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Boron neutron capture therapy of glioblastoma multiforme using the *p*-boronophenylalanine-fructose complex and epithermal neutrons

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1. INTRODUCTION

The amino acid analog *p*-boronophenylalanine (BPA) has been under investigation at Brookhaven National Laboratory (BNL) as a neutron capture agent for BNCT of glioblastoma multiforme (GBM) for the past six years. BPA, delivered orally, is effective in BNCT of the intracerebral 9L rat gliosarcoma[1]. Histologic examination of the brains of long-term BNCT survivors showed scar tissue replacing the tumor with no serious damage to the contiguous normal brain[2]. A series of 17 patients undergoing surgical removal of tumor (GBM or melanoma) received BPA orally as the free amino acid. Favorable tumor/blood boron concentration ratios were obtained but the absolute amount of boron in the tumor would have been insufficient for BNCT[3]. BPA can be solubilized at neutral pH by complexation with fructose[4]. BPA-fructose (BPA-F) is administered intravenously (iv) for thermal neutron-based BNCT of melanoma in Japan[5]. Intraperitoneal (ip) injection of the soluble BPA-F complex produces much higher tumor boron concentrations in the rat intracerebral 9L gliosarcoma than were previously possible using oral administration of BPA. Higher boron concentrations have allowed higher radiation doses to be delivered to the tumor while maintaining the dose to the normal brain vascular endothelium below the threshold of tolerance. This resulted in long-term survival of over 90% of BNCT-treated rats[6]. Surviving rats show no obvious neurologic deficit. We have measured the relative biological effectiveness (RBE) of the various high-linear energy transfer (LET) components of the total BNCT dose in both tumor and normal tissues. Our data indicate a therapeutic ratio (tumor dose/normal tissue dose) in excess of 5:1 (Coderre *et al.*, these proceedings). A biodistribution study of iv BPA-F in patients with high-grade gliomas is reported elsewhere in these proceedings (Bergland *et al.*, these proceedings).

An epithermal neutron irradiation facility has been designed and installed at the Brookhaven Medical Research Reactor (BMRR)[7]. The response of the normal dog brain to irradiation with epithermal neutrons in the presence of either BPA or BSH is consistent with the biological effectiveness factors determined in the rat (Huiskamp *et al.*, these proceedings). We have carried out a pilot study of BNCT in one patient with GBM. The results from the single patient study will be used in the design of a statistically controlled trial. The patient was given BPA-F i.v. and irradiated on September 13, 1994. This report summarizes the approach to BNCT used for the first patient and proposes a clinical research strategy for the first controlled test of BNCT for the post-debulking therapy of unilateral, unifocal GBM in adults.

2. METHODS

Clinical BNCT would comprise: 1) a BPA-F biodistribution study at the time of surgical debulking and 2) BNCT 2-5 weeks after surgery. In the pilot study, BNCT was delivered in a single fraction and with a single field. For each prospective patient, the biodistribution study with BPA will indicate whether the blood boron clearance kinetics for that patient are within the ranges observed to date. The blood clearance curve should be predictable to estimate the irradiation time and the dose to the normal brain. The tumor/blood boron concentration ratio measured at the time of the biodistribution study is assumed in calculating the tumor dose during the same patient's BNCT. BPA-F injection solutions were prepared at a concentration of 30 mg BPA/ml (0.14 M) by a modification of published procedures[4,8]. Briefly, BPA (^{10}B-enriched, L-isomer) was combined with a 10% molar excess of fructose in water (65% of the total volume needed to make a 0.14 M solution). The pH was adjusted to between 9.5 and 10.0 with NaOH for 2 - 3 minutes and then readjusted to 7.4 with HCl. The volume was adjusted with water to yield a 0.14 M solution. The solution was passed through a 5000 molecular weight-cutoff filter to remove endotoxins, and through through a 0.22 μm sterilization filter into empty, sterile infusion bags. A fresh solution of BPA-F was prepared for each individual patient study and was used within 48 hours. All BPA-F injection solutions are prepared and tested for sterility and pyrogenicity prior to use at BNL.

