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Tissue Distribution and Macromolecular Interactions of ^{14}[C-Ring] Melphalan in the Rat

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Summary. *The kinetics of uptake and elimination, covalent binding, and macromolecular interactions of ^{14}[C-ring] melphalan was studied after a single oral dose (20 mg/kg, 0.1 mCi/kg) in normal rats. Peak radioactivity level in tissues was observed at 2–4 h after administration. Uptake of label in most tissues was rapid, with a $t_{1/2}$ of less than 1 h. Elimination was biphasic. Tissues of the gastrointestinal tract showed the most rapid rates of elimination, with $t_{1/2}\beta$ of 13, 24, 18, and 19 h for stomach, duodenum, and small and large intestines, respectively. Bone marrow also showed a fast rate of elimination of radioactivity, with a $t_{1/2}$ β of 30 h. Tissues with the slowest rates of elimination were skin, eye, spleen, pancreas, and lung, with $t_{1/2}$ β of 333, 241, 149, 122, and 109 h, respectively. Covalent binding studies showed that melphalan, or its metabolites, bound irreversibly to all tissue macromolecular fractions. The percentage of covalently bound radioactivity increased with time in all tissues except kidney and eye, reaching up to 70%–80% of the total radioactivity remaining at 72 h. Elimination of covalently bound radioactivity was slower in the DNA fractions of the tissues of the gastrointestinal tract and heart compared with the elimination rate from lipid, protein, or RNA fractions. Slow elimination rates of ^{14}[C-ring] melphalan equivalents from the protein fraction were observed in the skin, eye, and brain. Accumulation, rather than elimination, of radioactivity in this fraction was most prominent in the pancreas. In the bone marrow accumulation of radioactivity was observed in the lipid fraction.*

Introduction

In a previous communication [2] we described the pharmacokinetics and macromolecular interactions of orally administered ^{14}[C-ring] melphalan (L-PAM) in rat blood. Melphalan was found to be covalently bound to plasma proteins and red cells, especially red cell membranes. This study describes the pharmacokinetics and macromolecular interactions of ^{14}[C-ring] melphalan in other tissues of the rat. Tissue distribution of melphalan was first studied by Cohn [4]. He showed that radioactivity from ^{14}C-DL-PAM was found in tumor, liver, blood, and kidney, with the highest radioactivity levels in the protein fraction of the kidney. Milner et al. [11], using ^{3}H-L-PAM, found that the radioactivity was ubiquitously distributed in tumor-bearing rats, with highest levels in liver and kidney. They also showed little binding of radioactive material to tumor DNA, RNA, or proteins. More recently, Furner and Brown [6] described the distribution of ^{14}[C-ring]- and ^{14}C-side chain-labeled melphalan in mice and monkey tissues. These reports [4, 6, 11] described the distribution of radioactivity at fixed time intervals. None of them, however, described the pharmacokinetics or rate of elimination of melphalan from these tissues and from the tissue macromolecules. The aim of this study is twofold: (a) To describe the tissue distribution and the rates of uptake and elimination of ^{14}[C-ring] melphalan or its equivalents in all tissues of the normal rat; and (b) to investigate the covalent binding of melphalan to tissue macromolecules and the rate of elimination of the irreversibly bound radioactivity from the lipid, protein, RNA, and DNA fractions. This study should provide insight on the

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Abbreviations used in this paper are: L-Pam, Melphalan, L-phenylalanine mustard 4-bis (2-chloroethyl) amino-1-phenylalanine; L-DOH, 4-bis (2-hydroxyethyl) amino-1-phenylalanine; L-MOH, 4-2 hydroxyethyl 2 chloroethyl amino-1-phenylalanine; HPLC, high-pressure liquid chromatography; TCA, trichloroacetic acid; AUC, area under the curve; GIT, gastrointestinal tract

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preferential sites of alkylation of melphalan in different tissues and tissue macromolecules.

