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Comparative brain and plasma pharmacokinetics and anticancer activities of chlorambucil and melphalan in the rat

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Summary. Equimolar doses of chlorambucil and melphalan (both 10 mg/kg) were administered i.v. to anesthetized rats, and the plasma and brain concentrations of chlorambucil, its metabolites 3,4-dehydrochlorambucil and phenylacetic mustard, and melphalan were determined by high-performance liquid chromatography from 5 to 240 min therafter. Chlorambucil demonstrated a monophasic disappearance from plasma, with a half-life of 26 min. The compound was 99.6% plasma-protein-bound. Chlorambucil underwent β-oxidation to yield detectable concentrations of 3,4-dehydrochlorambucil and substantial amounts of phenylacetic mustard in the plasma. Low concentrations of chlorambucil and phenylacetic mustard were detected in the brain. Calculated from the areas under the concentration-time curves, the brain:plasma concentration integral ratios of chlorambucil and phenylacetic mustard were 0.021 and 0.013, respectively. Melphalan demonstrated a biphasic disappearance from plasma, with half-lives of 1.9 and 78 min. The compound was approximately 86% plasma protein-bound. Low concentrations of melphalan were detected in the brain, and its brain:plasma ratio was 0.13. These data demonstrate that following the administration of chlorambucil and melphalan, only low concentrations of active drug are able to enter the brain. As a consequence, concentrations of both drugs that cause the complete inhibition of extracerebrally located tumor have no effect on those located within the brain. Further, the brain uptake of melphalan, although low, is greater than that of chlorambucil and its active metabolites, which coincides with its slightly greater intracerebral activity following the systemic administration of very high doses.

Introduction

Melphalan and chlorambucil are two structurally related anticancer drugs that are used to treat a wide variety of malignancies. Melphalan is effective in the treatment of multiple myeloma, ovarian carcinoma, as adjuvant chemotherapy of stage II breast carcinoma, and in the regional perfusion of nonresectable melanoma [9, 14, 15, 42, 44]. Chlorambucil is used in the treatment of chronic lymphocytic leukemia, carcinoma of the breast and ovary, and Hodgkin's and non-Hodgkin's lymphomas [9, 29, 42, 45]. Both drugs are classical bifunctional alkylating agents. Melphalan is a nitrogen mustard derivative of the large neutral amino acid L-phenylalanine, and chlorambucil is structurally similar to melphalan but lacks an amine moiety (Fig. 1).

Although these drugs have been used extensively in the clinic for some 20 years, relatively few studies have attempted to analyze their tissue distribution and pharmacokinetics. The majority of the tissue distribution studies employed [^{14}C]melphalan or [^{14}C]chlorambucil [7, 9, 16, 17, 28, 38], and consequently were unable to distinguish between the drug and its metabolites. Following the administration of melphalan to humans or rats, melphalan becomes hydrolyzed to its monohydroxy and dihydroxy products, the latter having no anticancer activity. Chlorambucil undergoes β-oxidation in vivo, to yield the active metabolite phenylacetic mustard via the active intermediate 3,4-dehydrochlorambucil [35, 36]. Recent studies have utilized high-performance liquid chromatography to quantitate both melphalan [2, 4, 46] and chlorambucil [36, 40] in biological fluids and differentiate them from, and in some cases separately quantitate, their metabolites. Such studies have provided valuable information regarding their plasma pharmacokinetics [2, 4, 36, 40], metabolism [35, 36], excretion and gastrointestinal absorption [1, 5]. Few studies, however, have related these to the brain and assessed the brain uptake and pharmacokinetics of these agents and then related these to their anticancer activity [21].

The brain is a major site in the metastatic cascade process [21, 23]. The incidence of metastases to the brain from cancer of the ovary, breast and melanoma, for which melphalan and chlorambucil are administered, are 5%, 10% and 40%, respectively [22]. The brain uptake and pharmacokinetics of both agents were therefore measured in the rat, and related to their plasma pharmacokinetics. In addition, their anticancer activity in the brain was assessed and related to that achieved against an extracerebral tumor implant following systemic drug administration. Preliminary pharmacokinetic data on melphalan has been published [25].

