Cancer Chemother Pharmacol (1992) 30: 81–85

Cancer Chemotherapy and Pharmacology



# In vivo distribution of [$^{11}$C]-busulfan in *cynomolgus monkey* and in the brain of a human patient*

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Received 12 October 1991/Accepted 23 January 1992

**Summary.** The in vivo distribution of the antileukemic agent busulfan labeled with the positron-emitting radionuclide carbon 11 was investigated in cynomolgus monkeys and in a human patient using positron emission tomography. After i.v. injection of the radiotracer, its regional uptake was monitored for about 1 h in the monkey's body and, in a separate experiment, in the monkey's brain. The concentration of radioactivity in the liver, which showed the highest levels of all the organs scanned, increased throughout the experiment and was 9-fold that in the brain at the end of the experiment. [$^{11}$C]-Busulfan rapidly crossed the blood-brain barrier. The radioactivity peaked in both the cortex and the white matter showing a ratio of 1.25, at 3 min but declined quickly to yield a ratio of approximately 1 after 30 min. In the human brain, radioactivity in the cerebellum, cortex, and white matter reached a maximum within 5 min showing a cortex: white matter ratio of 1.6. The activity in the cortex declined to yield a ratio of 1 within 30 min. Of the delivered dose, 20% penetrated into the brain.

## Introduction

The antileukemic agent busulfan [1,4-bis(methanesulfonoxy)-butane] has been the drug of choice for the treatment of chronic myelocytic leukemia since the 1950s. In the last decade, considerable interest has been focused on the use of busulfan as an alternative to total-body irradiation in myeloablative therapies, especially in combination with bone marrow transplantation (BMT [22]). The number of BMT/busulfan treatments performed annually has increased rapidly, especially in leukemia patients and individuals with hematopoietic disorders [1]. However, despite the increasing clinical use of high-dose busulfan, very little is known about the regional whole-body or cerebral distribution of the drug. Questions have also been raised concerning a possible connection between busulfan and the convulsions reported [18, 19, 27] during high-dose therapy, the ability of the drug to eradicate neoplastic cells in the CNS, especially in patients with CNS leukemia, and the risk of CNS damage in children treated with high doses.

In spite of the lack of direct in vivo observation, animal experiments have shown that considerable amounts of both busulfan and its metabolites are found ex vivo in the rat brain after i.p. administration [10]. It has been demonstrated that the use of anticonvulsants can protect mice from busulfan-induced seizures [5]. Recent studies in humans have shown that the busulfan concentrations in cerebrospinal fluid (CSF) were as high as those in plasma during the 4 days of treatment [11, 30]. Vassal and coworkers [31] have recently reported dose-dependent neurotoxicity for the drug in children.

Since the 1970s positron emission tomography (PET) has enabled the study of the in vivo distribution of endogenous substrates as well as drugs. Radiotracers in which an atom in the target molecule has been replaced by one of the positron-emitting radionuclides are chemically and biochemically identical to the original compound. We have recently synthesized busulfan [13] labeled with carbon 11 in the alkylating portion of the molecule [1-$^{11}$C]. In the present study, we investigated its in vivo distribution with time in monkeys and in a human patient so as to determine the extraction of busulfan across the blood-brain barrier (BBB), the apparent distribution volume of radiotracer with time between the brain and the blood, as well as the distribution of the drug to other organs in the body. Since some of the side effects (seizures) are associated with high-dose busulfan treatment, we also wished to examine whether the administration of tracer amounts of [$^{11}$C]-busulfan would result in a continuous cerebral accumulation throughout the PET examination.

*This work was supported by grants from the Swedish Cancer Society (2805-B90-01X) and the Swedish Medical Research Council (B9012X0827603A and B9012P0843102B)

*Abbreviations:* CSF, cerebrospinal fluid; AML, acute myelocytic leukemia; BMT, bone marrow transplantation; PET, positron emission tomography; BBB, blood-brain barrier

