Chlorpromazine Distribution in Hamsters and Mice Bearing Transplantable Melanoma


ABSTRACT

Chlorpromazine (CPZ) distribution was measured in tissues of Syrian golden hamsters bearing Greene melanoma and in BALB/c mice bearing Harding-Passey melanoma. Distribution was evaluated as a function of time (0.5 to 14 days) and as a function of single and multiple doses (up to five) of from 5 to 50 mg CPZ per kg body weight. Routes of administration (i.p., i.v., p.o.) were compared. The physiological behavior of CPZ is of interest as it is used extensively as a tranquilizing drug (Thorazine). Further, since CPZ binds to the pigment melanin, the possibility exists of using CPZ to transport diagnostic or therapeutic agents to melanoma. It was found that, at 2 days postinjection, tumor/tissue concentration ratios exceeded 10 for metabolizing organs, such as liver, and 100 for "background" tissues, such as blood and muscle. Absolute concentrations of CPZ in tumor exceeding 100 μg CPZ per g tumor were obtained with both single and multiple doses. This selective high concentration in tumor would make CPZ an ideal vehicle for the transport of boron to tumor for use in neutron capture therapy via the 10B(n,a)7Li reaction.

INTRODUCTION

The distribution of CPZ3 in tissues as a function of dose and time is of interest as CPZ is used extensively as a tranquilizing drug (Thorazine) (1, 11). The high therapeutic index of CPZ allows daily doses in humans varying from 25 to 5000 mg (12). A low percentage of patients subjected to chronic high doses develops melanosis (3). CPZ, as well as other nitrogen-substituted phenothiazines and related polycyclic aromatic compounds, binds to the pigment melanin. Pigmented tissues such as choroid are known to accumulate CPZ and to retain it with a biological t½ in the order of days, while a relatively rapid washout is observed for other tissues (1, 28). This physiological and selective localization of CPZ in pigmented tissues raises the possibility of utilizing CPZ to transport diagnostic or therapeutic agents to melanoma. In particular, the distribution of CPZ is of interest as it might be used as a vehicle for the transport of boron to tumors for NCT. Of the various polycyclic aromatic compounds of which the relative affinities for melanin have been listed by Potts (29), CPZ is advantageous because its toxicity is well known and because a borated analog is described in the literature (23).

Information is available regarding the early distribution (min) of CPZ (20) and for relatively low doses (5 mg/kg) out to 30 days (28). Less is known about the uptake in tumors, as this depends upon pigment (melanin) concentration and dilution effects due to tumor growth. The only data on tumor uptake of CPZ are those provided by Blois et al. (1), but they were not correlated with melanin content or presented in terms of absolute CPZ concentration. Consequently, it was decided to quantitate CPZ uptake in animal melanoma models. Given the absolute value of CPZ uptake, it then becomes possible to estimate its possible usefulness for transporting boron to tumors. A minimum of ~20 μg 10B per g tumor is needed (9).

CPZ distributions were evaluated for single doses of from 5 to 50 mg/kg. The higher level (50 mg/kg) approaches the value of ~70 mg/kg tolerated in daily doses by humans and also the mean lethal dose of 125 and 115 mg/kg found for hamsters and mice, respectively* (11). Multiple-dose distributions were also investigated in order to measure the effects of tissue loading and to try to optimize tumor CPZ content as well as tumor/tissue CPZ ratios.

Previous work has demonstrated that both Greene melanoma in Syrian golden hamsters and Harding-Passey melanoma in BALB/c mice are representative of human melanotic melanoma with respect to level of pigmentation (37). Thus, these 2 models were used. The distribution of CPZ is being evaluated in part on the assumption that information obtained might be applicable to a borated analog. It is recognized that the attachment of therapeutic elements on this chain may not adversely affect its toxicity. Nevertheless, the incorporation of nuclides into biochemicals or their precursors (such as halogenated pyrimidines and the labeling of certain proteins) has at times been successful (33).

At physiological pH, CPZ exists as a monoprotonated cation (13). The work of Larson and Tjäse (16) indicated that non-electrostatic forces are involved in the binding of CPZ to melanin (i.e., a charge transfer reaction with melanin as the electron donor). It is thought that binding is a property of the phenothiazine ring rather than the nitrogen side chain. Thus, additions, substitutions, and/or substitutions of diagnostic or therapeutic elements on this chain may not adversely affect binding. The distribution of CPZ as described in this paper should be useful in the evaluation of such CPZ analogs.