Materials and methods

Pharmacokinetic study

Adult male Wistar rats (Charles River Laboratories Inc., Wilmington, Mass) weighing approximately 200 g were

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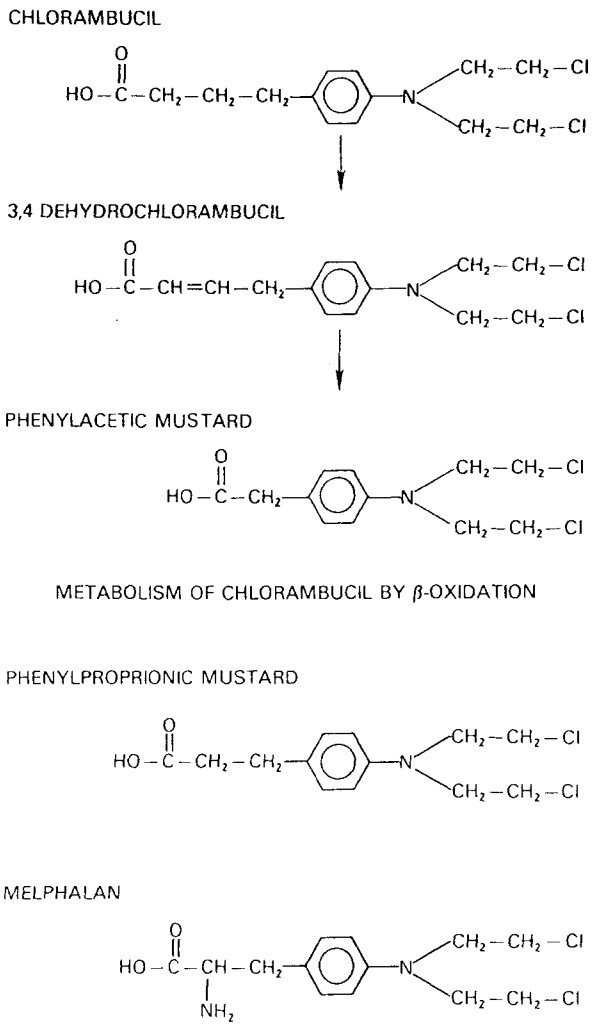

Fig. 1. Chemical structures of chlorambucil, its products of β-oxidation, 3,4-dehydrochlorambucil and phenylacetic mustard, and of phenylproprionic mustard and melphalan

anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The left saphenous vein was exposed, and melphalan 10 mg/kg (Sigma Chemical Co., St Louis, Mo), or chlorambucil 10 mg/kg (Sigma Chemical Co.) in buffered isotonic saline was injected i.v. (1 ml/kg). At intervals from 15 s to 4 h following melphalan administration and from 5 min to 4 h following chlorambucil administration, blood was collected by cardiac puncture and the brain was removed and placed on 0.9% NaCl, ice-chilled filter paper. At least five animals were killed per time point. The blood was centrifuged (7000 g, 1 min), and the plasma removed and stored immediately at −70° C. Plasma and brain samples from animals administered melphalan were analyzed for melphalan by high-performance liquid chromatography, HPLC, as described by Sweeney et al. [46]. Plasma and brain samples from animals administered chlorambucil were analyzed for chlorambucil and for its active metabolites, 3,4-dehydrochlorambucil and phenylacetic mustard, by HPLC.

Chlorambucil analysis procedure

Chemicals. Chlorambucil, 4(4-bis(2-chloroethyl)aminophenyl)butyric acid, was purchased from Sigma Chemical Co. (St. Louis, Mo). Phenylacetic mustard, 2(4-bis(2-chloroethyl)aminophenyl)acetic acid, and phenylproprionic mustard, 4(4-bis(2-chloroethyl)aminophenyl)proprionic acid, were obtained from the Pharmaceutical Resources Branch of the National Cancer Institute (Bethesda, Md). The latter is not a product of the in vivo metabolism of chlorambucil, and was used an internal standard during chlorambucil analysis (Fig. 1).

Instrumentation and conditions. HPLC analysis was performed using a Waters Assoc. system (Milford, Mass). This consisted of a Model 6000A solvent pump, a Wisp 710B automatic injector, and a model 480 variable wavelength UV detector, which was set at 254 nm wavelength. Separation was performed on a 10 μm Partosil 10 ODS 3 column (Ace Scientific, East Brunswick, NJ) with a guard column packed with pellicular C_{18} material (Waters Assoc.). Chromatograms were recorded on a Waters Model 720 data module. The mobile phase was a mixture of water, acetonitrile and acetic acid (59:39:2, v:v:v). The flow rate of the mobile phase was set at 1.8 ml/min, which produced a column pressure of 2000 psi. The retention times of phenylacetic mustard, the internal standard (phenylproprionic mustard), 3,4-dehydrochlorambucil, and chlorambucil were 4.9, 5.9, 6.4, and 7.3 min, respectively. Concentrations of chlorambucil and metabolites were calculated from the ratio of their peak height measurements to the internal standard. These were then quantified from calibration curves of six points, two samples per point, which were run daily and intermixed with the unknown samples.

Sample preparation and extraction. Plasma and brain samples were thawed and placed in an ice bath at 0° C. Then 2 ml chilled water and 6 ml ethylacetate were added to each plasma sample, which then was shaken and centrifuged (2000 g for 10 min at 4° C). The ethylacetate phase was removed and evaporated to dryness, after which 100 μl methanol containing 2% acetic acid was added. The samples then were injected onto the HPLC. For brain, 4 ml chilled water was added and the sample was sonicated (Model 225, Heat Systems-Ultrasonics Inc., Farmingdale, NY) for 30 s. It was then extracted as described for the plasma samples.