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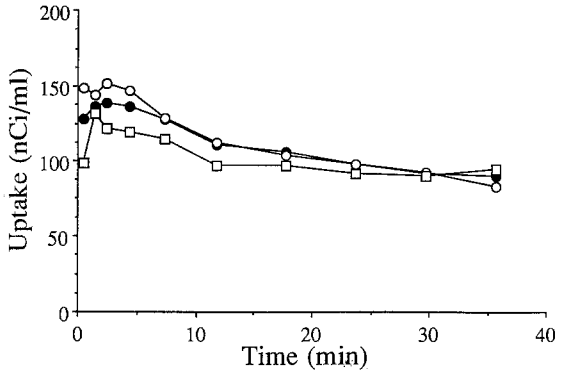


**Fig. 1.** Distribution of radioactivity in the monkey brain after an i. v. injection of 45 MBq [$^{11}$C]-busulfan as a function of time. ●, Frontal cortex; ○, occipital cortex; □, white matter

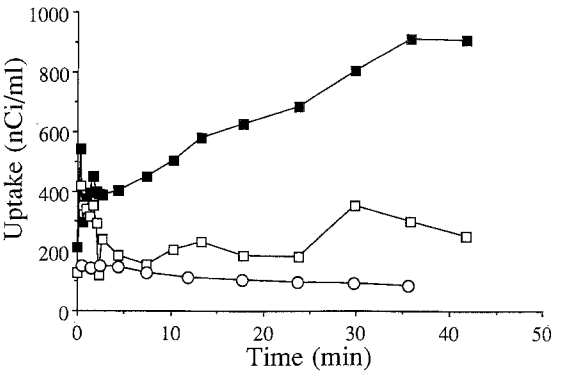


**Fig. 2.** Distribution of radioactivity in the brain of monkey 1 and in the liver and lungs of monkey 2 after the i. v. administration of 45 MBq [$^{11}$C]-busulfan to each monkey. ○, Brain; ■, liver □, lungs

## Subjects and methods

*Chemistry.* [$^{11}$C]-Busulfan was synthesized in a four-step procedure starting with [$^{11}$C]-ammonium cyanide as the radiolabeling precursor [13]. Aqueous [$^{11}$C]-cyanide was reacted with 3-bromo-1-propanol, which was followed by acidic hydrolysis to gamma-hydroxybutyric acid lactone. The lactone was isolated and reduced to 1,4-butanediol using lithium aluminum hydride. The diol was reacted with methanesulfonyl chloride to form [1-$^{11}$C]-1,4-bis(methanesulfonoxy)butane (busulfan). The product was isolated by semipreparative high-performance liquid chromatography (HPLC). Prior to injection, the identity of the radiotracer and the radiochemical purity (>99%) were determined by coelution with a reference compound on a separate radio-HPLC system. After filtration, a sterile solution that was free of pyrogens (limulus test) was available for injection: $CH_3$-$SO_2$-O-$^{11}CH_2$-$CH_2$-$CH_2$-$CH_2$-O-$SO_2$-$CH_3$ [1-$^{11}$C]-busulfan.

*PET.* The regional distribution of radioactivity was investigated using either the PC 384-7B or the PC 2048-15B Scanditronix camera. Both cameras are designed for brain studies and enable the observation of an axial distance of 10.5 cm divided into 7 or 15 slices, respectively. However, the opening in the camera is also large enough to permit scanning of the bodies of the cynomolgus monkeys used in the present study (4.5 kg). The spatial resolutions of the reconstructed images for these two systems are 7.6 and 4.5 mm, respectively. Attenuation correction in all studies using the PC 2048-15B device was performed via a rotating rod source provided by the manufacturer. In studies using the PC 384-7B camera, in which only the monkey brain was studied, the edge-finding technique was applied [3]. The characteristics of the cameras have been described elsewhere [16, 17].

*Experimental subjects.* The subjects studied included two male cynomolgus monkeys (4.5 kg) and a 32-year-old woman presenting with acute myelocytic leukemia (AML; 60 kg). The patient showed no CNS involvement and had not received radiotherapy or busulfan prior to the PET investigation.

*Experimental procedure.* The human research protocol was approved by the Ethics and Radiation Safety Committees of the Karolinska Hospital and the animal experiment was approved by the regional Animal Ethics Committee. The monkeys were anesthetized with i. v. ketamine (Ketalar, Parke-Davis; 4 mg $kg^{-1}$ $h^{-1}$). An i. v. cannula was inserted into the sural vein of one leg for injection of the radiotracer and for blood sampling. The animal was placed such that either the entire head (for the cerebral uptake studies) or a major portion of the body was within the positron camera. During the experiment, body temperature was maintained at 36°–37° C using a heating pad.

