than in rabbit tears, the change in binding capacity in disease states for rabbit tears is expected to be greater than in human tears.

Thus, based on the results of the present study, it is clear that to study the influence of drug–protein interaction on drug bioavailability in the eye, it is important to know: (*a*) the binding capacity of each major fraction of tear protein, and (*b*) the changes that occur in the major fractions during the disease state. This information will be of great help in predicting the loss of drug in pathological conditions due to drug–protein interaction.

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Phosphorus–Nitrogen Compounds XIX: Distribution of ^{32}P and Effect of an Active Oncolytic on Intracerebral Leukemia in Rodents

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Abstract ☐ *P,P*-Bis(1-aziridinyl)-*N*-1-adamantylphosphinic amide displayed oncolytic activity against intracerebral and intraperitoneal L-1210 leukemia. Administration of the isotope-labeled compound to rats shows ^{32}P distribution to the brain.

Keyphrases ☐ Organophosphorus compounds—adamantyl substituted, synthesis, oncolytic activity and tissue distribution screened ☐ Distribution, tissue—studied, adamantyl-substituted organophosphorus compounds ☐ Oncolytic activity—adamantyl-substituted organophosphorus compounds screened

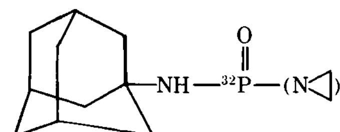
Of 19 adamantyl-substituted organophosphorus compounds synthesized and tested for anticancer activity (1, 2), only *P,P*-bis(1-aziridinyl)-*N*-1-adamantylphosphinic amide (NSC 166199, I) displayed oncolytic properties. Closely related to triethylenephosphoramide, I possesses a 1-adamantylamino moiety in lieu of one aziridinyl group. This modification produces a marked change in physical properties, with I having very low water solubility compared to triethylenephosphoramide, which is extremely soluble in water. The lipophilic nature of I suggests it may have the ability to overcome the blood–brain barrier and produce anticancer effect in the central nervous system (CNS). The eradication of leukemic cells in the CNS is a major goal of modern cancer chemotherapy.

To ascertain the ability of I to affect intracerebral leukemia, this agent was administered intraperitoneally to mice injected intracerebrally and intraperitoneally with L-1210 leukemia cells for survival time determination. The ^{32}P compound (II) of I was synthesized and administered intraperitoneally to rats to estimate its distribution to certain tissues and organs, including the brain.

EXPERIMENTAL

Chemistry—A mixture of 0.14 g (0.9 mmole) of ^{32}P-phosphorus oxychloride (4.47 mCi) and 10.3 g (67 mmoles) of phosphorus oxychloride in 50 ml of ether was added dropwise with stirring and cooling (0–5°) to a solution of 22.0 g (146 mmoles) of 1-aminoadamantane in 250 ml of ether. After standing for 24 hr, the reaction mixture was filtered and the filtrate containing *N*-1-adamantylphosphoramidic dichloride (3) was used for the *in situ* preparation of II.

The ethereal solution was added dropwise with stirring to a solution of 18 g (420 mmoles) of aziridine in 50 ml of ether, with a re-



II

sultant rise in temperature of 5° and formation of a white precipitate. After standing for 24 hr, the reaction mixture was filtered and the precipitate was washed with water until the washings no longer gave a positive test for chloride ion when treated with silver nitrate solution. When a hot acetone solution of the residue was cooled, white crystals of II, mp 177–178°, formed.

To test the radiochemical purity of II, an acetone solution was spotted on TLC plates, developed with ethanol–chloroform (1:9), and exposed to film[1] for 17 hr. The film showed a single spot at $R_f \sim 0.21$.

Tissue Distribution—Nine female Sprague–Dawley rats, 90–120 g, were administered 2.5 μCi of II (approximately 200 mg/kg) by intraperitoneal injection of a carboxymethylcellulose suspension of II. Each animal was then placed in a plastic metabolism cage for separation and collection of urine and feces.