Materials and Methods

^{14}[C-ring] L-PAM (specific activity 8.0 mCi/mmole, 24.8 μCi/mg) was obtained from the National Cancer Institute, NIH. Unlabeled L-PAM, pharmaceutical grade, was provided by Burroughs Wellcome Co. (Research Triangle Park, NC), USA. Mono- and dihydroxy analogs of L-PAM were prepared in 0.1 *M* NaOH at 70° C for 1 h and confirmed by high-performance liquid chromatography (HPLC) as previously described [1]. All other reagents were analytical grades obtained from various commercial sources.

Treatments and Sample Collections. Male albino Sprague-Dawley rats (Charles River, Wilmington, MA) ranging in weight from 190 to 200 g were placed in groups of three in metabolic cages to collect urine and feces samples. The animals were provided with standard lab chow and tap water ad libitum. A stock solution of 4.0 mg L-PAM/ml (containing 20 μCi ^{14}[C-ring] L-PAM/ml) was freshly prepared prior to administration in 0.12 *N* HCl. A dose of 20 mg/kg (0.1 mCi/kg) was orally administered to each rat. One group of rats was sacrificed at each time interval of 0.5, 1, 1.5, 2, 4, 8, 12, 24, 48 and 72 h after drug administration. The tissues were excised, pooled, weighed, immediately frozen, and stored at −70° C.

Determination of Radioactivity in Tissues. A known weight of each tissue was homogenized in five volumes of 0.25 *M* sucrose. Tissues with low levels of radioactivity were homogenized in one volume of 0.25 *M* sucrose. The radioactivity contents of 0.1−0.5 ml aliquots of homogenates or urine pooled from three rats were assayed by combustion in the sample oxidizer for ^{14}C analysis (Model B306 Tri-Carb, Packard, Downers Grove, IL, USA). Small-size tissues, e.g., adrenal glands, were burned whole in the sample oxidizer without prior homogenization. ^{14}Carbon dioxide was trapped into scintillation vials each containing 8 ml Carbosorb (Packard, Downers Grove, IL, USA) mixed with 12 ml Permafluor. The samples were then counted in a liquid scintillation system (Mark IV, 6880, Searle Analytic, Inc.) equipped with an external standard ratio technique to calibrate DPM.

Determination of Covalently Bound Radioactivity. A known weight of each tissue was chemically fractionated according to the method of Trams et al. [12] into trichloroacetic acid (TCA)-soluble fraction containing the unbound (free) drug or its metabolites and TCA-insoluble fraction containing the bound drug. The latter fraction was further separated into lipid, protein RNA, and DNA fractions by the method of Trams et al. [12]. The precipitate obtained after TCA was made alkaline with Na_2CO_3. Lipids were extracted by 10 volumes ethanol-ether (3 : 1) at 55° C, followed by centrifugation and extraction of the supernatant with 10 volumes methanol-$CHCl_3$ (1 : 2). Total lipids were estimated by weighing the residue obtained after evaporating the methanol-$CHCl_3$ extract to dryness under vacuum. Following the extraction of the lipids, the precipitate was treated with 10 volumes 1 *N* KOH for 16 h. The precipitated proteins were determined by the method of Lowry [9]. The supernatant, which contained the nucleic acids, was treated with 1 *N* perchloric acid, pH 2.0. RNA in the supernatant was determined by the orcinol method of Kuchler [8]. The precipitate containing the DNA fraction was further treated with 5% NH_4OH, centrifuged, and the supernatant acidified with HCl. DNA in the precipitate was determined by the method of Burton [3]. Aliquots (0.1 ml) of each fraction were taken and burned in a sample oxidizer as described above and the radioactivity of each fraction determined. In every macromolecular fraction the specific activity was calculated as nmol melphalan equivalents/mg macromolecule.