An individually adjusted plaster helmet was made for the patient's use together with a head-fixation system in both computerized tomography (CT) and PET. This fixation system standardized the positioning of the head of the patient in the PET system, thereby enabling the exact anatomical localization of the PET images by direct comparison with CT images. One venous cannula was inserted into the antecubital vein and one arterial cannula was inserted into the radial artery.

A saline solution containing 10% ethanol and [$^{11}$C]-busulfan was injected as a bolus. The cannula was flushed with saline. Radioactivity in the brain (human and monkey) or in the body (monkey) was measured by preprogrammed sequential PET scans. In the human study, blood was drawn from the contralateral artery and radioactivity was measured in a well counter.

*Calculations.* Regional radioactivity was measured for each sequential scan, corrected for decay, and plotted versus time. In the human study, the total brain radioactivity at 5 min after the i. v. injection was used to calculate the percentage of injected radioactivity that penetrated into the brain. To calculate the total brain radioactivity, the mean radioactivity was measured for each of the 15 slices examined. The volume of each slice was used to calculate the average radioactivity in the brain volume (950 ml) covered by 15 slices. This value, expressed in nanocuries per milliliter, was multiplied by an assumed total brain volume of 1350 ml to obtain an estimate of the total radioactivity in the brain.

## Results

### *Monkey study*

After the i. v. injection of [$^{11}$C]-busulfan (45 MBq) in the absence of a carrier, the radioactivity in the brain reached a peak within 2–4 min (Fig. 1) and subsequently declined. There was initially more radioactivity in the cortex than in the white matter (ratio, 1.25) during the first 10 min. The radioactivity in the cortex decreased more rapidly than that in the white matter. These levels reached essentially the same values within 30–35 min. At the end of the scanning period, the radioactivity remaining in the brain was approximately 50% of the initially extracted dose. No selective regional distribution was observed at the end of the scanning time.

At the end of the cerebral scan, the monkey was repositioned in the positron camera and the radioactivity in the body was scanned for 10 min such that the regional distribution in the body could be related to the cerebral uptake. At that time (50–60 min after the i. v. injection), the radioactivity measured in the liver of the monkey was approximately 9-fold that found in the brain at 35 min.

In a separate experiment, whole-body dynamic scans were performed on the second monkey following an i. v.

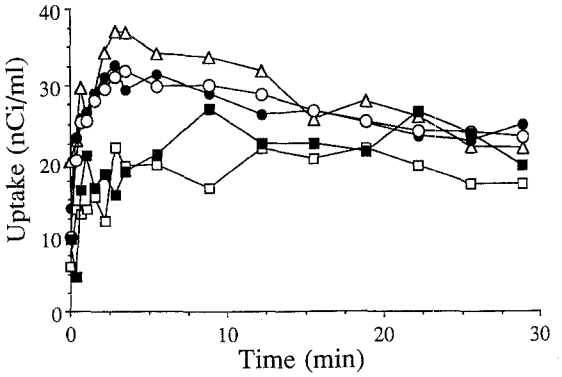


**Fig. 3.** Distribution of radioactivity in the human brain as a function of time after an i.v. injection of 95 MBq [$^{11}$C]-busulfan. △, Cerebellum; ●, right cortex; ○, left cortex; ■, right white matter; □, left white matter

injection of [$^{11}$C]-busulfan (45 MBq). The largest regional accumulation of radioactivity, observed in the liver and lungs, was compared with the cerebral uptake observed in the first experiment. Liver uptake was initially rapid and essentially increased throughout the entire experiment. A tendency of the values to level off during the last few minutes of the investigation was observed. The uptake of radioactivity in the lungs was 25%–45% of that found in the liver of the same monkey and was 1.4- to 3-fold that measured in the brain of the second monkey, which received the same dose (Fig. 2).