Three animals were decapitated at each of three time periods: 3, 6, and 12 hr after injection of II. Blood was collected, and the spleen and brain were dissected from the carcass. The spleen was weighed, and a portion (100–200 mg) was placed in a counting vial containing 1 ml of a tissue solubilizer[2]. The remainder of the carcass was homogenized[3], and portions were prepared for counting in a similar manner.

Urine was diluted with water to 10 ml, and an aliquot was taken. Feces were suspended in water prior to sampling. All vials containing tissue or feces were placed in a 50° water bath until dissolution was complete and then decolorized with benzoyl peroxide. Radioactivity in each sample was determined by liquid scintillation counting[4].

An aliquot of each urine sample was spotted on a silica gel plate and developed in the same manner as that previously described for II.

Antileukemia Testing—L-1210 leukemia cells were injected intraperitoneally (10^5 cells) or intracerebrally (10^4 cells) to groups of 10 mice. Each mouse was given various doses of I by intraperitoneal injection on Day 1 only or on Days 1, 5, and 9, and survival times were determined.

RESULTS AND DISCUSSION

The literature reports few examples of *in vivo* distribution studies involving radioisotopic organophosphorus antitumor agents. Crossley *et al.* (4) followed the distribution of ^{32}P-triethylenephosphoramide (III) and found a greater concentration of radioactivity in testes and less in the bone than with ^{32}P-dibasic sodium phosphate. Craig and Jackson (5), using III and rats implanted with Walker carcinosarcoma or Jensen sarcoma, showed that only a small part of the administered radioactivity was retained after 24 hr. Other investigators studied the distribution of the oncolytic agent ^{32}P-oxapentamethylenediethylenethiophosphoramide in the rat (6) and human (7) and found no evidence of selective uptake in any tissues, including the tumors, by any mode of administration. A degree of success at selective uptake by a phosphorus isotopic-labeled agent was reported by Fletcher *et al.* (8) who found that ^{32}P-diethylstilbestrol was taken up by breast tumors to an extent twice that of ^{32}P-dibasic sodium phosphate. None of these investigations involved determination of radioactivity in the brain.

In this present study, approximately one-third of the recovered activity appeared in the urine and one-half was found in the feces 12 hr after intraperitoneal injection of II. Thus, 80% of the activity was excreted within 12 hr. TLC of the urine resulted in the appearance of two radioactive spots, one with the same R_f value as II and a second that remained at the origin.

Levels of ^{32}P in all tissues sampled and in plasma were highest at the 3-hr time period and declined thereafter. A comparison of these results with those of Craig and Jackson (5), who also used a single intraperitoneal injection, shows that relatively more ^{32}P from III (0.18 and 0.08% after 3.5 and 12 hr, respectively) than from II (0.09 and 0.006% after 3 and 12 hr, respectively) was present in plasma. Spleen values, however, were essentially the same after the first time period (0.23% for III and 0.25% for II) and higher in the case of III (0.12%) compared to II (0.02%) after 12 hr.

The brain level of ^{32}P from the administration of II was about half that in the plasma after 3 hr compared to values reported for III (0.04 *versus* 0.09%) and one-third after 6 hr (0.01 *versus* 0.03%). This latter determination indicates a degree of penetration of II, or a metabolite, into the CNS, which may correlate with the activity found in intracerebral L-1210 testing.

Against intraperitoneal L-1210 cells, II administered on Day 1 only gave one 45-day survivor and a 50% increase in lifespan at 40 mg/kg. When this dose was given on Days 1, 5, and 9, the percent increase in lifespan was 75. The same dosage regimen against intracerebral leukemia cells gave 40 and 30% increases in lifespan for the single and triple injections, respectively.

As expected, II had better activity against intraperitoneal than intracerebral L-1210, but the margin was surprisingly narrow, at least for the single treatment. Although the activity of II is considered minimal compared to such oncolytics as 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, the ability of this agent to pass the blood–brain barrier in a cytotoxic form is encouraging; these data may prove helpful in the design of future compounds. The water solubility of II is possibly too low (less than 1 mg/liter) to provide the correct hydrophilic–lipophilic balance for optimum absorption and/or distribution.

[1] Type 57 Polaroid film.
[2] NCS.
[3] Waring blender.
[4] Packard Tri-Carb liquid scintillation spectrometer.

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