Annals of Nuclear Medicine Vol. 12, No. 5, 267–273, 1998

Characterization of neuronal damage by iomazenil binding and cerebral blood flow in an ischemic rat model

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I-123-iomazenil is a SPECT probe for central benzodiazepine receptors (BZR) which may reflect intact cortical neuron density after ischemic insults. We evaluated whether neuronal damage in rats could be characterized by iomazenil as compared with cerebral blood flow (CBF). Serial changes in I-125-iomazenil for BZR and I-123-IMP for CBF were analyzed after the unilateral middle cerebral artery occlusion in rats by using an *in vivo* dualtracer technique. Uptake ratios of affected to contralateral regions were calculated. The iomazenil as well as IMP were decreased in all regions except for the cerebellum (remote area). Both iomazenil and IMP increased over time except in the temporal region (ischemic core). The iomazenil uptake was higher than IMP except in the ischemic core between 1 and 3–4 wk when iomazenil was lower than IMP. Iomazenil showed a moderate decrease in the proximal and middle parietal regions (peri-infarct areas) at 3–4 wk. The triphenyl-tetrazolium-chloride (TTC) stain at 1 wk demonstrated unstained tissue in the temporal region indicating tissue necrosis. With hematoxylin-eosin (HE) stain at 1 wk, widespread neuronal necrosis with occasional intact neurons were found in the proximal parietal region, and isolated necrotic neurons were represented in the distal parietal region. Iomazenil correlated well with the neuron distribution and the finding of a discrepancy between iomazenil and IMP might be useful in evaluating the neuronal damage.

Key words: ischemic penumbra; benzodiazepine receptor; cerebral blood flow, iomazenil; neuronal damage

INTRODUCTION

I-123-iomazenil is a single photon emission computed tomography (SPECT) probe for central benzodiazepine receptors (BZR).[1] The central BZR, the gamma-amino butyric acid (GABA)-ergic complex exists on the postsynaptic membrane of virtually all cortical neurons.[2] Because, BZR density has been shown to reflect intact synapses, it may be used as a marker for viable neuron density.[3] BZR imaging is therefore expected to provide information on neuron-specific damage after ischemic insult in patients with cerebral infarction[4–6] and cerebral hemorrhage,[7] whereas previously established imaging techniques such as CT, MRI and perfusion/metabolism measurements with SPECT or positron emission computed tomography (PET) may not do so.

Ischemic penumbra[8] was originally defined with the CBF criteria as the tissue where CBF values are between the functional and morphological thresholds, but this concept has recently been extended to refer to ischemically affected but still viable tissue, but whether this tissue will eventually become infarcted or recover is uncertain.[9] The knowledge of the extent of ischemic penumbra in the acute phase of cerebral infarction is a crucial factor in designing effective thrombolytic therapy.[10] Although cerebral blood flow (CBF) has been used for this purpose,

Received March 30, 1998, revision accepted July 27, 1998.
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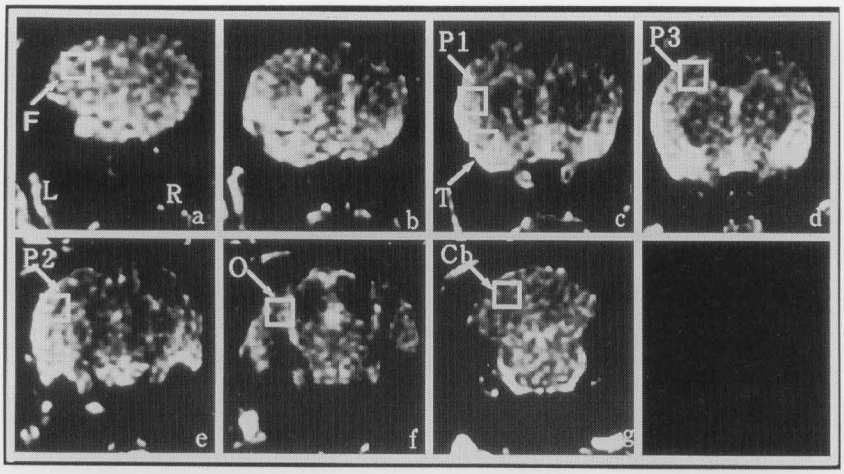

Fig. 1 T2 weighted coronal MRI 1 wk after left MCA and CCA occlusion. Note that the hyperintense areas represent cerebral infarction. Each ROI of the ischemic core (temporal region; T), the peri-infarct areas (proximal parietal region; P1, middle parietal region; P2, distal parietal region; P3), the intrahemispheric remote areas (frontal region; F, occipital region; O), and remote area (cerebellum; Cb) was shown.

