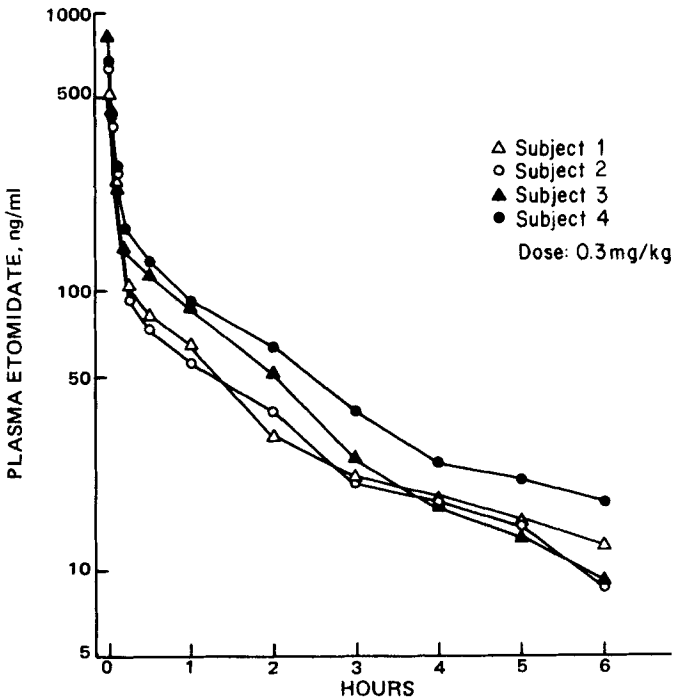

Figure 3—*Plasma concentrations following 0.3 mg of etomidate sulfate/kg iv. Concentrations in the 1-min samples from Patients 1 and 4 were 1170 and 1200 ng/ml, respectively.*

the vascular volume. Plasma etomidate concentrations in these patients at 6 hr after the injection were still well above the limits of assay sensitivity. Therefore, etomidate is detectable in plasma for several hours longer than previously indicated (7).

The semilogarithmic plot of these plasma level data (Fig. 3) indicates that there are at least three phases in the plasma disappearance of etomidate. This assay provides the sensitivity necessary to measure terminal phase plasma concentrations important in drug kinetic studies (12). A pharmacokinetic study involving a larger number of patients is currently in progress.

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ˣ To whom inquiries should be directed.

In Vitro Adsorption of Doxorubicin Hydrochloride on Insoluble Calcium Phosphate

ROY J. STURGEON, CYNTHIA FLANAGAN *, DATTA V. NAIK ‡, and STEPHEN G. SCHULMAN ˣ

Abstract □ The adsorption of doxorubicin hydrochloride, a potent antitumor agent, on solid tribasic calcium phosphate was studied *in vitro*. A Langmuir adsorption isotherm at pH 7.4 and the maximum adsorption capacity of tribasic calcium phosphate were established. Tribasic calcium phosphate was chosen as a model for solid bone samples, which are stained with doxorubicin in patients who have received long-term doxorubicin therapy.

Keyphrases □ Doxorubicin hydrochloride—adsorption on solid tribasic calcium phosphate *in vitro* □ Adsorption—doxorubicin hydrochloride on solid tribasic calcium phosphate *in vitro* □ Calcium phosphate, tribasic—adsorption of doxorubicin hydrochloride *in vitro*

Doxorubicin (I), an anthracycline antibiotic, has demonstrated antitumor activity against various solid tumors (1) as well as certain hematologic malignancies (2). Some serious complications encountered in the use of I are related to bone marrow depression, cardiac toxicity, stomatitis, and alopecia (3). Perhaps the most significant is bone marrow depression, primarily of leukocytes, which requires careful monitoring. In patients who have received extended I therapy, autopsies have shown that cross sections of bone tissue are stained with I (2, 4). Since solid bone samples are also stained, the process by which I is deposited in these areas is of interest.

The I molecule contains an anthraquinone moiety. Some mono- and dihydroxyanthraquinones are good chelating agents for several metal ions (5, 6). However, these compounds only form calcium chelates at pH 10–12, much greater than physiological pH. It was originally assumed that I would chelate a calcium ion in solution *in vivo* and be deposited in the outer layers of solid bone. Preliminary studies showed that I does not interact appreciably with calcium ions in solution at pH < 10 even when calcium ions are present in large excess.

To approximate bone samples, solid tribasic calcium

phosphate was added to solutions of varying concentrations of I buffered at pH 7.4. A substantial fraction of I was removed from solution. Compound I has a tendency to self-aggregate at concentrations greater than about 5×10^{-5} *M*; therefore, an adsorption isotherm (7) was established for only a very limited concentration range where only the monomer prevails. Comparisons of solutions of varying concentration and with varying amounts of adsorbent added are used to show that I can be deposited on bone by an adsorption mechanism.

EXPERIMENTAL

The pH measurements were made on a pH meter[1] employing a combination silver–silver chloride glass electrode[2]. Electronic absorption spectra were taken in 1-cm silica cells on a grating-type spectrophotometer[3]. Fluorescence measurements were taken on a fluorescence spectrophotometer[4] whose monochromators were calibrated against the line emission spectrum of xenon.

Doxorubicin hydrochloride[5] was used without further purification. Tribasic calcium phosphate[6] was triturated in a mortar until a relatively uniform particle size was obtained and then dried in an oven overnight. Phosphate buffers at pH 7.4 with a total buffer concentration of 0.05 *M* and the ionic strength adjusted to 0.16 with sodium chloride were used.