MATERIALS AND METHODS

CPZ. [35S]CPZ hydrochloride (t½, 87.2 days) was used as obtained from Amerahem/Searle Corp. (Arlington Heights, Ill.) (radiochemical purity, 99%). Specific activity at the time of purchase was ~15 mCi/μg. (Work supported by National Cancer Institute Grant R01-CA22749 and United States Department of Energy Contract DE-AC02-76CH00016.)

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2 To whom requests for reprints should be addressed, at Medical Dept., Brookhaven National Laboratory, Upton, N. Y. 11973.

3 The abbreviations used are: CPZ, chlorpromazine; NCT, neutron capture therapy; WBAR, whole-body autoradiography.

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* R. Fairchild and S. Packer, unpublished data.
mmol. High-dose-level studies of CPZ were carried out with lower-level activity material such that absorbed doses to tumor (as calculated from measured count rates) were estimated to be <10 rads/day.

**Animal Models.** Previous work has shown that Greene melanoma in Syrian golden hamster (melanin content, ~0.4% by weight) and Harding-Passey melanoma in BALB/c mice (~0.7% by weight melanin) were representative of human melanotic melanoma with respect to melanin content (0.1 to 0.9% by weight melanin; average, ~0.35%). Further, the average melanin content of hamster whole eye (~0.45% by weight) is analogous to that in human melanoma and may indeed serve as a more useful model of the latter which has a doubling time of ~42 days (37). The rapid tumor volume doubling time of Greene melanoma (~2 days) makes interpretation of long-term drug studies difficult. The longer and less variable doubling time of the Harding-Passey model permitted acquisition of data which were much more reproducible with respect to CPZ uptake. Both models were used, however, in order to facilitate intercomparison with other work, as data from models have been reported extensively in the literature. Hamsters weighed ~130 g; mice weighed ~20 g.

**Counting Procedures.** Multiple tissue samples were taken when possible. Each data point represents the average of 3 to 4 animals. Tissue samples with wet weights between 100 and 200 mg were prepared for liquid scintillation counting. A mixture of perchloric acid (70%) and hydrogen peroxide (30%) was used for wet oxidation of samples (18). Samples were counted in a Beckman LS 300 counter (Beckman Instruments, Inc., Mountainside, N. J.). The external standard method was used for efficiency correction. Correlation between the external standard and reduction in efficiency from chemical and color quenching was made through experimental quenching curves obtained for each tissue type (internal standard method). Each experiment incorporates data from the same or adjacent tumor passages; no significant change in tumor characteristics (i.e., histological appearance, pigmentation, doubling time) was observed during the course of this work.

**Dosage.** Single and multiple doses (up to 5) of from 5 to 50 mg CPZ per kg body weight were administered i.p. Routes of administration (p.o., i.v.) were investigated at low doses (5 mg CPZ per kg). Unless otherwise noted (i.e., single-dose distribution as a function of time), animals were sacrificed 48 hr after the last injection.

**WBAR.** The technique described by Ullberg (36) and developed by Fand and McNally (10) was used to produce WBARS of 30-µm sections from tissues of BALB/c mice sacrificed at 6 and 48 hr postinjection of $^{35}$S]CPZ. Doses of 5 µg CPZ per g tissue were given i.v. with an activity of 6 µCi per 100 g.

**RESULTS**

**Route of Administration.** Clinically, CPZ is generally given p.o., while with animal experiments (and in the present work), i.p. injections are often used for convenience. Tissue distribution in hamsters was measured at 2 days after injection of 5 mg/kg. No consequential difference in distribution between i.p. and p.o. routes of administration was noted. Similarly, p.o., i.p., and i.v. routes were investigated with BALB/c mice carrying Harding-Passey melanoma. Again, little variation was observed among routes. Uptake in analogous tissues of hamster and mouse was found to be similar when results were expressed in terms of µg CPZ per g tissue.

**Single Dose as a Function of Time (Hamsters).** CPZ distribution in various tissues is shown in Chart 1, from 0.5 to 14 days (administered dose, 5 µg CPZ per g tissue; total dose, 650 µg). Excretion is seen to be mainly through the bladder; major accumulations occur in lung and liver. From these data, it is clear that 2 days should be allowed to elapse for CPZ to accumulate in pigmented tissues and to clear other tissues. At 2 days, most organs (with the exception of liver) are lower than whole eye by ~10-fold (liver lower by ~4-fold) with "background" tissues, such as blood and muscle, lower by ~100-fold. Tumor uptake at 2 days averaged ~0.3% g but varied by factors of 2 between tumors, or between multiple samples of the same tumor, due in part to dilution effects caused by rapid and uneven growth.