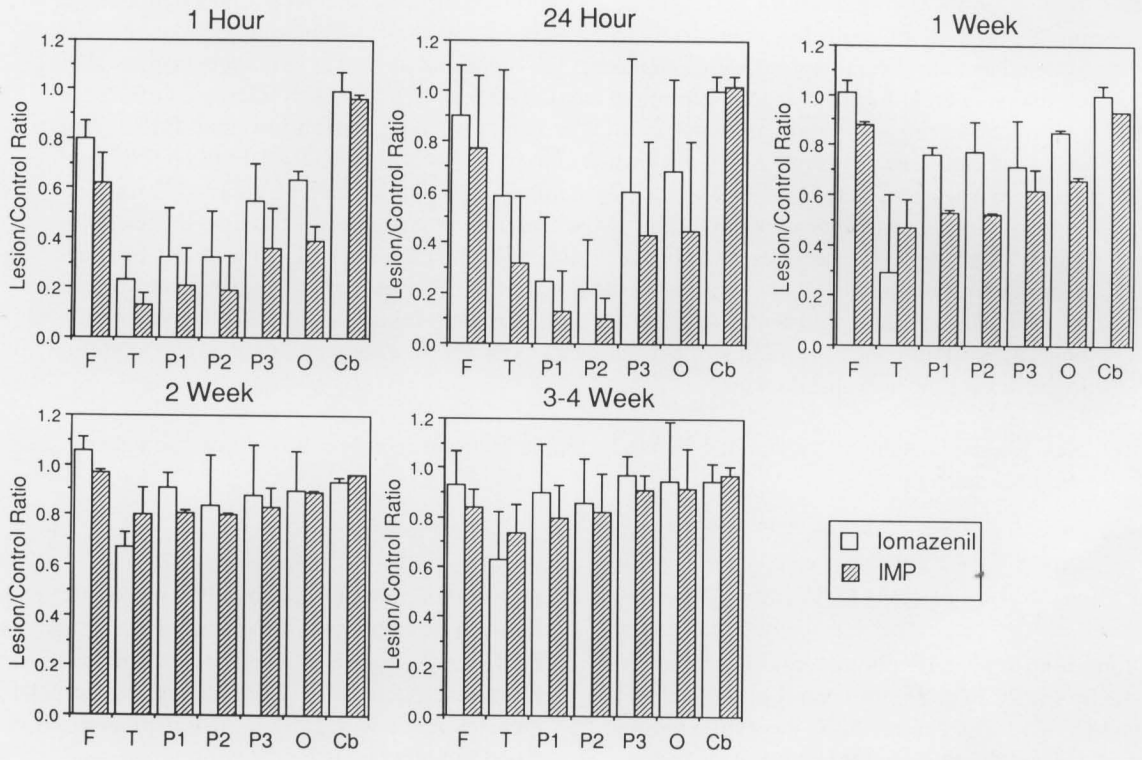

Fig. 2 Serial changes of I-125 iomazenil (Iomazenil) and I-123 IMP (IMP) in each ROI. The values were expressed mean ratio values ± 1 s.d. of Iomazenil and IMP uptake in the lesioned hemisphere to that in contralateral hemisphere. Abbreviations on the x axis refer to the ROIs on MRI templates in Fig. 1.

CBF changes do not allow reliable discrimination between the infarct core and ischemic penumbra.[11] Multitracer studies with metabolic indexes measured by PET may provide much more accurate information in predicting cell death and the extent of infarct,[10] but such measurements are not yet feasible with SPECT.