Analysis of Data. The estimations of apparent rates of uptake and of distribution and elimination rate constants of ^{14}[C-ring] melphalan equivalents in tissues and macromolecules were achieved through the nonlinear regression program, NONLIN [10]. The profiles of ^{14}[C-ring] melphalan equivalent levels in the tissues were best fitted by a two-compartment model as described by equation (a). The area under the curve (AUC, concentration X time) was also calculated. Uptake and release from macromolecules were best fitted by a one-compartment model as described by Eq. (b).

$$C_t = L \cdot e^{-t} + M \cdot e^{-\beta t} + N \cdot e^{-K_a t}, \quad \text{(a)}$$

$$C_M = \mathrm{INT} \cdot (e^{-K_1 t} - e^{-K_2 t}). \quad \text{(b)}$$

In Eq. (a), C_t represents the concentration of the drug equivalents per gram of tissue; C_M, the concentration per mg macromolecule. The constants K_a, α, and β reflect the apparent rate of absorption of the drug into the tissue, its rate of distribution within the tissue, and its rate of elimination from the tissue, respectively. *L*, *M*, and *N* are the respective Y-intercepts for those portions of the curve represented by α, β, and K_a. In Eq. (b) K_1 is the apparent rate of drug uptake by macromolecules and K_2 it its apparent rate of elimination. INT is the Y-intercept for the portions of curve represented by K_1 and K_2.

Results

The distribution of radioactivity in rat tissues within 72 h after the oral administration of ^{14}[C-ring] melphalan is shown in Table 1. The contents and tissues of the gastrointestinal tract (GIT) showed the highest concentration of ^{14}[C-ring] melphalan equivalents. During the first 30 min the stomach and stomach contents showed the highest concentration of melphalan equivalents. This was followed by a rapid decline of radioactivity and its concomitant increase in the small and large intestines. Peak levels occurred between 1 and 2 h and 8 and 12 h for the small and large intestines, respectively. This seems in accordance with the passage of materials through the GIT. Fecal excretion of radioactivity occurred 12−24 h after ^{14}[C-ring] melphalan administration.

Radioactivity was detected in most tissues at 0.5 h after administration, indicating a fast absorption and rapid distribution of the drug (Table 1). Peak radioactivity levels in most tissues were observed at 2 h after administration. Organs that contained the highest concentrations of radioactivity at this time were the kidney, pancreas, bone marrow, lung, and liver. No appreciable levels of radioactivity were detected in adipose tissue at any time interval. The decline of radioactivity from all tissues was biphasic.

Table 1. Tissue distribution of ^{14}(*C*-ring) melphalan equivalents in normal rats after oral administration of 0.1 mCi/kg

Tissue	nmol Melphalan equivalents/g									
	0.5 h	1 h	1.5 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h
Stomach	163.17	99.54	36.32	54.56	82.66	40.42	2.64	3.83	1.07	0.51
Duodenum	31.91	14.52	18.92	16.83	19.81	26.12	16.17	10.65	15.72	1.11
Small intestine	42.32	47.91	32.74	46.13	16.94	12.63	11.92	6.85	8.95	0.52
Large intestine	6.97	11.81	13.02	8.31	35.81	53.84	38.83	11.44	15.47	1.52
Esophagus	0.84	1.36	1.45	2.05	1.68	1.09	0.89	0.56	0.86	0.31
Pancreas	6.47	8.73	7.66	12.38	5.63	5.63	7.38	4.61	3.72	4.44
Liver	3.92	5.96	5.29	6.47	5.29	5.91	3.53	2.91	2.14	1.53
Spleen	1.36	1.86	1.86	2.43	1.92	2.61	2.12	1.67	1.54	1.63
Blood[a]	2.46	3.19	3.43	3.46	2.67	2.81	2.41	1.73	1.19	1.02
Bone marrow	2.52	3.01	5.03	8.75	3.75	6.44	5.68	5.68	1.63	1.11
Heart	1.23	1.97	1.91	3.24	1.75	1.86	1.63	1.22	0.84	0.73
Lung	1.63	1.97	1.63	7.54	1.46	1.69	1.13	0.90	0.67	0.90
Brain	0.17	0.23	0.28	0.39	0.34	0.45	0.34	0.28	0.11	0.11
Kidney	12.50	16.05	15.54	16.67	19.98	25.4	22.97	12.73	10.81	6.87
Eye	0.39	0.90	1.52	1.63	2.7	2.65	2.87	2.19	1.86	1.80
Skin	2.19	3.21	2.87	4.05	3.89	4.34	3.99	2.42	2.42	2.08
Thymus	0.90	1.29	1.63	2.42	1.75	2.19	1.63	1.18	1.07	3.60
Testes	0.45	0.79	0.79	1.07	1.01	0.67	0.62	0.39	0.23	0.23
Adipose tissue	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Stomach contents	514.36	727.87	176.18	177.98	324.38	123.19	14.98	51.58	2.48	0.79
Duodenum contents	69.48	26.24	21.34	34.52	25.39	35.36	62.61	13.34	30.74	0.96
Small intestinal contents	79.39	122.58	121.79	92.96	51.58	36.49	13.12	19.88	17.34	0.73
Large intestinal contents	2.70	6.47	6.81	3.72	180.63	200.39	264.02	140.59	102.08	2.42
Urine[a]	14.11	34.93	66.89	118.64	219.14	204.67	37.48	16.84	10.02	9.12
Feces	0.01	0.02	0.29	0.37	0.22	0.29	17.64	520.72	480.58	30.53