2.1 Radiobiology

The mixed radiation field produced during BNCT comprises radiations with different LET and different RBEs. RBE is a complex factor which is dependent upon a number of parameters including radiation dose, dose rate, number of dose fractions, physical radiation quality (LET), the choice of biological system, and the radiation effect that is monitored in the biological system. In BNCT there is an additional parameter to be considered. The short ranges of the two high-LET products of the $^{10}B(n,\alpha)^{7}Li$ reaction make the microdistribution of the boron relative to target cell nuclei of particular importance. The compound-adjusted biological effectiveness (CBE) for the $^{10}B(n,\alpha)^{7}Li$ reaction is defined as the product of the true, geometry-independent, RBE for these particles multiplied by a "boron compound localization factor", which will most likely be different for each particular boron compound. To express the total BNCT dose in a common unit, and to compare BNCT doses with the effects of conventional photon irradiation, multiplicative factors (RBEs and CBEs) are applied to the physical absorbed radiation doses from each high-LET component. The total effective BNCT dose is then expressed as the sum of RBE-corrected physical absorbed doses with the unit Gray-equivalent (Gy-Eq). The RBEs of the beam components and the CBE for BPA-based BNCT have been assessed in the rat brain tumor model[9], in the rat spinal cord model[10], in rat skin[11] and in human skin[11]. Table 1 summarizes the radiobiological parameters used in calculation of the dose during BNCT with the BMRR epithermal beam.

Table 1....insert

2.2 Definition of Brain Regions

The following terms are useful in BNCT dosimetry. The *tumor volume* is defined as the contrast enhancing volume evident in CT or MRI images. The *target volume* is defined as the tumor

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volume plus a 2 cm margin: a zone likely to contain infiltrating tumor cells. The thermal neutron fluence from the epithermal beam reaches a maximum at approximately 1 cm depth in the brain. The *absolute peak dose* is the Gy-Eq dose to a 1 cm^3 volume (a single "voxel"in the treatment planning software). Within 1-2 cm deeper than this contour, the fluence drops to 90% of the maximum. The *peak-dose volume* is defined as the volume of tissue that receives a dose between 90% and 100% of the absolute peak dose. For the current epithermal beam collimation (8 cm aperture) the *peak dose volume* corresponds roughly to an ellipsoid with a minor diameter (depth) of 1 cm. The major diameters (perpendicular to the beam axis) are approximately 2 cm each. The volume of this ellipsoid is about $\approx$ 15 cm^3, *i.e.*, about 1% of the brain volume.

2.3. BNCT Treatment Planning

The BNCT treatment planning software was developed at the Idaho National Engineering Laboratory (see Nigg *et al.*, these proceedings). It requires input of the boron concentration in the blood and tumor as well as the RBE and CBE. The treatment plan defines the dose rate to the normal brain vasculature per unit concentration of boron in the blood per megawatt-minute of reactor irradiation. It is assumed that the residual tumor accumulates boron to the same degree as the primary tumor. The duration of the irradiation is based on the blood boron concentration at the end of the infusion, 20 - 30 minutes after the end of the infusion, at the beginning of the BNCT irradiation, and at the irradiation midpoint. The irradiation time will be that required to deliver the prescribed normal brain endothelium dose in the peak-dose volume.

2.4 BNCT Treatment Conditions

The prescribed BNCT radiation doses to the tumor and the normal tissues in the pilot study were based on literature reports of single-fraction irradiation tolerance of the human brain, single-fraction photon tumor-control doses, and on the BNCT (all single fraction) literature. The dose to the peak-dose volume ($\approx$15 cm^3) should be $\leq$ 10 Gy-Eq. The minimum tumor dose should be $\geq$ 23 Gy-Eq. The minimum dose to the target volume (tumor plus 2 cm) should be $\geq$ 15 Gy-Eq. The average whole brain dose should be $\leq$ 7.5 Gy-Eq at a dose rate $\leq$ 27 cGy-Eq/min.