*Human study*

To investigate the regional cerebral distribution of [$^{11}$C]-busulfan in humans, the radiotracer (95 MBq) was given in the absence of a carrier as a bolus i.v. injection to the human subject, who was positioned in the PC 2048-15B camera as described above. The radioactivity in the arterial blood reached a peak within 3 min and subsequently declined. As observed in the monkey, the tracer rapidly penetrated into the brain (Fig. 3). Initially, more radioactivity distributed into the gray matter than into the white matter (cortex: white matter ratio 1.6 at 5–7 min). During the first 5 min, the distribution of radioactivity between the brain and the blood was 1, which compares well with the previously reported results of rat studies using the $^{14}$C-labeled tracer [10]. Radioactivity in the white matter increased to a maximum during the first 5–10 min and remained at essentially that level throughout the remainder of the experiment. Radioactivity in the cortex declined more rapidly, reaching the level in the white matter at 20 min after the injection. The uptake in the cerebellum was slightly higher than that in the cortex. At 5 min, the cerebellum: cortex ratio was approximately 1.2, but this value had also declined to essentially the same level found in the white matter by 20 min postinjection. The amount (%) of the total injected dose of [$^{11}$C]-busulfan that penetrated into the brain was calculated to be 20%.

## Discussion

Busulfan is a bifunctional alkylating agent of the methanesulfonic acid ester type. Since its introduction in myeloablative therapy, many side effects have been reported, such as hemorrhagic cystitis and veno-occlusive disease [2, 28]. It has also been reported that some patients undergoing long-term treatment with this drug develop lung fibrosis, generally known as "busulfan lung" [23]. The whole-body monkey investigation showed that radioactivity rapidly distributes to the lungs after i.v. administration, displaying a tendency to accumulate in this tissue with time (Fig. 2). Although the chemical identity of the radioactive species was not determined, previous studies [21, 29] indicate that only 8%–14% of the dose of [$^{14}$C]-labeled busulfan was exhaled as [$^{14}$C]-$CO_2$ within 24 h after i.v. injection, with very small amounts being exhaled within the 1st h. This in vivo observation of the accumulation of radiotracer in the lungs confirms the ex vivo findings previously reported for [$^{14}$C]-busulfan in rats [21].

Dynamic scans of the uptake of radioactivity in the liver over 45 min showed a continuous accumulation for up to at least 35 min (Fig. 2). This observation is consistent both with the rapid metabolism of busulfan via the liver as previously demonstrated in the perfused rat liver [8] and with the levels of metabolites found in urine from both rats and humans [9, 12]. The high hepatic uptake of busulfan and/or its labeled metabolites observed in the present study might possibly be involved in the development of veno-occlusive disease in patients who have been preconditioned for bone marrow transplantation (BMT) using high-dose therapy with busulfan [2].

Many leukemic patients show CNS involvement, which may cause a relapse after BMT. Patients with CNS leukemia usually receive methotrexate intrathecally, since it penetrates into the CNS very poorly when given by i.v. injection [25]. This poor cerebral penetration is typical of many alkylating agents, such as melphalan and chlorambucil [6, 7]. In contrast, [$^{11}$C]-busulfan was shown in the present study to be highly taken up into the brain after i.v. administration, which is consistent with previous studies of concentrations in human CSF [11, 30] and in rat-brain tissue [10]. The high cerebral uptake of busulfan could be explained by its low molecular weight, its lipophilicity [10], and the low extent to which it binds to proteins [4, 12].

In the present monkey and human studies using [$^{11}$C]-busulfan, more radioactivity was found in the gray matter than in the white during the first few minutes after injection, indicating a flow-dependent delivery of the tracer. However, within 20 min, the radioactivity in the cortex and cerebellum decreased and this regional difference disappeared (Figs. 3, 4). Cerebral radioactivity declined at a half-life larger than the 30-min observation period used. The plateau observed in both the monkey and the human brain is in accordance with the rather long elimination half-life previously reported for busulfan (2.5 h) and its metabolites (8 h) in the rat brain [10].