[a] Values are expressed as nmol melphalan equivalent/ml

Table 2. Pharmacokinetic parameters of the two-compartment model following oral administration of ^{14}(*C*-ring) melphalan in rat tissues

Tissue	$t^1/_2$ Ka (h)	$t^1/_2$ α (h)	$t^1/_2$ β (h)	AUC nmol melphalan equivalent · h/g
Stomach	0.09	1.8	13.2	466.2
Duodenum	0.09	0.5	23.7	710.2
Small intestine	0.33	0.9	18.4	528.0
Large intestine	1.53	1.1	18.6	974.6
Esophagus	0.97	2.7	52.9	67.6
Pancreas	0.49	1.1	109.2	940.3
Liver	0.37	6.8	62.8	339.5
Spleen	0.60	14.7	149.1	433.1
Blood	0.27	9.3	92.8	251.4
Bone marrow	1.58	2.8	30.6	373.5
Heart	0.47	5.8	85.7	174.5
Lung	0.73	1.8	122.1	205.5
Brain	1.50	7.2	54.3	26.8
Kidney	0.67	23.8	41.8	1352.9
Eye	2.09	5.8	241.1	765.1
Skin	0.66	13.5	333.4	1115.5
Thymus	1.35	1.9	79.0	187.4
Testes	1.27	1.4	48.9	48.4

The kinetics of absorption and elimination ($t_{1/2}$ K_a, $t_{1/2}$ α and $t_{1/2}$ β) are shown in Table 2. The rate of uptake in most tissues was very rapid, the $t_{1/2}$ of absorption rate (K_a) being less than 1 h. The slowest rates of absorption were in the eye, brain, testes, bone marrow, and thymus. Elimination rates varied widely from one tissue to the other. Thus the fastest rates of elimination were in the tissues of the GIT and bone marrow. The $t_{1/2}\beta$ was 13.2, 23.4, 18.4, 18.6, and 30.6 h for stomach, duodenum, small intestine, large intestine, and bone marrow, respectively. Tissues with slow elimination rates were the skin, eye, spleen, lung, and pancreas, for which the $t_{1/2}\beta$ values were 333.4, 241, 149, 122 and 109 h, respectively. The $t_{1/2}\beta$ for all the other tissues ranged between 40 and 90 h.

Table 2 also shows the AUC for each tissue, which is an indicator of the amount of radioactive material passing through each tissue during the 72-h period. The highest levels of radioactive material passed through the kidneys, skin, pancreas, and large intestine. The brain and testes showed the smallest AUC.

Urinary excretion of ^{14}[C-ring] melphalan equivalents occurred 0.5 h after administration. The excretion rate gradually increased by time, reaching a maximum at 24 h. Less than 50% of the total radioactivity excreted in the urine was accounted for as L-DOH, L-MOH, and L-PAM (data not shown). This indicates that more than 50% of melphalan is excreted in the form of other metabolites, which remain to be identified. Fecal elimination of radioactive material was slow during the first 12 h and gradually increased until it reached a maximum at 24 h.