Two ^{238}U-enriched fission chambers are mounted above and below the beam aperture, behind the collimator, at the irradiation port in a non-perturbing and non-perturbable configuration. A change in the symmetry, position or spatial distribution of the beam due to shutter position can be detected by comparing the chambers' readings. Integral chamber readings and appropriate ratio calculations are displayed and recorded at 20-sec intervals. Dosimeters were placed on the patient to provide post-BNCT verification of the dose to the rest of the body. Lithium-6-depleted, slow neutron-shielded, thermoluminescent dosimeters (gamma dose) and gold wires (thermal neutron flux) were placed on the patient's neck and at the xiphoid process to provide an estimate of the whole-body equivalent dose.

3. RESULTS

3.1 BPA-fructose Biodistribution Study

For the pilot study, 100 mg BPA/kg was infused before surgical debulking, 33 days prior to

BNCT. Boron concentrations in blood, normal brain, scalp and tumor are shown in Figure 1. The blood boron concentration during debulking was 6.0 μg ^{10}B/g. Boron concentrations in normal brain were 3.8 μg ^{10}B/g (n=2). The boron concentrations (mean $\pm$ sd) in tumor were 19.2 $\pm$ 3.2 μg ^{10}B/g (n=10). The range of tumor boron concentrations, 15.2 to 26.6 μg ^{10}B/g, correlates qualitatively with the degree of microscopic necrosis. The sample showing 26.6 μg ^{10}B/g was exceptionally cellular with little or no necrosis. Scalp samples obtained at 0.7, 1.0 and 2.2 hours after the end of the infusion contained 7.3, 6.6 and 8.4 μg ^{10}B/g, respectively.

Figure 1...insert

Figure 1. Boron concentrations in blood, tumor, scalp and normal brain as a function of time after the start of the infusion. BPA-F (100 mg of BPA/kg body weight) was delivered iv over a period of 1.75 hours. Craniotomy was started $\approx$ 15 min after the end of infusion.

3.2 BNCT

On the day of BNCT, the patient received 244 mg BPA/kg body weight infused (as BPA-F) over a 2-hr period. Blood boron concentrations are shown in Figure 2. Blood samples were obtained every 20 minutes during the 2 hr infusion, at 20 minutes after the end of the infusion, at the start of the irradiation (3 hrs, 10 minutes after the start of the infusion), at the mid-point of the irradiation and at the end of the irradiation. The boron concentration in the blood during the infusion (mean $\pm$ sd) was 11.2 $\pm$ 1.4 μg ^{10}B/g. The neutron irradiation was carried out at reduced reactor power (2 MW) to not exceed the specified dose rate limit of 27 cGy-Eq/min. The neutron irradiation was given in two parts with about a 10 minute break for blood sampling. The first part of the irradiation was 20 min, the second part, 27 min in duration.

Figure 2...insert

Figure 2. Boron concentrations in blood and a scalp biopsy as a function of time after the start of the BPA-F infusion.

3.3 Dose Evaluation Summary

The peak thermal neutron fluence was 3.6 x 10^{12} n_{th}/cm^2. The average ^{10}B concentration in the blood during the irradiation was 11.2 $\pm$ 1.4 μg ^{10}B/g. The total irradiation time was 47 minutes at 2 MW power (20 minutes, $\approx$ 10 minute break, 27 minutes). The absolute peak physical dose (1 cm^3 voxel) to normal brain was 8.0 Gy, comprised of 2.77 Gy from the $^{10}B(n,\alpha)^7Li$ reaction, 4.50 Gy gamma, 0.42 Gy from the $^{14}N(n,p)^{14}C$ reaction, and 0.35 Gy from fast neutrons. Using the values listed in Table 1, the physical dose of 8.0 Gy corresponds to 10.5 Gy-Eq. The average dose to the peak dose volume (90-100% iso-dose contours) was 10.0 Gy-Eq. The estimation of tumor dose depends on the value assumed for the tumor/blood ratio. The mean boron concentration in the biodistribution study (19.2 μg ^{10}B/g) gave a tumor/blood ratio of 3.2:1. The maximum tumor dose was 42.0 Gy-Eq, based on an assumed boron concentration in the tumor of 35.8 μg ^{10}B/g (11.2 μg ^{10}B/g in blood x assumed tumor/blood ratio of 3.2:1). The minimum dose to the tumor volume was 55% of the absolute peak dose (see Figure 3A), 22.2 Gy-Eq. The minimum dose to the target volume was 45% of the absolute peak dose (see