During the acute and subacute stages of an ischemic stroke, a limited loss of neurons in the peri-infarct region (incomplete infarction)[6,12,13] does not result in structural changes detectable by CT or conventional MRI, but this

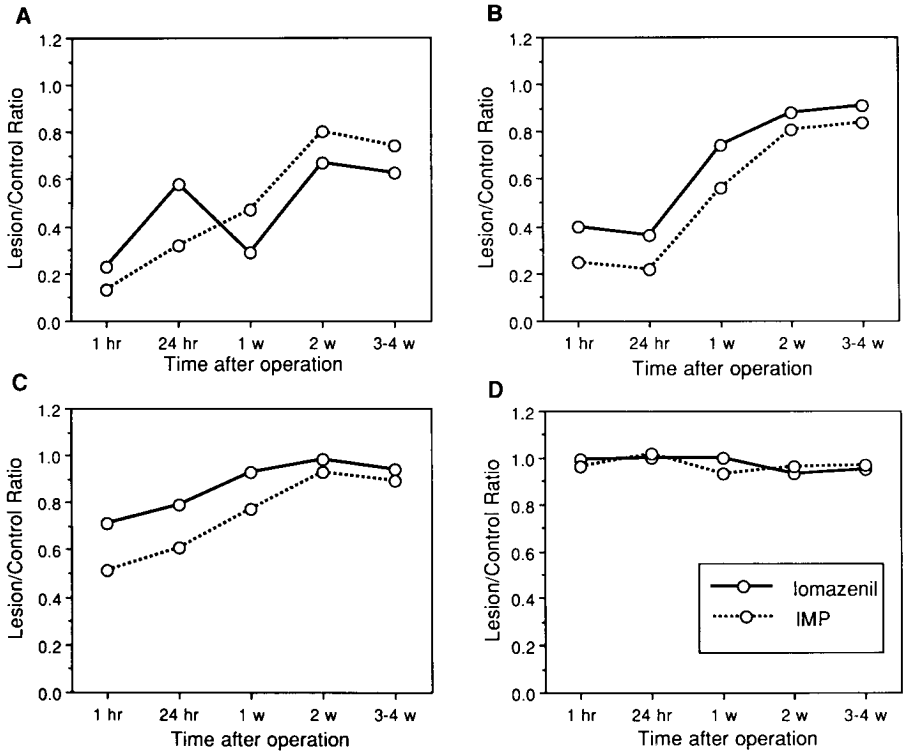

Fig. 3 Serial changes of I-125 iomazenil (iomazenil) and I-123-IMP (IMP) in ischemic core (temporal region) (A), peri-infarct areas (proximal to distal parietal regions) (B), intrahemispheric remote areas (frontal and occipital regions) (C), and remote area (cerebellum) (D). The values were expressed mean ratio value of Iomazenil and IMP uptake in the lesioned hemisphere to that in contralateral hemisphere. For clarity, these figures do not show error bars and are meant to convey only trends.

incomplete infarction may be demonstrable *in vivo* by measuring changes in BZR.[6,12,13]

Recently, a PET study in cats reported relationships between sequential early changes in flow/energy metabolism and deficits in C-11-flumazenil (a PET BZR ligand) uptake in stroke. And the reduction in flumazenil binding reflected irreversible neuronal damage that otherwise could only be detected by multitracer studies,[14] but serial changes in iomazenil uptake as well as serial relationships between iomazenil uptake and CBF in ischemic core and peri-infarct areas together with histopathological examinations have not yet been reported.

To characterize neuronal damage, in other words, viable tissue (ischemic penumbra), we evaluated whether cortical neuronal damage could be characterized by concurrently examining serial changes in BZR binding with I-125-iomazenil and CBF with I-123-IMP (N-isopropyl-p-iodoamphetamine) in experimental brain ischemia in rats. The reason for examining CBF simultaneously with iomazenil uptake was to evaluate whether there exists a relationship between CBF and tissue viability in a zone of potentially salvageable tissue,[14] to estimate tracer delivery through the blood-brain barrier, and to assess the feasibility of combined analysis of iomazenil and IMP which was used in a clinical trial for a human SPECT study in Japan. Hematoxylin-eosin (HE) and triphenyltetrazolium chloride (TTC) stain[15] tissue sections were also studied to see if iomazenil uptake correlates with neuronal distribution and damage.