As noted above, hamster whole eye has a melanin content similar to the average value found for human melanocytic melanoma. Thus, the value for eye in Chart 1 may be representative of drug uptake in human melanoma. The tumor volume doubling time for human melanoma of 6 to 7 weeks (25, 32) is "long" compared to the study duration of 0.5 to 14 days, while the rapid doubling time (~2 days) of Greene melanoma made data beyond 2 to 6 days postinjection difficult to evaluate.

**Single-Dose Distribution as a Function of Dose Magnitude (Hamsters).** Percentage of uptake is shown in Chart 2 for injected doses of 5, 25, and 50 µg/g (total doses of 650, 3250, and 6500 µg). The higher dose approaches the maximum daily dose advised for humans and the median lethal dose of ~120 mg/kg for i.p. injection in mice and hamsters. The median lethal dose for p.o. administration would be 3-fold higher (11).

These data were obtained at 2 days postinjection in order to allow clearance from nonpigmented tissues as indicated in the time distribution study. The effects of tissue loading are evident. As the injected dose is increased by a factor of 10, from 5 to 50 mg/kg, the fraction taken up by pigmented tissues (eye, tumor) is reduced by ~25%, while the fraction taken up by nonpigmented tissues is reduced by a factor of 4. (Concomitantly, the fraction in urine is seen to rise.) Clearly, the loading of pigmented tissues with CPZ would best be accomplished by multiple large doses administered

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**Chart 1.** CPZ distribution in hamsters for various times following single doses of 5 µg/g. Each point represents the average of 3 to 4 animals. Experimental error is similar to that shown in Tables 1 and 2; bars have been omitted for simplicity.
every 2 days. Large doses would increase pigmented/nonpigmented tissue ratios, while the 2-day wait would allow tissue clearance and reduce physiological trauma.

**Multiple-Dose Distribution (Hamsters).** In an effort to increase absolute uptake, the distribution following multiple doses was evaluated. Five doses of 50 μg CPZ were administered every 2 days, and 5 doses of 25 μg CPZ per g were given daily. Results are displayed in Table 1 in terms of absolute CPZ uptake. The molecular weight of CPZ hydrochloride is 355; the sodium salt of the borated compound described in Ref. 23 would be 472 (twelve 10B atoms). Assuming a molecular uptake of borated CPZ equivalent to CPZ, the uptake of boron can be estimated. Projected boron levels were obtained which are adequate for therapy in both Greene melanoma and whole eye (i.e., ≥20 μg 10B per g tumor). To simplify comparison, results were expressed in terms of percentage of dose per g in Charts 1 and 2. Absolute uptake may be obtained directly by using animal weights of 130 g for hamsters (20 g for BALB/c mice).

**Single- and Multiple-Dose Distribution (Mouse).** Because of persistent problems in obtaining reproducible results with Greene melanoma, another established model, the Harding-Passey melanoma in BALB/c mice, was evaluated.

Multiple-dose distributions measured at 2 days following i.p. injection are given in Table 2 for 5, 25, and 50 μg/g. Reproducibility of CPZ uptake in tumor was satisfactory, with experimental S.D.s similar to those of other tissues. At low doses (5 μg CPZ per g), uptake in normal tissue was about the same for both hamster and mouse (Chart 2; Table 2), while CPZ concentration in Harding-Passey melanoma was significantly higher. At higher doses, tissue uptake increased per unit administered dose, indicating perhaps effects of reduced metabolic rate.

Multiple-dose distributions are shown in Table 2 for 5 doses of 25 μg CPZ per g (2 days between doses) and 3 doses of 25, 50, and 50 μg CPZ per g, given every 2 days. Animals were sacrificed 2 days after the last dose. Projected borated CPZ accumulations in tumor are more than adequate for therapy.

**WBAR.** WBARs obtained following doses of 5 μg CPZ per g tissue are shown in Fig. 1; Fig. 1A (6 hr) shows a fairly ubiquitous distribution as would be suggested from Chart 1. Early tumor distribution is nonuniform, while at 48 hr (Fig. 1B), activity is concentrated in the tumor with a fairly homogeneous distribution in tumor. WBAR is a powerful technique which allows visualization and quantitative evaluation of CPZ distribution among all organs as well as distribution within the organ itself. When 35S radioactivity equivalents were measured densitometrically in WBARs from the 48-hr animal, melanoma tissue showed on average 11.6 times greater activity than did liver (Table 3). Absolute values may be obtained from Table 2. From the WBAR shown in Fig. 1 and the other WBARs studied, it is apparent that no tissues concentrate CPZ to the extent seen in the tumor.