Figure 3A), 18.1 Gy-Eq. The measured thermal neutron fluence at the neck was 8.2 x 10^9 n_{th}/cm^2 (0.2% of peak). The measured thermal neutron fluence at the xiphoid process was 2.0 x 10^9 n_{th}/cm^2 (0.06% of peak). The measured gamma dose at the neck was 35 cGy. The measured gamma dose at the xiphoid process was 13 cGy. The estimated dose to the skin in the 8 cm irradiation field (for 18 μg ^{10}B/g in the skin) was 12.5 Gy-Eq. The whole-body dose equivalent was $\approx$ 0.4 Sv.

Figures 3A and 3B show the treatment plan (isodose contours) for tumor and normal brain, respectively. The tumor volume and the target volume are indicated on the images. The minimum doses to tumor and target volume were 55% and 45% of the maximum dose, respectively (**Figure 3A**). Examination of the isodose contours on MRI images at different levels in the brain allowed an estimation of the dose to other vital structures. These are expressed as a percentage of the absolute peak dose, 10.5 Gy-Eq: ipsilateral basal ganglia, 65%; hypothalamus, 50%; cerebral midline, 25%; optic chiasm, $\leq$ 20%; retina, $\leq$ 10%.

Figure 3...insert

Figure 3. **(3A)** Iso-dose contours (% of absolute peak dose) for brain tumor. The tumor and target volumes are enclosed within a solid line and a dotted line, respectively. The peak dose-equivalent to the tumor was 42.0 Gy-Eq. **(3B)** Iso-dose contours (% of absolute peak dose) for normal brain endothelium. The absolute peak dose to normal brain endothelium was 10.5 Gy-Eq.

4. DISCUSSION

It is not possible to determine the boron concentration in residual tumor at the time of BNCT with clinically proven non-invasive techniques. The estimation of the BNCT dose received by the tumor is based on the assumption that the tumor/blood ratio will remain the same as was determined in the biodistribution study of a particular patient at the time of prior surgical debulking. The boron content of the tumor samples obtained from the pilot study patient showed considerable variation (mean=19.2 $\pm$ 3.2 μg ^{10}B/g; range=15.2-26.6 μg ^{10}B/g). Histologically, the samples with lower amounts of boron correlate qualitatively with a higher fraction of necrosis. The maximum value may be more representative of the BPA accumulation in residual, so-called "healthy" tumor, or in small nests of cells (or individual cells) in the normal brain parenchyma. To be conservative, we have used the mean value of tumor boron concentration from the biodistribution study for estimation of the dose to the tumor during BNCT. It is thus probable that calculated BNCT doses to the tumor and to the target volume are underestimated by 25% or more. We assume that the use of previously observed blood/normal brain boron concentration ratios will not seriously underestimate the normal brain endothelial dose, even in the edematous brain, since BPA does not respect the normal blood-brain barrier.

The starting dose of BPA and the prescribed doses to the normal brain and the tumor were chosen with consideration given to both safety and efficacy. With only $\approx$ 11 weeks of follow-up, it is premature to judge from this single patient whether the BNCT was more effective against the tumor than other treatments might have been, or safe as regards late effects in the

brain. The procedure produced no untoward acute or sub-acute effects in the brain. There has been some scalp hair loss in and around the field without erythema or dry desquamation.