The high cerebral uptake of busulfan (one-ninth that of the critical organ, the liver) as well as the lack of regional differences in its distribution in the human brain could

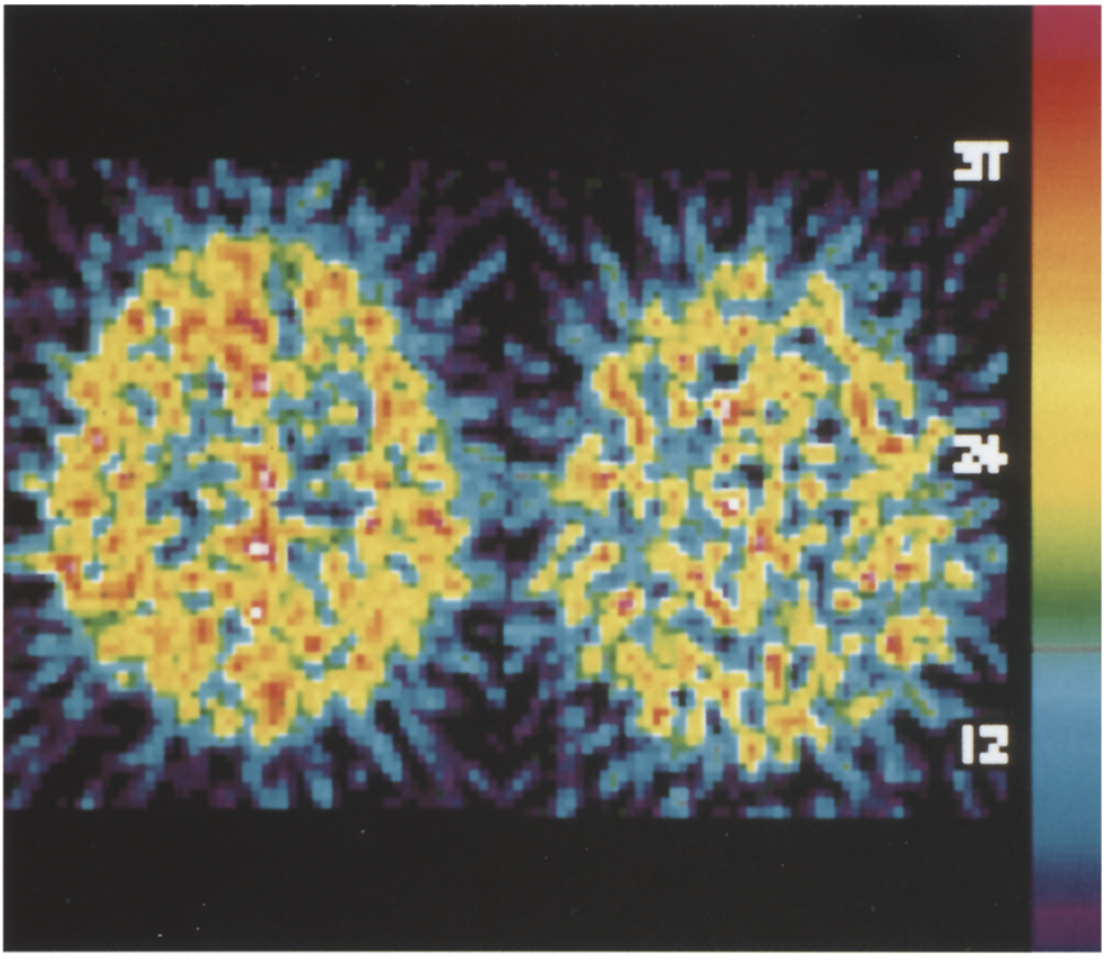

**Fig. 4.** PET image of the uptake of [$^{11}$C]-busulfan in the human brain as integrated from 0 to 5 min (*left*) and from 25 to 35 min (*right*) after an i. v. injection

have implications for both treatment with and side effects resulting from therapy with the drug. Our study shows the distribution of only trace amounts of busulfan. However, no accumulation of this drug has previously been observed in the CSF of patients given therapeutic doses [11], indicating that our results may also be valid at therapeutic levels.

