies are planned to examine the antitumor activity of SGM when administered as a prolonged infusion. Additional data are needed to determine whether selective protection from pyrrolopyrimidine toxicity can be obtained. The present studies show that SGM and other pyrrolopyrmidine derivatives are more cytotoxic as drug exposure is prolonged, and that early inhibition of RNA synthesis appears to protect against the lethal effects of these drugs. Our studies further demonstrate that sustained levels of the natural competitive analog may also obtain protection against the cytotoxic effects of pyrrolopyrimidines. These data are consistent with previous obser-

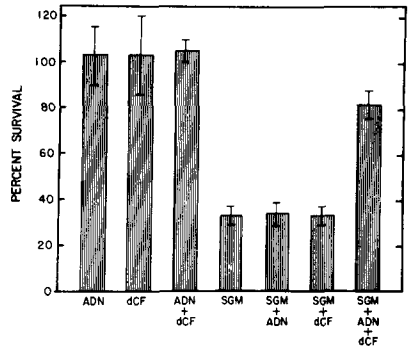

Fig. 3. Effects of adenosine (ADN) and 2′-deoxycoformycin (dCF) on clonogenic cell survival following 1-hr exposure, alone and together with sangivamycin (SGM), at final drug concentrations of 5×10^{-6} M.

vations suggesting that fraudulent incorporation into RNA may be responsible for pyrrolopyrimidine lethality.

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