Based on the reasonable result of the pilot study to date, we plan to irradiate a series of 10-15 patients using the same approach. Each patient will undergo a BPA-F biodistribution study at the time of surgical debulking and will receive BNCT using the protocol as used for the pilot study: 250 mg BPA/kg; 10.5 Gy-Eq absolute peak dose to normal brain. We have devised a statistical analysis that will allow us to determine the effectiveness of this BNCT dose level. We will compare the clinical outcome in the BNCT patients to that of an equal (or greater) number of prognostically-matched recent historical (or concurrent) reference cases treated with standard post-debulking photon therapy. We will use a statistical analysis that requires ranking the patients with a morbidity-mortality index and evaluation of the result using the Wilcoxon two-sample test. This approach is analogous to that described for the analysis of BPA-based BNCT of subcutaneous murine melanomas[13] and should allow a determination, within 6 months after the treatment of the last patient, as to whether BNCT, at the proposed starting dose level, is better, the same, or worse in outcome than the reference group treated with conventional post-debulking photon therapy.

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Table 1. Biological effectiveness of the high-LET dose components produced in tissue during BNCT.

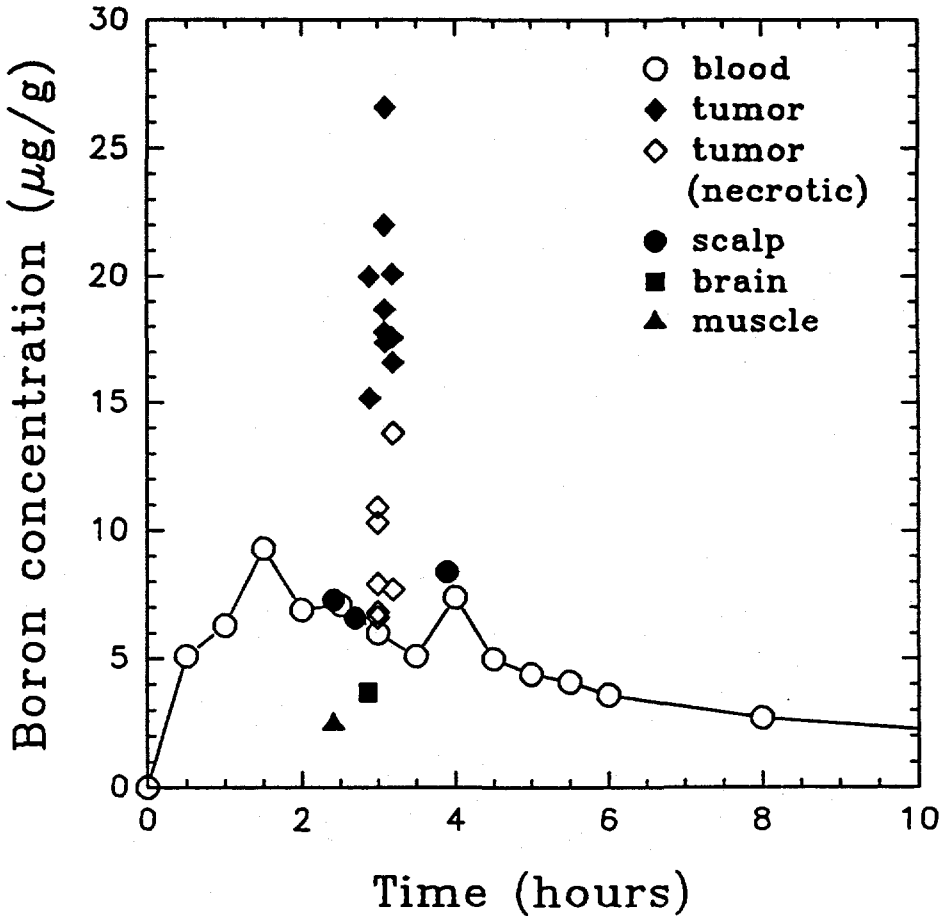
Dose component	Biological effectiveness factor: (RBE or CBE)
$^{10}B(n,\alpha)^{7}Li$ reaction (BPA-fructose)	tumor endpoint[a] = 3.8 CNS (spinal cord) endpoint[b] = 1.35 rat skin; moist desquamation endpoint = 3.7[c] rat skin; dermal necrosis endpoint ≈ 1.0[c] human skin; moist desquamation ≈ 2.5[d]
beam protons [$^{14}N(n,p)^{14}C$ and $^{1}H(n,n')p$]	3.2[a,b]
gamma photons from the reactor and from the $^{1}H(n_{th},\gamma)^{2}H$ reaction	1.0

[a] Determined from the results of *in vivo/in vitro* clonogenic assays of the rat 9L gliosarcoma as described in Reference 9.