Table 3 shows the amount of covalently bound radioactivity as nmol melphalan equivalents/mg tissue and as a percentage of the total melphalan

Table 3. Covalently bound melphalan equivalents in rat tissues following the administration of 0.1 mCi ^{14}(*C*-ring) melphalan/kg

Tissue	nmol Melphalan equivalent/g									
	0.5 h	1 h	1.5 h	2 h	4 h	8 h	12 h	24 h	48 h	72 h
Stomach	21.05	14.63	6.14	11.51	17.11	11.76	1.38	1.20	0.65	0.34
	(12.9)[a]	(14.7)	(16.9)	(21.1)	(20.7)	(29.1)	(51.7)	(31.4)	(60.5)	(67.01)
Duodenum	3.16	2.13	1.55	1.80	2.54	2.77	1.46	0.88	1.07	0.3
	(9.9)	(14.7)	(8.2)	(10.7)	(12.8)	(10.6)	(9.0)	(8.4)	(6.8)	(27.1)
Small intestine	6.81	7.62	4.32	7.52	2.64	2.09	1.57	0.90	1.02	0.13
	(16.1)	(15.9)	(13.2)	(16.3)	(15.6)	(16.6)	(13.2)	(13.2)	(11.4)	(26.4)
Large intestine	1.26	1.82	1.76	1.51	5.98	6.41	5.82	1.48	2.21	0.54
	(18.2)	(15.5)	(13.5)	(18.2)	(16.7)	(11.9)	(15.0)	(12.9)	(14.3)	(35.4)
Esophagus	0.35	0.68	0.76	1.19	0.83	0.70	0.66	0.43	0.73	0.25
	(41.7)	(49.7)	(52.2)	(57.9)	(49.3)	(64.4)	(73.9)	(76.9)	(84.6)	(80.9)
Pancreas	0.69	1.07	1.13	2.19	1.15	2.02	1.86	1.84	1.95	2.44
	(10.6)	(12.2)	(14.7)	(17.7)	(20.4)	(35.9)	(24.2)	(39.9)	(52.5)	(54.9)
Liver	0.67	1.54	1.33	1.84	2.18	2.64	1.98	1.68	1.32	1.10
	(17.2)	(25.9)	(25.2)	(28.4)	(41.3)	(44.7)	(56.1)	(57.6)	(61.9)	(71.9)
Spleen	0.31	0.43	0.49	0.79	0.75	0.83	0.96	0.74	0.78	0.75
	(22.2)	(23.2)	(26.5)	(32.4)	(39.3)	(43.1)	(45.1)	(44.2)	(50.9)	(46.2)
Bone marrow	0.73	0.95	1.67	4.38	1.46	2.90	2.68	3.25	1.15	0.78
	(28.8)	(31.7)	(33.2)	(50.1)	(38.9)	(45.1)	(47.1)	(57.2)	(70.7)	(70.1)
Heart	0.31	0.56	0.56	1.29	0.51	0.84	0.94	0.65	0.64	0.56
	(25.2)	(28.6)	(29.4)	(39.7)	(28.9)	(45.3)	(57.8)	(53.2)	(76.5)	(76.1)
Lung	0.37	0.54	0.45	3.66	0.63	0.81	0.63	0.52	0.43	0.56
	(22.5)	(27.2)	(27.8)	(48.6)	(43.2)	(48.1)	(56.1)	(57.9)	(64.3)	(62.6)
Brain	0.03	0.05	0.05	0.11	0.10	0.17	0.16	0.14	0.07	0.08
	(18.2)	(21.3)	(18.4)	(27.0)	(30.2)	(37.4)	(46.9)	(51.3)	(61.3)	(75.0)
Kidney	4.11	5.02	5.64	5.60	5.41	7.59	6.25	3.55	3.19	2.49
	(32.9)	(31.3)	(36.3)	(33.6)	(27.1)	(29.9)	(27.2)	(27.9)	(29.6)	(36.6)
Eye	0.09	0.23	0.11	0.13	0.11	0.09	0.06	0.06	0.09	0.03
	(22.9)	(25.4)	(6.9)	(7.9)	(4.1)	(3.7)	(2.0)	(3.0)	(5.2)	(1.4)
Skin	0.07	0.99	0.87	1.33	1.19	2.16	1.27	1.06	1.48	1.22
	(3.1)	(31.1)	(30.3)	(32.8)	(30.6)	(49.7)	(31.9)	(43.7)	(61.3)	(58.8)
Thymus	0.21	0.31	0.43	0.89	0.68	0.92	0.78	0.44	0.48	2.35
	(22.9)	(24.4)	(26.4)	(37.1)	(39.0)	(42.2)	(47.7)	(37.7)	(41.8)	(65.5)
Testes	0.06	0.13	0.15	0.21	0.29	0.21	0.27	0.17	0.12	0.13
	(12.8)	(17.0)	(18.9)	(19.7)	(19.2)	(31.8)	(44.0)	(43.2)	(52.1)	(57.0)