Seizures are observed primarily in connection with high-dose busulfan treatment. The present results do not indicate that there is a specific regional accumulation of the drug (Fig. 4). Busulfan has been shown to react with glutathione in the liver [8], a reaction involving glutathione-S-transferase. The amount of glutathione in the brain (2 μmol/g wet tissue [15]) is comparable with the levels in the liver (0.5–1.7 μmol/g [26]). The reaction of busulfan with glutathione could reduce the glutathione pool in the brain, which in turn could affect the concentration of L-glutamate and δ-aminobutyric acid [24], resulting in seizures [20]. The sulfonium ion formed in the reaction between busulfan and glutathione [8] is a hydrophilic, charged species that might also contribute to disturbances of brain function. However, an elucidation of the mechanisms underlying the convulsions observed following high-dose therapy with busulfan requires additional studies. Our results indicate that such side effects cannot be explained by any regional accumulation of the drug in the brain.

The present study showed that about 20% of the injected activity was taken up into the brain after i. v. administration. It remains to be shown whether the amount of drug penetrating into the brain tissues is sufficient to eradicate leukemic cells in the CNS or whether, as proposed by Kalifa and co-workers [14], busulfan can be used for the treatment of brain tumors.

*Acknowledgements.* The technical assistance provided by Mr. G. Printz, Mr. F. Hamnqvist, and Ms. K. Antilla and by the members of the Stockholm PET group during the PET experiments is gratefully acknowledged.

## References

1. Anderson CC, Goldstone AH, Linch DC, Jones HM, Franklin IM, Boughton BJ, Cawley JC, Richards JDM (1987) Autologous bone marrow transplantation for patients with acute myeloid leukaemia and acute lymphoblastic leukaemia – a comparison. Bone Marrow Transplant 1: 271
2. Atkinson K, Biggs J, Noble G, Ashby M, Concannon A, Dodds A (1987) Preparative regimens for marrow transplantation containing busulphan are associated with haemorrhagic cystits and hepatic veno-occlusive disease but a short duration of leucopenia and little oro-pharyngeal mucositis. Bone Marrow Transplant 2: 385