Protein binding studies

The plasma protein-binding of melphalan and of chlorambucil were measured at concentrations from 1 nmol/ml to 150 nmol/ml by centrifugal ultrafiltration. Amicon centrifree micropartition systems (Amicon Corp., Danvers, Mass) were used for the rapid preparation of protein-free ultrafiltrates of melphalan and of chlorambucil in human plasma and pooled rat plasma. Each micropartition device contained an Amicon YMT membrane with a molecular weight cutoff of 25000 daltons. Prior to experimentation, the membranes and supporting structures of each device were presoaked with the freshly prepared test melphalan or chlorambucil solution (5 min, 0° C), blotted and assembled into the micropartition system. One milliliter of the test drug solution then was applied to the membrane, whereupon the device was equilibrated to 37° C for 5 min and centrifuged for 15 min at 2000 g at 4° C. Approximate-

ly 100 µl protein-free ultrafiltrate was produced. This and a sample of the original drug solution were stored at −70° C prior to melphalan or chlorambucil analysis. Drug-membrane binding was measured with solutions of melphalan or chlorambucil in isotonic physiological buffer. The recovery of both drugs was approximately 100%.

Anticancer activity studies

Male Wistar rats (Charles River Laboratories Inc.), approximately 120 g weight, were anesthetized with Na pentobarbital (40 mg/kg, i.p.). Walker 256 carcinosarcoma tumor cells (1×10^5) in 10 µl Dulbecco's modified Eagle's tissue culture medium (Gibco Laboratories, Chagrin Falls, Ohio) then were injected intracerebrally into the parietal cortex through a 30-gauge needle attached to a Hamilton syringe. Into other rats, 0.2 ml freshly minced viable Walker 256 carcinosarcoma tumor was injected s.c. into the left flank.

With the exception of control animals, which received injections of vehicle alone, all animals received either melphalan (0.025–9 mg/kg) or chlorambucil (0.25–16 mg/kg) i.p. at 36 h, 3 days and 7 days after tumor implantation. Drug activity against the intracerebrally implanted tumors was assessed by comparing the mean sruvival time of the drug-treated animals with that of the controls. Activity against s.c.-implanted tumors was assessed by excising the tumors surgically 9 days after their implantation and comparing their mean wet weight with that of the controls. Controls were run concurrently with treated animals in all studies; a minimum of seven animals were used in each control and treatment group. Animal weight was measured to estimate drug toxicity. This was judged to have occurred by the drug-related death of one or more animals. Postmortems and light microscope tissue examinations were undertaken to confirm drug-related deaths.

Calculations

Pharmacokinetic study. Brain concentrations of melphalan, chlorambucil and its active metabolites were calculated from the net regional brain concentration, as measured with HPLC, by subtracting the intravascular volume at the time of death (T). The intravascular concentration equalled the plasma concentration of the compound (nmol/ml) at time T, multiplied by the regional blood volume (ml/g brain).

Regional blood volume was measured in four adult male Wistar rats (Charles River Laboratories Inc.), 200–250 g weight, following i.v. injection of [^{125}I]human serum albumin (100 µCi/kg, Amersham Corp., Arlington Heights, Ill). The [^{125}I]human serum albumin was determined to be 99.9% pure by gas-liquid chromatography. Blood and brain samples were collected at 2 min as described for melphalan, and samples of plasma, cerebellum and cerebral cortex were assayed for [^{125}I] activity (Gamma 4000, Beckman Instruments, Palo Alto, Calif). [^{125}]Human serum albumin is restricted to the cerebral vasculature during the 2-min experiment [24]. The regional blood volumes of the cerebellum and cerebral cortex were calculated by dividing the [^{125}I] activity of the brain samples by that of the plasma (dpm.g^{-1}/dpm.ml^{-1}) and were equal to 2,4% and 1.7%, respectively.

The melphalan and chlorambucil concentration-time data were fitted by nonlinear regression analysis [31, 32] to either a single or a biexponential equation, where

$$C = Ae^{-at} \text{ or } C = Ae^{-at} + Be^{-bt}$$

The brain data, the peak concentration and the drug levels terminal to it, were fitted to the single exponential equation. In these equations, C equals the concentration of melphalan (nmol/ml or nmol/g) at time t (min). A and B are defined as the theoretical zero-time concentration in a central and peripheral compartments, respectively, and a and b are apparent first-order elimination rate constants (min^{-1}). Plasma half-lives were calculated from the parameters by the general formula

$$\text{Half-life}_x = \frac{0.693}{x}$$

where x = a or b. Areas under the concentration-time profiles were calculated by the trapezoidal rule.