[a] Values in between brackets are the covalently bound equivalents represented as percent of the total remaining at that time

Table 4. Half-life of elimination of covalently bound radioactivity from the macromolecular fractions of rat tissues after the administration of 0.1 mCi ^{14}(*C*-ring) melphalan/kg

Tissue	$t^1/_2$ β (h)[a]			
	Lipid	Protein	RNA	DNA
Stomach	6.8	7.3	3.3	9.2
Duodenum	10.9	1.5	7.04	76.5
Small intestine	22.5	2.8	11.3	100.0
Large intestine	16.0	30.7	10.1	72.7
Esophagus	263.3	–	13.6	73.7
Pancreas	19.0	⊤	56.1	277.7
Liver	16.1	45.0	10.9	52.9
Spleen	37.6	54.7	14.1	0.76
Bone marrow	⊤	15.4	13.1	63.1
Heart	28.3	120.0	25.8	558.8
Lung	16.2	24.9	15.8	47.2
Brain	63.7	389.2	21.2	25.6
Kidney	40.3	36.5	65.9	39.4
Eye	26.2	270.9	78.7	73.2
Skin	32.6	1993.0	409.8	408.9
Thymus	44.9	30.4	80.4	62.0
Testes	19.6	52.8	5.6	35.4

⊤ Indicates a positive slope and therfore accumulation of radioactivity rather than elimination

[a] The rate elimination was calculated from the slope obtained by plotting the nmol melphalan equivalents bound/mg macromolecule versus time. The data (not shown) were fitted in a one-compartment model and the rate of elimination was calculated by the nonlin program as described in *Materials and Methods*

equivalents (Table 1) at each time interval. In most tissues the percentage of bound radioactivity increased with time and was highest by 72 h. Thus, in the heart, liver, bone marrow, esophagus, and brain, 70%–80% of the radioactivity remaining at 72 h was irreversibly bound to tissue macromolecules. For most of the other tissues, irreversibly bound radioactivity constituted 40%–60%. Covalently bound radioactivity in the kidney was fairly constant over the 72-h time period, averaging about 32% of the total radioactivity. The small intestine, duodenum, and large intestine had 26%, 35%, and 27% of the total radioactivity remaining at 72 h in the bound form.

Table 4 shows the elimination rates of the covalently bound melphalan equivalents to lipids, proteins, RNA, or DNA fractions as fitted to the one-compartment model described before. In the tissues of the GIT, covalently bound radioactivity appears to be mainly in the DNA fraction. The $t_{1/2}$ of elimination was slowest in DNA compared with the elimination rate from the RNA, protein, and lipid fractions. In the pancreas, there appears to be an accumulation of radioactivity in the protein fraction. Accumulation rather than elimination of radioactivity was also observed in the lipid fraction of bone marrow. In the eye and skin, slow elimination rates were observed in the protein fraction, with $t_{1/2}$ = 270 and 1 993 h, respectively.