3. Bergström M, Litton J, Eriksson L, Bohm C, Blomqvist G (1982) Determination of object contour from projections for attenuation correction in cranial positron emission tomography. J Comput Assist Tomogr 6: 365
4. Ehrsson H, Hassan M (1984) Binding of busulfan to plasma proteins and blood cells. J Pharm Pharmacol 36: 694
5. Fitzsimmons WE, Ghalie R, Kaizer H (1990) The effect of hepatic enzyme inducers on busulfan neurotoxicity and myelotoxicity. Cancer Chemother Pharmacol 27: 226
6. Gouyette A, Hartmann O, Pico J-L (1986) Pharmacokinetics of high-dose melphalan in children and adults. Cancer Chemother Pharmacol 16: 184
7. Greig NH, Sweeney DJ, Rapoport SI (1988) Comparative brain and plasma pharmacokinetics and anticancer activities of chlorambucil and melphalan in the rat. Cancer Chemother Pharmacol 21: 1
8. Hassan M, Ehrsson H (1987) Metabolism of [$^{14}$C]-busulfan in isolated perfused rat liver. Eur J Drug Metab Pharmacokinet 12: 71
9. Hassan M, Ehrsson H (1987) Urinary metabolites of busulfan in the rat. Drug Metab Dispos 15: 399
10. Hassan M, Ehrsson H, Wallin I, Eksborg S (1988) Pharmacokinetic and metabolic studies of busulfan in rat plasma and brain. Eur J Drug Metab Pharmacokinet 13: 301
11. Hassan M, Ehrsson H, Smedmyr B, Tötterman T, Wallin I, Öberg G, Simonsson B (1989) Cerebrospinal fluid and plasma concentrations of busulfan during high-dose therapy. Bone Marrow Transplant 4: 113
12. Hassan M, Öberg G, Ehrsson H, Ehrnebo M, Wallin I, Smedmyr B, Tötterman T, Eksborg S, Simonsson B (1989) Pharmacokinetic and metabolic studies of high-dose busulphan in adults. Eur J Clin Pharmacol 36: 525
13. Hassan M, Thorell J-O, Warne N, Stone-Elander S (1991) $^{11}$C labelling of busulphan. Appl Radiat Isotopes 42: 1055
14. Kalifa C, Hartmann O, Vassal G, Valteau D, Lemerle J (1988) Preliminary results of a phase II study of high-dose busulfan and thiotepa in children with recurrent brain tumors. Pediatr Neurosci 14: 154
15. Lajtha AL, Maker HS, Clarke DD (1981) Metabolism and transport of carbohydrates and amino acids. In: Siegel GJ, Albers RW, Agranoff BW, Katzman R (eds) Basic neurochemistry, 3rd edn. Little, Brown and Company, Boston, pp 329–353
16. Litton JE, Bergström M, Eriksson L, Bohm C, Blomqvist G, Kesselberg M (1984) Performance study of the PC-384 positron camera system for emission tomography of the brain. J Comput Assist Tomogr 8: 74
17. Litton JE, Holte S, Eriksson L (1990) Evaluation of the Karolinska new positron camera system; the Scanditronix PC2048-15B. IEEE Trans Nucl Sci 37: 743
18. Marcus RE, Goldman JM (1984) Convulsions due to high-dose busulphan. Lancet II: 1463
19. Martell RW, Sher C, Jacobs P, Monteagudo FCP (1987) High-dose busulfan and myoclonic epilepsy. Ann Intern Med 106: 173
20. Peterson DW, Collins JF, Bradford HF (1983) Transmitter amino acids and their antagonists in epilepsy. In: Hertz L, Kvamme E, McGeer EG, Schousboe A (eds) Glutamine, glutamate, and GABA in the central nervous system, vol 7. Alan R. Liss, New York, pp 643–652
21. Roberts JJ, Warwick GP (1961) The mode of action of alkylating agents: III. The formation of 3-hydroxytetrahydrothiophene-1 : 1-dioxide from 1 : 4-dimethanesulphonyloxybutane (Myleran), *S*-β-L-alanyltetrahydydrothiophenium mesylate, tetrahydrothiophene and tetrahydrothiophene-1: 1-dioxide in the rat, rabbit and mouse. Biochem Pharmacol 6: 217
22. Santos GW, Tutshka PJ, Brookmeyer R, Saral R, Beschorner WE, Bias WB, Braine HG, Burns WH, Elfenbein GJ, Kaizer H, Mellits D, Sensenbrenner LL, Stuart RK, Yeager AM (1983) Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. N Engl J Med 309: 1347
23. Schallier D, Impens N, Warson F, Van Belle S, De Wasch G (1983) Additive pulmonary toxicity with melphalan and busulfan therapy. Chest 84: 492
24. Shank RP, Campbell GL (1983) Metabolic precursors of glutamate and GABA. In: Hertz L, Kvamme E, McGeer EG, Schousboe A (eds) Glutamine, glutamate, and GABA in the central nervous system, vol 7. Alan R. Liss, New York, pp 355–369
25. Shapiro WR, Young DF, Mehta BM (1975) Methotrexate: distribution in cerebrospinal fluid after intravenous, ventricular and lumbar injections. N Engl J Med 293: 161
26. Siegers C-P, Bossen KH, Younes M, Mahlke R, Oltmanns D (1982) Glutathione and glutathione-S-transferases in the normal and diseased human liver. Pharmacol Res Commun 14: 61
27. Sureda A, Pérez de Oteyza J, García Laraña J, Odriozola J (1989) High-dose busulfan and seizures. Ann Intern Med 111: 543
28. Thomas AE, Patterson J, Prentice HG, Brenner MK, Ganczakowski M, Hancock JF, Pattinson JK, Blacklock HA, Hopewell JP (1987) Haemorrhagic cystitis in bone marrow transplantation patients: possible increased risk associated with prior busulphan therapy. Bone Marrow Transplant 1: 347
29. Trams EG, Nadkarni MV, DeQuattro V, Maengwyn-Davies GD, Smith PK (1959) Dimethanesulphonoxybutane (Myleran), preliminary studies on distribution and metabolic fate in the rat. Biochem Pharmacol 2: 7
30. Vassal G, Gouyette A, Hartmann O, Pico JL, Lemerle J (1989) Pharmacokinetics of high-dose busulfan in children. Cancer Chemother Pharmacol 24: 386
31. Vassal G, Deroussent A, Hartmann O, Challine D, Benhamou E, Valteau-Couanet D, Brugiéres L, Kalifa C, Gouyette A, Lemerle J (1990) Dose-dependent neurotoxicity of high-dose busulfan in children: a clinical and pharmacological study. Cancer Res 50: 6203