Bicarbonate buffers and sodium hydroxide solutions were used in studying the binding of calcium ions to I in the pH 7–12 range. Aliquots, 25 ml, of 1.00×10^{-1} *M* calcium perchlorate were added to 25 ml of 1.00×10^{-5} *M* I at varying pH's. The absorption and fluorescence spectra of these solutions were compared with those to which no calcium ions were added.

Accurately weighed amounts of tribasic calcium phosphate, 10–170 mg, were transferred to 25-ml test tubes with screw caps. After 25 ml of 1.00×10^{-5} *M* I was added to each test tube, the calcium phosphate suspensions were continuously mixed for 4 hr in a constant-temperature shaker at 37°. The suspensions were then centrifuged rapidly, and the clear supernate was assayed fluorometrically at 556 nm. Appropriate control solutions also were run and assayed to ensure control over experimental conditions.

The amount of I adsorbed on the calcium phosphate was determined on the basis of a previously established standard curve. The values used to construct the Langmuir isotherm were each the average of three separate measurements, and the standard deviation was no larger than ±0.05 unit in any set of measurements.

The solids from the centrifuged samples were collected by filtration and dried overnight. These solids were then reshaken in the phosphate buffer solutions, heated to 60°, and placed in an ultrasonic cleaning device for 5 min to desorb I partially. The absorption and fluorescence spectra of each solution after centrifugation were taken to ensure that I had not undergone any chemical reaction.

RESULTS AND DISCUSSION

Figure 1 illustrates the Langmuir adsorption isotherm for the uptake of I by tribasic calcium phosphate suspended in solution at pH 7.4. The reciprocal of the slope of the line gives the maximum binding capacity of tribasic calcium phosphate at pH 7.4. The wavelength absorption maxima of I at pH 7.0, 11.5, and 11.5 with 5.0×10^{-2} *M* Ca^{2+} were 495 (log ϵ = 3.97), 554 (3.76), and 556 (3.69) nm. For 1,8-dihydroxyanthraquinone, the wavelength absorption maxima at pH 7.0, 11.5, and 7.0 with 5.0×10^{-2} *M* Ca^{2+} were 415 (log ϵ = 3.74), 494 (3.91), and 500 (3.97) nm.

Since the absorption and fluorescence spectra of the monocation of I at pH 7 should show a dramatic red shift upon chelation of a metal ion such as Ca^{2+}, the lack of any perturbation of the spectra is indicative of no interaction. At physiological pH, the absorption and fluorescence spectra of I showed no changes in the presence of excess calcium ions. At pH 11.5 (where I is in the singly charged anionic form) in the presence of excess calcium ions, the absorption spectrum of I was slightly red shifted (1–2 nm) and showed a slight hypsochromic effect indicating that chelation took place at high pH. The hydroxyanthraquinones showed substantial shifts of their longest wavelength absorption bands upon binding to a metal ion. For example, the longest wavelength absorption band of 1,8-dihydroxyanthraquinone at pH 7.0 shifted from 410 to 500 nm upon the addition of excess calcium ions.

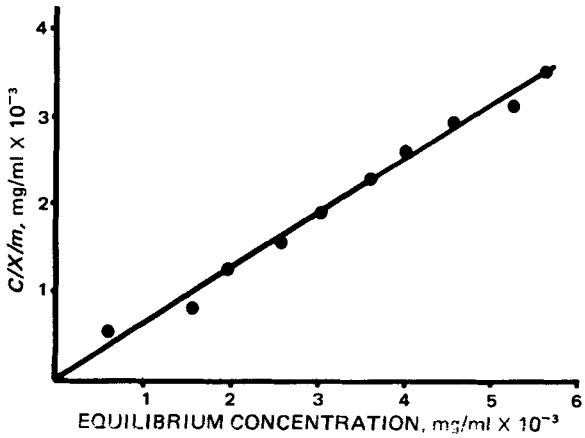

Figure 1—*Langmuir isotherm for the adsorption of I on tribasic calcium phosphate at pH 7.4; C is the equilibrium concentration in milligrams of doxorubicin per 100 ml of solution and* X/m *is the amount in milligrams of doxorubicin as the hydrochloride adsorbed per gram of calcium phosphate.*

The data indicate that the singly charged cation of I is adsorbed on the surface of the tribasic calcium phosphate. The ionic and lipophilic moieties of this drug probably promote drug adsorption through hydrophobic and electrostatic interactions with the surface of the calcium phosphate. The surface of the calcium phosphate crystal differs from the interior, and it is possible that the triply charged phosphates at the surface can contribute to the electrostatic part of the adsorption process. However, steric limitations on the chelation of calcium by I at the surface are imposed by the orbital structure of calcium. The forces of adsorption, whether they are electrostatic or hydrophobic, are certainly much weaker than the forces normally involved in coordinate–covalent bonding. The substantial dislodgement of I by gentle heating and sonication support these statements.

Under the given experimental conditions, the maximum adsorption capacity of calcium phosphate was estimated at 1.60 mg of I/g of calcium phosphate in suspension at pH 7.4. That the deposition of I on calcium phosphate is a physical adsorption process is further supported by the desorption of I from the tribasic calcium phosphate, which shows that no chemical alteration of I occurred. These results strongly suggest that the adsorption of I on solid bone does occur *in vivo*, resulting in the staining of bone samples upon extended treatment with this drug.

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* Summer Research Assistant, Florida Foundation for Future Scientists, 1975. Present address: California Institute of Technology, Pasadena, Calif.

‡ Present address: Manhattanville College, Purchase, N.Y.

ˣ To whom inquiries should be directed.

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[2] Markson 888.
[3] Beckman model 25.
[4] Perkin-Elmer MPF-2A.
[5] Adria Laboratories, Wilmington, Del.
[6] Merck & Co., Rahway, N.J.