**DISCUSSION**

Greene melanoma was evaluated as a model, because it has been used extensively to test melanin-affinic diagnostic and therapeutic agents (22, 27). Poor reproducibility of CPZ uptake was experienced repeatedly; tumor uptake variability exceeded experimental error in all other tissues. Variation in pigment concentration is not believed to be enough to account for this variable uptake (37). Tumor volume doubling times were found to vary from ~1 to 5 days during experiments; the latter fact no doubt contributed to the observed variation in CPZ uptake, due to dilution effects. Such variability in doubling time and in uptake of melanin-seeking agents has been observed by others with Greene melanoma (22, 27).

While variability is expected in human clinical trials with spontaneous neoplasms, such characteristics are not useful in a laboratory test system used to define limits and illuminate trends.

It is felt that the improved reproducibility obtained with Harding-Passey melanoma makes this a more useful test system.
Table 2

Table 2 CPZ distribution in mice following single and multiple doses

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Single dose (µg/g)</th>
<th>Multiple dose (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Tumor</td>
<td>8.5 ± 1.2±(2.9)²</td>
<td>55 ± 11 (19)</td>
</tr>
<tr>
<td>Liver</td>
<td>1.02 ± 0.09</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.14 ± 0.04</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.26 ± 0.01</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Lung</td>
<td>0.12 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.07 ± 0.03</td>
<td>0.42 ± 0.15</td>
</tr>
<tr>
<td>Blood</td>
<td>0.023 ± 0.002</td>
<td>0.31 ± 0.09</td>
</tr>
<tr>
<td>Brain</td>
<td>0.012 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>Eye</td>
<td>0.024 ± 0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± S.D.

Numbers in parentheses, boron content (µg/g) projected from CPZ content.

Table 3

Table 3 Densitometric comparison of 35S radioactivity in Harding-Passey melanoma and liver tissue 48 hr after i.v. administration of [35S]CPZ

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>Tumor</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.86</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>1.16</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>1.06</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>0.94</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>1.19</td>
<td>1.04</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*dpm/sq mm = 39.5*

*dpm/sq mm = 3.4

Further, correlation of CPZ uptake with melanin content (Chart 3) supports the thesis that CPZ uptake is proportional to melanin content (26). Included in Chart 3 are data from B-16 melanoma in C57/BL mice and hamster and mouse whole eye. Melanin contents are from Ref. 37. CPZ uptake in Greene melanoma is evidently not consistent with the 4 other pigmented tissues.

The accumulation of CPZ in pigmented tissues is accompanied by a rapid and almost complete clearance from blood and a washout from other (nonpigmented) tissues over a period of a few days, as has been reported by others (1, 28, 35). At 2 days, tumor/tissue ratios exceed 10 for metabolizing organs, such as liver, and 100 for background tissues, such as blood and muscle. Thus, CPZ would appear ideal as a vehicle for diagnostic or therapeutic agents. Dosing procedures involved one to 5 doses at a concentration of 5 to 50 µg/g every 2 days. These results are in general agreement with the work of Blois (1). In the latter case, a spontaneous tumor (doubling time, ~2 weeks) was used and produced CPZ uptakes in tumor equal to that of whole eye (C3H mice). Tumor pigmentation was given as twice that of C3H whole eye. Our work with C3H mice indicated an uptake in whole eye of 4.6 µg CPZ per g tissue for an injected dose of 5 µg/g (C3H mouse whole eye melanin content = 0.4% by weight, Ref. 37) This is consistent with uptakes of 5.2 and 8.5 µg CPZ per g tissue for hamster whole eye and Harding-Passey melanoma. Efforts with the original tumor used by Blois revealed that, by the 57th passage, it had lost its pigmentation (37).

While absolute uptake per g was similar for hamster and mouse at low doses, high doses produced effects of tissue loading for hamsters and an evident accumulation in mouse tissues due perhaps to reduced metabolic rate. Hamsters were found to recover from high doses of CPZ (50 µg/g) within 12 to 24 hr, while BALB/c mice remained groggy at 48 hr. Particular sensitivity was noted in mice ≤6 weeks old. Data here were from mice ≥10 weeks.