[b] Determined from dose-response studies of the irradiated rat spinal cord with an endpoint of limb paralysis within 7 months as described in Reference 10.

[c] Determined from the response of rat skin irradiated with thermal neutrons as described in Reference 11.

[d] Determined from thermal neutron-based BNCT of cutaneous melanoma in patients as described in Reference 12.


Boron concentration (μg/g)
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25
20
15
10
5
0
0
2
4
6
8
10
Time (hours)
blood
tumor
tumor (necrotic)
scalp
brain
muscle

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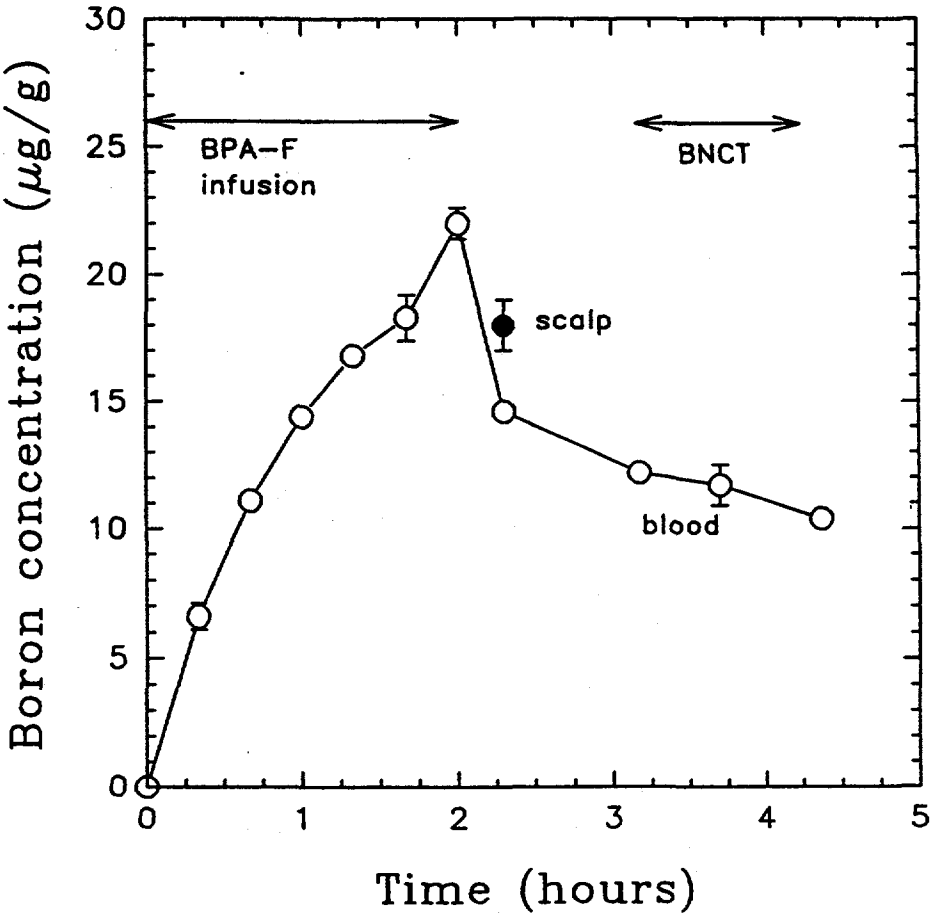
Boron concentration (μg/g)
30
25
20
15
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5
Time (hours)
BPA–F
infusion
BNCT
scalp
blood