Statistical analysis. A two-tailed Student's *t*-test was performed to compare two means. When more than two means were compared, one-way analysis of variance and the Bonferroni multiple test were used [37]. Statistical significance for all tests was taken as $P < 0.05$. Means ± SEM are given routinely, unless otherwise stated.

Results

Pharmacokinetics

Chlorambucil. Figure 2 illustrates the plasma and brain concentrations of chlorambucil, 3,4-dehydrochlorambucil and phenylacetic mustard, following i.v. administration of

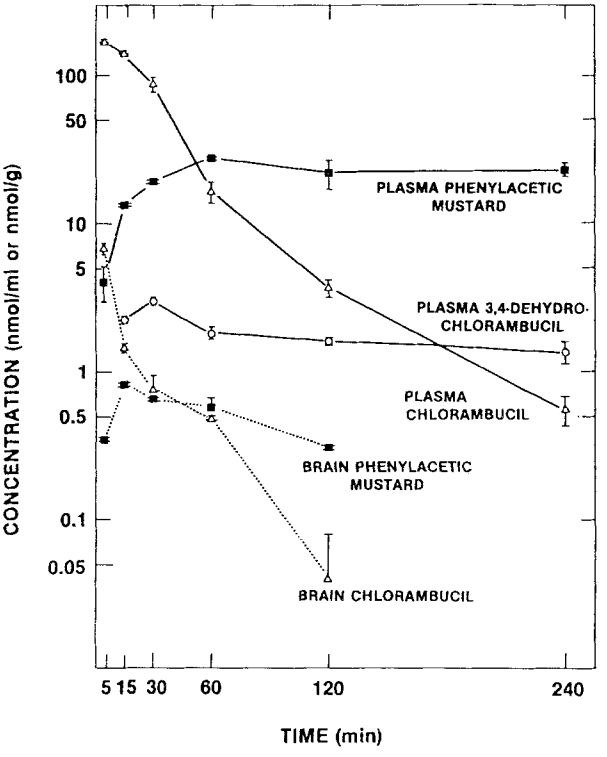

Fig. 2. Plasma and brain concentrations (± SEM) of chlorambucil, 3,4-dehydrochlorambucil and phenylacetic mustard following i.v. administration of chlorambucil (10 mg/kg) to the rat

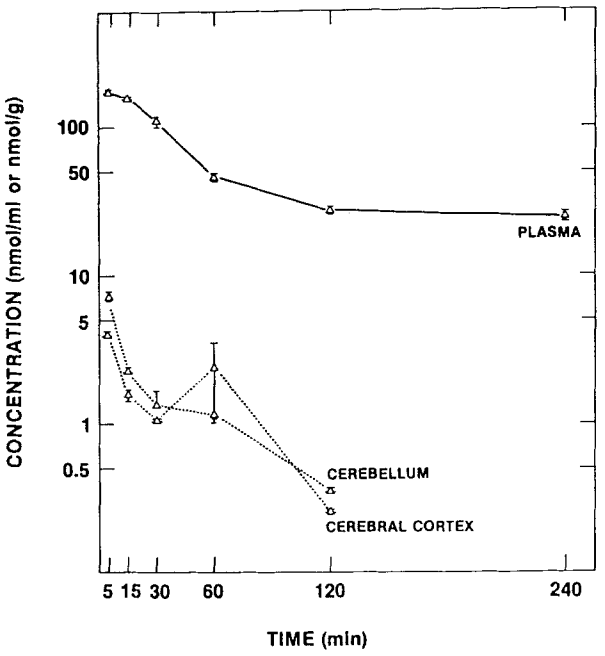

Fig. 3. Plasma and brain concentrations (± SEM) of chlorambucil plus its active metabolites following i.v. administration of chlorambucil (10 mg/kg) to the rat

chlorambucil (10 mg/kg). Chlorambucil demonstrated a monophasic disappearance from plasma during the period of the study, from 5 to 240 min, with a half-life of 26 min. Low concentrations of the intermediate metabolite of chlorambucil derived by β-oxidation, 3,4-dehydrochlorambucil, were detected in the plasma at and after 15 min. A peak concentration of 3.05 nmol/ml was achieved at 30 min. Phenylacetic mustard, the end product of chlorambucil β-oxidation, was detected in the plasma in substantial amounts throughout the study. Phenylacetic mustard concentration peaked at 27.8 nmol/ml at 60 min, and remained approximately unchanged throughout the rest of the study; declining to 23 nmol/ml at 240 min.

Low concentrations of chlorambucil and phenylacetic mustard were found within the brain (Fig. 2). Chlorambucil reached a peak concentration of 3.95 nmol/g in the cerebral cortex and 6.8 nmol/g in the cerebellum at 5 min. Thereafter, chlorambucil concentrations declined in both brain areas and were undectable at 240 min. Phenylacetic mustard reached a peak concentration of 0.62 nmol/g and 0.81 nmol/g in the cerebral cortex and cerebellum, respectively, at 15 min, declined thereafter, and was undetectable at 240 min. 3,4-Dehydrochlorambucil could not be detected within the brain at any time.