Discussion

The results of this study show that the distribution of radioactivity after oral administration of ^{14}[C-ring] melphalan in rats was ubiquitous. Radioactivity was detected in all tissues examined, in agreement with previous studies [4, 6, 11]. The highest levels of radioactivity were found in the tissues of the GIT. These high levels are attributed to the presence of the radioactive drug in direct contact with these tissues following oral administration.

The rates of uptake of ^{14}[C-ring] melphalan equivalents in the stomach and duodenum were similar. In addition, they were the most rapid rates of uptake of all the tissues studied. This may indicate that absorption of melphalan occurs equally well from both stomach and intestine. The rapid absorption of melphalan is in contrast to its low lipid solubility. Low levels of melphalan equivalents were detected in adipose tissue or tissues with high fat content, e.g., brain and testes. It is quite conceivable that melphalan, being an amino acid analog, is absorbed via the carrier-mediated amino acid transport system from both stomach and intestine. This indeed has been shown by Vistica et al. [13, 14] to be the case for the transport of melphalan in L1210 cells in vitro.

Studies on elimination rates from the different tissues have yielded several important aspects of melphalan distribution and macromolecular interactions, namely:

a) Melphalan and/or its metabolites seem to bind, possibly by alkylation, to all tissues and their elimination is not rapid.

b) It was observed that the $t_{1/2}$ of melphalan equivalents in bone marrow and gastrointestinal tissues was lower than in any of the other tissues studied, indicating that elimination is most rapid from these tissues. This, however, is not surprising since the gastrointestinal epithelium and bone marrow are tissues with rapid cellular turnover and therefore faster rates of elimination of melphalan equivalents from these tissues would be expected. In this respect, it is interesting to note that the covalent binding of ^{14}[C-ring] melphalan equivalents was more apparent in the DNA fraction of the gastrointestinal tissues, and thus appears to be preferentially alkylated. In the bone marrow, covalent binding was more apparent in the lipid fraction, where accumulation of covalently bound radioactivity was observed. Lipids in the bone

marrow are more of a stationary compartment than the cellular compartment. The RNA, DNA, or protein fractions are more representative of the cellular compartment, which is constantly released into the circulation. The role of accumulation of melphalan equivalents in the lipid fraction of the bone marrow and its relationship to its myelotoxicity is not readily apparent.

c) The $t_{1/2}$ β of melphalan equivalents in the hematopoietic compartment was 30 h, 92 h, and 149 h, for the bone marrow, whole blood, and spleen, respectively. In a previous study [2] we have shown that melphalan binds covalently to red cells, especially red cell membranes. Therefore, it appears that melphalan may be binding to bone marrow cells, which are perfused by the blood to the general circulation and are finally trapped in the spleen. The longer half-life in the spleen may indicate certain defects of the red cell membranes due to melphalan binding, which would allow them to be captured by this organ.

d) The slowest elimination rates were in the skin and the eye. The skin and eye have a high content of thiol-containing groups, most of which are protein sulfhydryl [7]. These would be likely alkylation sites for melphalan. Thus, we observed a slow rate of elimination from the protein fraction of the skin and eye compared with the RNA, DNA, or lipid fractions of these organs.

e) In the pancreas, there appears to be an accumulation of melphalan in the protein fraction. Melphalan may be incorporated as a false amino acid precursor in this organ, which has active protein synthesis for the various digestive enzymes and hormones. Accumulation of melphalan in the pancreas may prove to be of potential value in the treatment of pancreatic tumors.

f) In the kidney, most of the radioactivity is in the unbound form, which indicates that it is the form of soluble metabolites for excretion.

The results of this study show that melphalan, or its equivalents, binds to all target and non-target tissue macromolecules. This fact may cast some doubt on the proposed mechanism of action of melphalan as being solely through the alkylation of DNA base pairs [5]. It is quite possible that alkylation of other macromolecular fractions may be involved. On the other hand, if DNA is indeed the target for melphalan, then it appears that as major fraction of the drug is lost via alkylation of the other macromolecules. Further studies are required to investigate the chemical nature of the binding species as well as to correlate covalent binding to various macromolecules and the alterations, if any, of their function.

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