Route of administration experiments indicate p.o. administration would be preferable for clinical use, because toxicity is reduced without significant loss (i.e., less than ~20%) of absolute uptake. With animals, 2 days must elapse between high doses to allow recovery and self-nourishment. The same restriction would not necessarily obtain for human use. Significant reduction in sensitivity was obtained by predosing with one half-strength dose, as shown in Table 2, where maximum uptake of CPZ was obtained.

From the data given above, it follows that, if the borated
analogs behave in the same way, the accumulation of CPZ obtained in both Greene and Harding-Passey melanoma models should be adequate for NCT.

The consensus is that natural melanins are highly irregular 3-dimensional polymers made up mainly of indole units at different oxidation levels with free radicals trapped in segments of the structure (2, 31). Melanin is thought to serve as an electron trap (17, 19), while CPZ is an electron donor (14). In the presence of melanoproteins, CPZ has been found to form a radical ion (CPZ)\(^+\) (3, 5). It has been suggested that the binding of CPZ to melanin is a result of a completed charge transfer reaction between CPZ and the free radical of melanin (4, 16, 29). Studies with various substituted phenothiazines have led to the assertion that the nature of the nitrogen side chain or the position 2 substituent does not determine whether or not storage in pigmented tissues occurs, and thus, binding to melanin may be a property of the phenothiazine ring (28).

Melanin is known to be rich in metals such as zinc, copper, iron, and manganese (6). The strong affinity for metal ions has been ascribed to electrostatic forces between the cations and anionic sites on the melanin polymer (presumably carboxyl groups) (16). Melanin cation affinity increases with the cation atomic weight. While there appears to be little or no affinity between melanin and cations of the alkali metals (15, 30), on a molar basis (μmol/mg melanin), heavy multivalent metal cations are bound to the same degree as CPZ. For example, the total binding capacity of melanin for Ni\(^2+\) and CPZ was found to be 0.69 and 0.77 μmol/mg melanin, respectively (16). This is in basic agreement with the value of ~0.5 μmol CPZ per mg melanin found by Potts (29) (based on 50% melanin per mg pigment granules). Of the various metal ions investigated in vitro, Gd\(^3+\) is bound with the greatest avidity (15). The binding of CPZ has been found to be predominantly nonelectrostatic and to occur at different sites than the metal ions, with different association constants (16). Thus, heavy metal cations might be bound simultaneously with CPZ. \(^{155}\)Gd, \(^{157}\)Gd, or \(^{149}\)Sm (ε(ν,γ) = 61,000, 250,000, and 42,000 barns, respectively) would make these isotopes prime candidates for both dose enhancement with neutron therapy and melanoma detection with prompt γ analysis. \(^{164}\)Dy (ε(ν,γ) = 2600 barns; \(^{165}\)Dy t\(^\frac{1}{2}\) = 2.35 hr; γ = 95 keV) might allow conventional imaging.

If one can accumulate boron in tumor, irradiation with neutrons releases charged particles in tumor via the \(^{10}\)B(n,α)\(^7\)Li reaction. In such a circumstance, NCT would provide optimal conditions for radiotherapy, because the short range and high linear energy transfer of the reaction products allow selective irradiation of tumor cells and a greater effectiveness against hypoxic cells as the oxygen enhancement ratio is ≥1. A review of various high linear energy transfer “particle” beam radiotherapeutic modalities has concluded that potentially, NCT offers a uniquely powerful system for cancer therapy (8). However, the consensus is that previous efforts with NCT have suffered from borated compounds which failed to clear the blood (34). The possible advantage afforded by a borated analog of CPZ is based on its specificity and high concentration in melanoma and its essentially complete clearance from blood. It has been demonstrated that CPZ provides differential and absolute concentrations in melanoma which are more adequate for therapy, assuming borated analogs behave similarly. While a borated analog of CPZ has been reported in the literature (21, 23, 24), attempts to make such a compound available in this country have so far been unsuccessful. The possibility of a robust \(^{10}\)B concentration coupled with a nearly complete clearance from blood and other tissues would make continued efforts to synthesize a borated CPZ analog of prime importance.

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Fig. 1. WBAR of BALB/c mice carrying Harding-Passey melanoma. CPZ dose was 5 μg/g with a specific activity of 6 μCi/100 g (i.v. injection). A, distribution 6 hr postinjection; B, distribution at 48 hr.
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