Figure 3 shows the total plasma and brain concentrations of 'compounds possessing anticancer activity' following i.v. administration of chlorambucil (10 mg/kg). This term was calculated by summing the concentrations of chlorambucil, 3,4-dehydrochlorambucil and phenylacetic mustard in each individual sample. The total concentration of compounds possessing anticancer activity in plasma demonstrated a biphasic disappearance, declining from 172 nmol/ml at 5 min to 24.8 nmol/ml at 240 min, with serial half-lives of 28 and 228 min. Chlorambucil represented the major component of the total concentration at times up to 30 min, whereas phenylacetic mustard represented the major component at and after 60 min. Within the cerebral cortex and cerebellum, the total concentration of compounds possessing anticancer activity declined from 3.95 nmol/g and 7.1 nmol/g, respectively, at 5 min to 0.25 nmol/g and 0.35 nmol/g, respectively, at 120 min.

The areas under the concentration-time curves measured between 5 and 240 min in plasma, cerebral cortex and cerebellum for chlorambucil, 3,4-dehydrochlorambucil, phenylacetic mustard and the total compounds possessing anticancer activity are shown in Table 1. Calculated from these, the mean brain:plasma concentration integral ratio for chlorambucil, 3,4-dehydrochlorambucil, phenylacetic mustard and the total compounds possessing anticancer activity were 0.021, 0.00, 0.013 and 0.017, respectively (Table 2).

Melphalan. Figure 4 shows the plasma and brain pharmacokinetics of melphalan following i.v. administration (10 mg/kg). Melphalan demonstrated a biphasic disappearance from plasma, with an initial half-life of 1.9 min and a secondary half-life of 78 min. Its concentration peaked to 230 nmol/ml at 5 min and fell to 8.9 nmol/ml

Table 1. Pharmacokinetic parameters of melphalan and of chlorambucil and its active metabolites following i.v. administration of melphalan and chlorambucil (both 10 mg/kg) to the rat

	Melphalan	Chlorambucil	3,4-Dehydro-chlorambucil	Phenylacetic mustard	Total active compounds from chlorambucil
Plasma half-life (min)	1.9 and 79	26			28 and 228
Peak plasma concentration (nmol/ml)	75 at 5 min	168 at 5 min	3.0 at 30 min	27.8 at 60 min	172 at 5 min
AUC plasma (nmol · min/ml)	4829.4	5679.6	406.0	5207.7	11293.3
Peak brain concentration (nmol/g)	4.5 at 60 min	6.8 at 5 min	0	0.8 at 15 min	7.2 at 5 min
AUC cerebral (nmol · min/g)	405.9	136.6	0	57.7	193.8
AUC cerebellum (nmol · min/g)	886.1	100.9	0	80.1	181.4

AUC, area under the concentration versus time curve, from 5 to 240 min

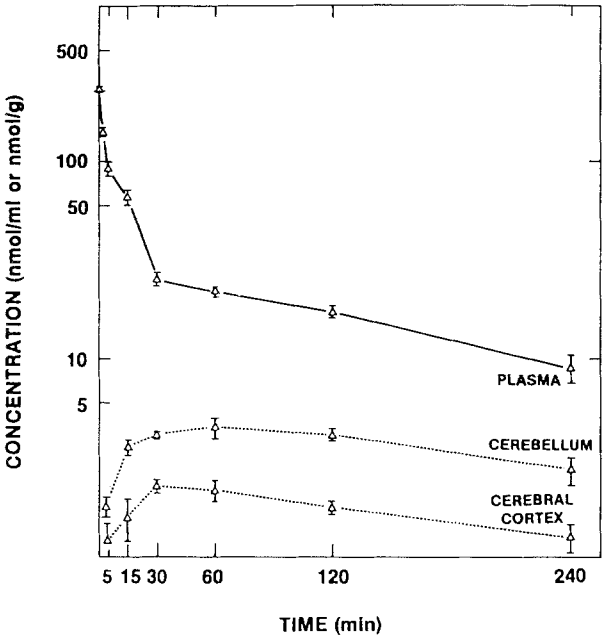

Fig. 4. Plasma and brain concentrations (± SEM) of melphalan following i.v. administration of melphalan (10 mg/kg) to the rat

by 240 min. Melphalan slowly entered the brain reaching a maximum concentration of 2.3 nmol/g in the cerebral cortex at 30 min. Its disappearance from cerebral cortex was monophasic, with a half-life of 128 min. A maximum melphalan concentration of 4.4 nmol/g was achieved in the cerebellum at 60 min. Its disappearance from the cerebellum was similarly monophasic, with a half-life of 174 min.