Fig 7

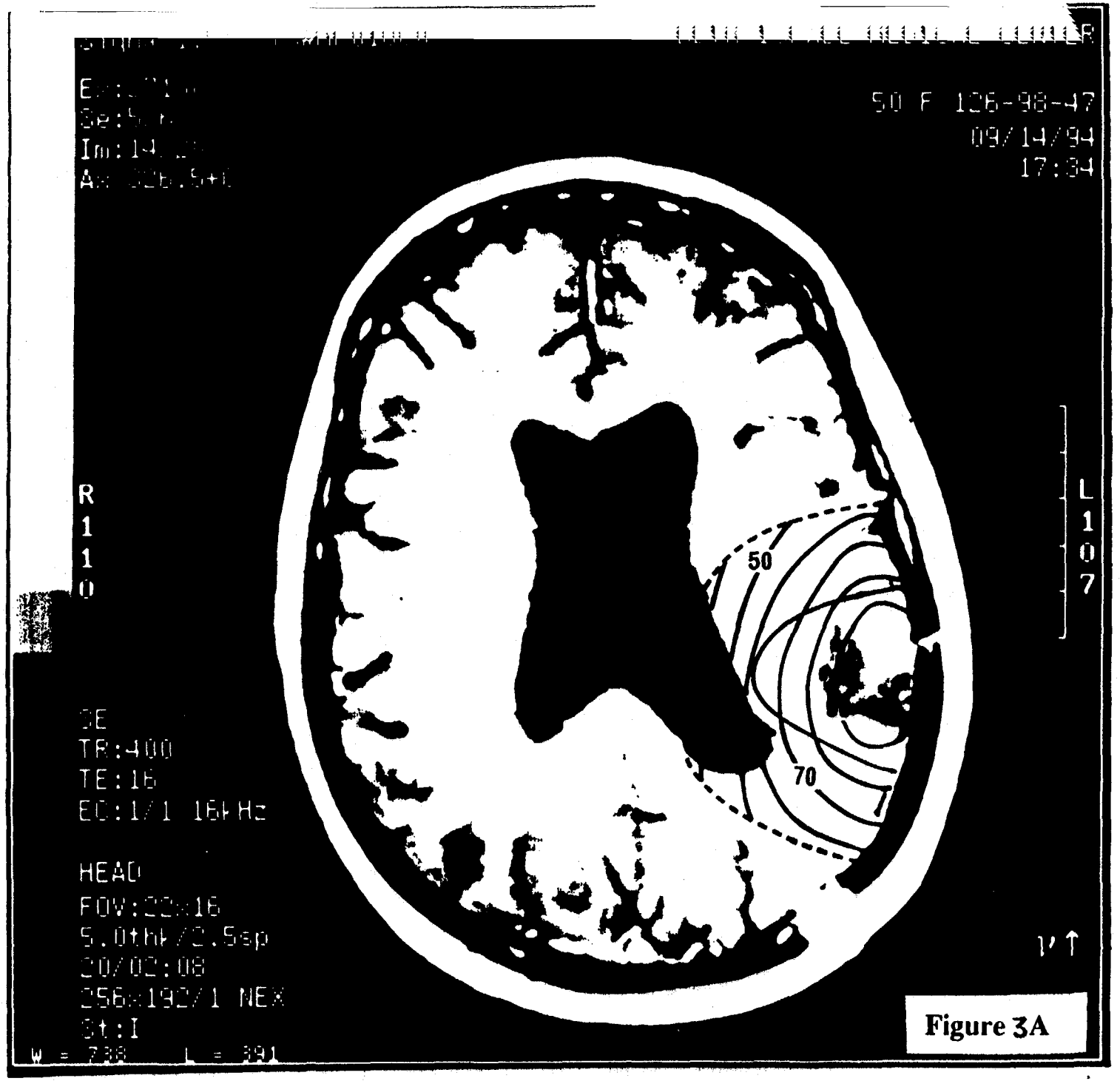
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Figure 3A