The areas under the concentration-versus-time curves, between 5 and 240 min, for melphalan in plasma, cerebral cortex and cerebellum, were 4829 nmol min/ml, 406 nmol m/g and 886 nmol min/g, respectively (Table 1). Calculated from these concentration integrals, the cerebral cortex:plasma ratio for melphalan was 0.084 and the cerebellum:plasma ratio was 0.18, giving a mean brain:plasma integral ratio of 0.13 (Table 2).

Plasma protein binding

The binding of chlorambucil to plasma proteins was not dependent on concentration. The drug was 99.6% bound at concentrations from 1 to 100 nmol/ml. The binding of melphalan to plasma proteins, in contrast, demonstrated concentration dependence and varied from 76%±0.4% at 230 nmol/ml, to 88.1%±0.15 at 1 nmol/ml. The percentage of drug that bound to plasma proteins at melphalan concentrations in excess of 70 nmol/ml was significantly less than that which bound at concentrations of 3 nmol/ml and less. At melphalan concentrations achieved in the pharmacokinetic study (8.9 to 230 nmol/ml), concentration-dependent plasma protein binding only occurred during the initial 5 min after drug administration.

Anticancer activity

Figure 5 shows the effect of chlorambucil and melphalan, both administered on a days 1, 3 and 7 schedule, on growth inhibition of s.c.-implanted Walker tumor and on survival of rats with intracerebral implants of Walker tumor. Chlorambucil completely inhibited the growth of s.c.-implanted Walker tumor at a dose of 2 mg/kg i.p. (days 1, 3, and 7), and 50% growth inhibition was achieved at a dose of 1 mg/kg i.p. (days 1, 3, and 7). With the exception of a dose of 9 mg/kg i.p. (days 1, 3, and 7), which increased the survival time of animals with intracerebral tumors to 149% that of untreated controls, chlorambucil had little effect on the survival of animals bearing brain tumors. The administration of chlorambucil in dose in excess of 9 mg/kg i.p. (days 1, 3, and 7) resulted in drug-induced toxicity.

Melphalan totally inhibited the growth of s.c. Walker tumor at a dose of 1 mg/kg i.p. (days 1, 3, and 7), whereas 50% inhibition of growth was achieved with a dose of approximately 0.4 mg/kg i.p. (days 1, 3, and 7). Similar doses had no significant effect on the survival of animals bearing brain tumors. However, 6 mg/kg i.p. (days 1, 3, and 7) significantly lengthened the survival of animals with brain tumors, to 220% that of untreated controls. The administration of melphalan in doses of 9 mg/kg i.p. (days 1, 3, and 7) caused toxicity.

Discussion

Several factors co-determine the concentration of a drug that is eventually achieved in the brain following its systemic administration [23, 24]. One is the plasma concentration-time profile of the compound, which is related to its systemic metabolism and distribution. Another is the percentage of the drug that binds to plasma constituents such as serum albumin and α1-acid glycoprotein, and whether this binding is restrictive or non-restrictive for brain drug uptake [24]. Only free, unbound drug is available to enter the brain. A third factor is the compound's ability to penetrate the blood-brain barrier, which restricts water soluble and ionized drugs [24, 43]. A final factor is the rate of cerebral blood flow, which is critical for lipophilic agents [24, 43].

Following i.v. administration of 10 mg/kg melphalan or chlorambucil to the rat, significant concentrations of

Table 2. Brain: plasma concentration integral ratios* of melphalan, and of chlorambucil and its active metabolites, following i.v. administration of 10 mg/kg to the rat

	Melphalan	Chlorambucil	3,4-Dehydro-chlorambucil	Phenylacetic mustard	Total active compounds from chlorambucil
Cortex: plasma	0.084	0.024	0	0.011	0.017
Cerebellum: plasma	0.183	0.018	0	0.015	0.016
Mean brain: plasma	0.134	0.021	0	0.013	0.017

* Calculated from the areas under the concentration versus time curves from 5 to 240 min (see Table 1)

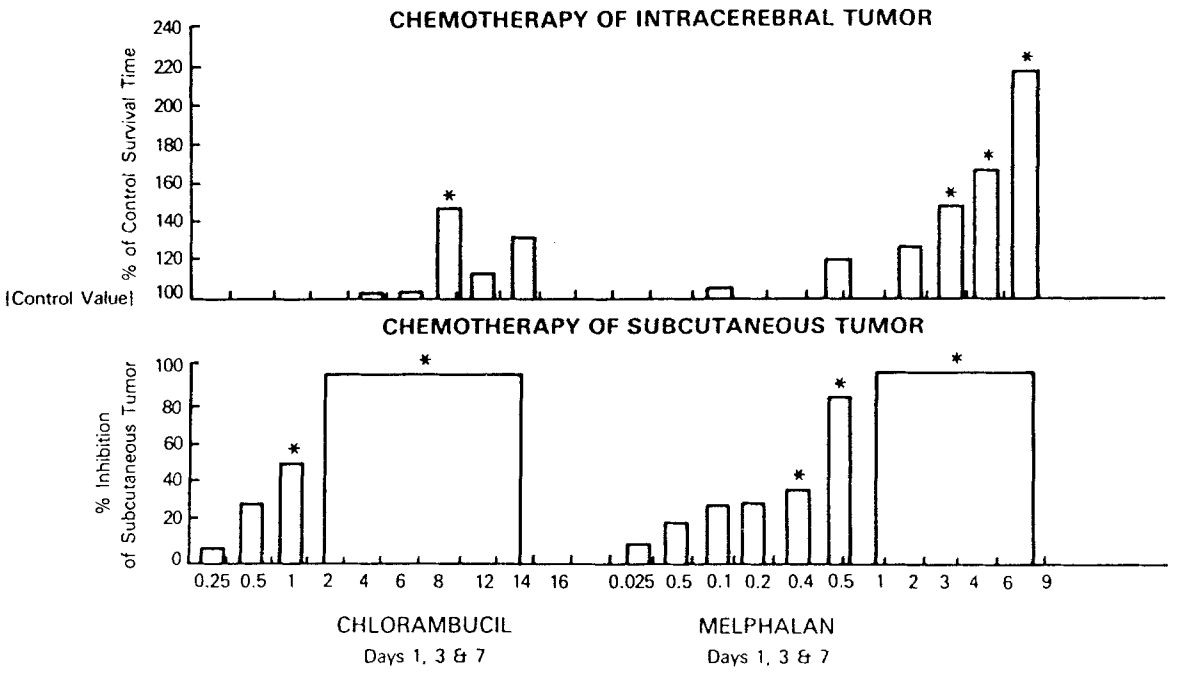

Fig. 5. Anticancer activities of melphalan and chlorambucil, administered i.p. on days 1, 3, and 7 after tumor implantation, against s.c. (*bottom*) and intracranial (*top*) implants of Walker 256 carcinosarcoma tumor in the rat. Each group contained at least 7 animals. $*P < 0.05$ for difference between test and control animals

both compounds were present in the plasma throughout the time course of the study, and thus were available to enter the brain. The equimolar administration of both agents resulted in approximately similar areas under the drug concentration versus time curves (integrated plasma concentrations) of each. The plasma elimination half-lives of the compound were different, however; this can be related to their different routes of disappearance. Melphalan had a plasma elimination half-life of 78 min, which compares favorably with previous studies [2, 4]. There is little evidence for the metabolism of melphalan. Its disappearance has been accounted for by the formation of its mono- and dihydroxyethyl hydrolysis products [9], the latter having no anticancer activity. Chlorambucil demonstrated a plasma elimination half-life of 26 min, which has not been reported previously in the rat after i.v. drug administration. The compound was metabolized to its phenylacetic acid analogue via its 3,4-dehydro intermediate. The relatively low plasma concentrations of this intermediate indicate that its further metabolism occurs fairly rapidly in the rat, as it does in humans [36, 40]. Both 3,4-dehydrochlorambucil and phenylacetic mustard have demonstrated anticancer activities similar to that of chlorambucil against human tumor cells in vitro [19], and similar or greater activities in vivo in rats and mice [9, 16, 17]. The formation of active metabolites, as a consequence of β-oxidation of chlorambucil, which cannot occur for melphalan, results in (1) an extended plasma half-life of active drug-products, compared to chlorambucil alone or to melphalan, and (2) a doubling of the area under the concentration-time curve. Effective plasma concentrations of active drug, either chlorambucil or drug derived from chlorambucil, are therefore maintained for a longer duration.

Although the area under the drug concentration-time curve of chlorambucil plus its active metabolites was 2.3-fold greater than that of melphalan, the drugs demonstrated approximately similar therapeutic activity against s.c.-implanted tumors, and the total amount of melphalan that entered the brain was considerably greater than that of chlorambucil plus its active metabolites. Thus, although high doses of both drugs are required to achieve activity against brain implants of tumor, melphalan achieves higher brain concentrations and a higher therapeutic activity against intracerebral tumor than does chlorambucil, at equimolar dose.

Binding measurements demonstrated that chlorambucil was very highly bound, 99.6%, to plasma proteins, whereas melphalan was 86% bound. These results agree with previous reports [7, 11, 12, 26], which have additionally demonstrated that phenylacetic mustard is 96.6% plasma-protein-bound [11]. As a result, free drug concentrations of melphalan were higher than those of chlorambucil plus its active metabolites, possibly accounting for the higher brain uptake of melphalan. Plasma protein binding limits the hydrolysis and inactivation of both drugs as melphalan and chlorambucil are approximately 3- and 100-fold more stable, respectively, when bound to plasma proteins than when unbound [11, 12].