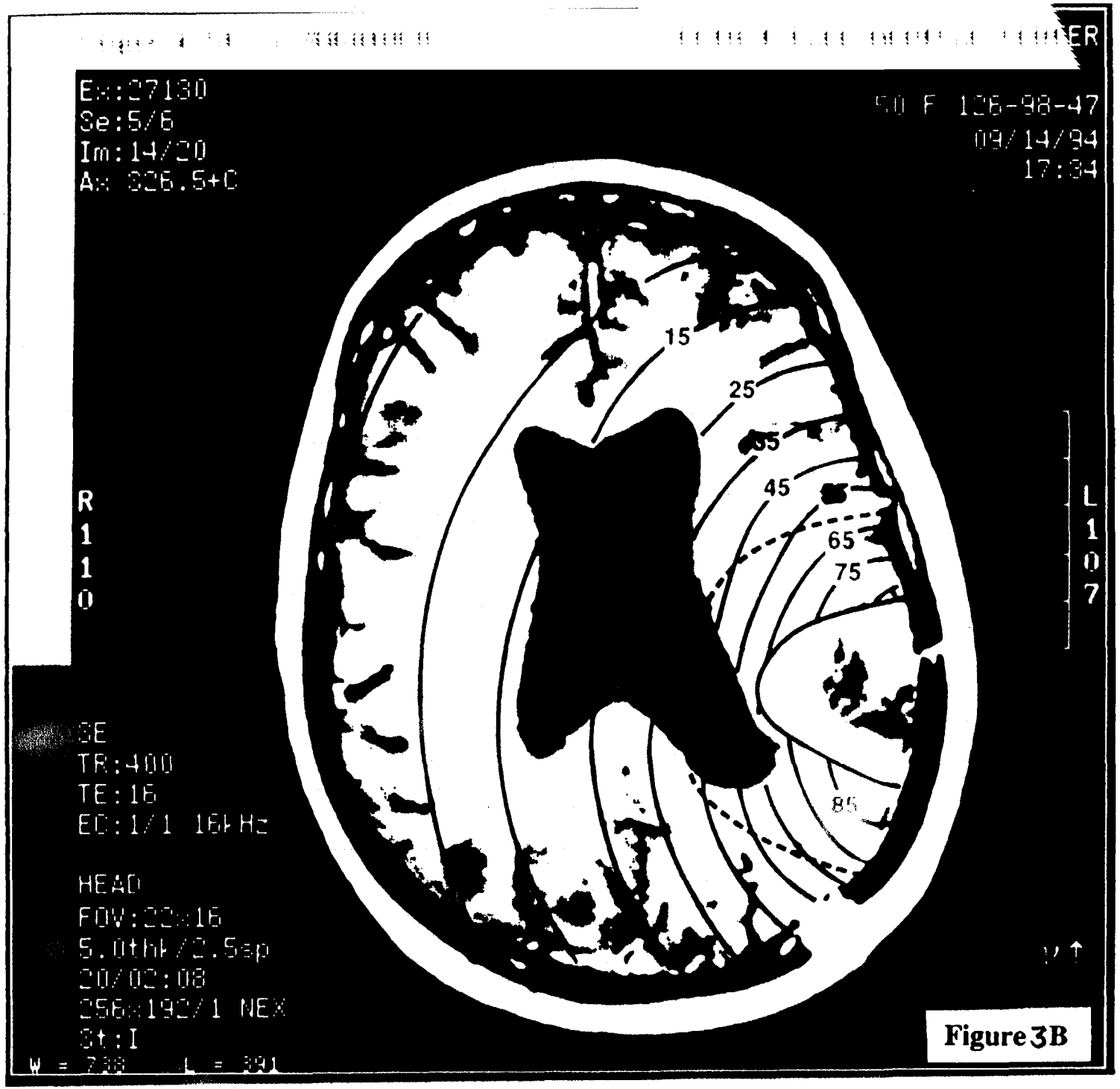
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Figure 3B