The free, unbound plasma concentrations of both agents, chlorambucil in particular, were low in the rat and, therefore, can be predicted to be even lower in cancer patients, as less drug per square meter of body surface area is normally administered to humans [9]. Further, both chlorambucil and melphalan are mainly ionized at physiological pH. The pKa of chlorambucil is 5.6 [9], and as a consequence, approximately 95% of the free, unbound drug is ionized at a plasma pH of 7.4. A similar or greater fraction of phenylacetic mustard probably exists in its ionized form. Melphalan is a zwitterion; the pKa values are available for the phenylalanine moiety alone and are 1.83 and 9.13 [13]. Therefore melphalan also exists almost totally in an ionized state at physiological pH. According to the pH-

partition hypothesis, the diffusion of ionized, charged forms across the blood-brain barrier is very limited, and hence the brain uptake of chlorambucil and melphalan would be expected to be extremely low [24, 43]. Our pharmacokinetic and activity studies demonstrated this low uptake. The brain:plasma concentration integral ratios of chlorambucil and phenylacetic mustard are extremely low and comparable to ratios of other water-soluble anticancer agents, whose activities against brain-sequestered neoplasms are likewise minimal [21]. Interestingly, the concentration of melphalan that was achieved in the brain and its subsequent anticancer activity, following its i.v. administration, although low, were higher than expected.

Greig et al. [25] recently demonstrated the concentration-dependent and saturable transport of melphalan into the brain, utilizing an isolated brain perfusion technique. This suggests that, in addition to simple diffusion, melphalan is transported across the blood-brain barrier and into the brain by a saturable, facilitated mechanism. The additional demonstration that L-phenylalanine completely inhibits this transport [25] incidates that melphalan, like L-dopa administered to patients with Parkinson's disease [43, 50], utilizes the large neutral amino acid carrier system located at the blood-brain barrier [41, 43].

Amino acid transport systems for melphalan exist in tissues other than brain [30, 39]. Adair and McElnay [1] recently demonstrated that the gastrointestinal absorption of melphalan, as opposed to that of chlorambucil, is reduced by metabolic inhibitors, as well as by amino acids [5]. This likewise suggests that, in addition to simple diffusion across the gut wall, melphalan utilizes an active transport system at the gastrointestinal epithelium. Finally, in vitro studies of melphalan uptake into tumor cells have clearly demonstrated melphalan's ability to utilize transport systems for essential endogenous substrates, specifically the sodium-independent L-amino acid system [3, 18, 34, 48, 49] and possibly the ASC system [3, 18], for specific neutral amino acids [47]. The uptake of chlorambucil into tumor cells, on the other hand, occurs by passive diffusion alone [27, 28].

Facilitated uptake of melphalan by tumor cells [3, 18, 34, 48, 49] may account for the similar or slightly greater anticancer activity of melphalan than of chlorambucil against s.c.-implanted rat tumor, when the area under the drug concentration-time curve of the former was considerably less than that of chlorambucil plus its active metabolites. Further, although Greig and colleagues [23–25] reported that the affinity of melphalan for the large neutral amino acid transport system at cerebral capillaries is less than that of the endogenous L-amino acids, with which it competes, and is not concentrative, the facilitated transport of melphalan at the blood-brain barrier and the less extensive plasma protein binding of melphalan than of chlorambucil and phenylacetic mustard could account for melphalan's greater than expected brain uptake and activity in the rat.

The doses of both chlorambucil and melphalan that were administered to rats in this study were considerably larger than those that are routinely given to cancer patients during therapy. It is therefore unlikely that therapeutic cerebral concentrations of chlorambucil plus its active metabolites or, indeed, of melphalan, are achieved clinically. This would account for the development of brain metastases in patients whose extracerebral primaries and metastases respond to these drugs. It should be noted, however, that reports of chlorambucil-associated reversible brain side-effects, myoclonic seizures, have appeared in the literature [6, 10, 20, 51]. These are extremely rare and have been related to accidental drug overdoses. Such cases, nevertheless, suggest that high doses of melphalan or chlorambucil [8], together with autologous bone marrow rescue, might elevate free, unbound plasma drug levels sufficiently to achieve therapeutic brain concentrations in clinical studies. Greig et al. [22] recently demonstrated that the binding of melphalan to human plasma proteins is concentration-dependent at drug levels that are achieved during high-dose therapy. As chlorambucil is even more extensively (99.6%) bound to plasma proteins, a relatively minor decrease in its binding at high concentrations, which may not be statistically significant, might substantially increase its free, unbound drug concentration [33] and hence the amount of drug that enters the brain during high-dose therapy. Whether or not this would result in therapeutic brain concentrations clinically remains to be determined. This study suggests that neither drug should be considered for the first-line treatment of primary or metastatic brain tumors.

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