MATERIALS AND METHODS

Under pentobarbital sodium (Nembutal) anesthesia (0.09 g/kg), the left common carotid artery (CCA) and the ipsilateral middle cerebral artery (MCA) of male Sprague-Dawley rats (250–300 g) were exposed. Simultaneous coagulation in the left MCA between the olfactory tract and rhinal fissure and ligation of the ipsilateral CCA were carried out by the modified method of Hakim[11] and Brint[16] to make neocortical infarction.

At 1 and 24 hr, 1, 2 and 3–4 wk after the MCA + CCA occlusion (3–5 rats, respectively), saline solution containing 10 μCi (0.37 MBq) of I-125-iomazenil and 45 min later 100 μCi (3.7 MBq) of I-123-IMP were injected. Both tracers were obtained from Nihon Medi-Physics Co. (Nishinomiya, Japan). The animals were allowed to recover from anesthesia prior to the administration of the tracers. Under ether anesthesia, the rats were decapitated and the brains were removed 1 hr after injection of I-125-iomazenil. Our preliminary *in vivo* kinetic study showed

Table 1 Mean ± S.D. ratio values of I-125-iomazenil (Iomazenil) and I-123-IMP (IMP) in the lesioned hemisphere to that in contra-lateral hemisphere in the ischemic core (temporal region), the peri-infarct areas (proximal to distal parietal regions), the intrahemispheric remote area (frontal & occipital regions), and the remote area (cerebellum) at 24 hr, 1 and 3–4 wk

	Time after operation					
	24 hr		1 wk		3–4 wk	
	Iomazenil	IMP	Iomazenil	IMP	Iomazenil	IMP
Ischemic core						
temporal	0.58 ± 0.50	0.32 ± 0.26	0.29 ± 0.31	0.47 ± 0.11	0.63 ± 0.19*	0.74 ± 0.11
Peri-infarct areas						
proximal parietal	0.25 ± 0.25	0.13 ± 0.16	0.76 ± 0.03*	0.53 ± 0.01	0.90 ± 0.20*	0.80 ± 0.13
middle parietal	0.22 ± 0.19	0.10 ± 0.08	0.77 ± 0.12	0.52 ± 0.01	0.86 ± 0.18*	0.82 ± 0.16
distal parietal	0.60 ± 0.53	0.43 ± 0.37	0.71 ± 0.19	0.62 ± 0.08	0.97 ± 0.08*	0.91 ± 0.06
Intrahemispheric remote area						
frontal & occipital	0.79 ± 0.29*	0. 61 ± 0.34	0.93 ± 0.09*	0.77 ± 0.12	0.94 ± 0.19*	0.89 ± 0.12
Remote area						
cerebellum	1.00 ± 0.06	1.02 ± 0.04	1.00 ± 0.04	0.93 ± 0.00	0.95 ± 0.07	0.97 ± 0.04

Significant differences between Iomazenil and IMP uptake ratios in each lesion (Student's paired t-test): *$p < 0.05$

enough specific binding of I-125-iomazenil to the rat cerebral cortex at 1 hr after injection (Matsumura, Toyama, et al., unpublished results).

We performed T2 weighted coronal magnetic resonance imagings (MRI) of representative rat models at 1 wk postocclusion in order to construct morphological templates (Fig. 1). Based on the T2 weighted MRI images, the temporal region containing high intensity signals was considered as an ischemic core, the proximal parietal region, the middle parietal region and the distal parietal region served as peri-infarct areas. The frontal region within the anterior cerebral artery (ACA) territory and the occipital region within the posterior cerebral artery (PCA) territory were considered as intra-hemispheric remote areas of infarction. The cerebellum served as a remote area.[4] Brain tissues within the regions of interest (ROIs) {frontal region, temporal region, parietal regions 1–3, occipital region and cerebellum} defined by MRI templates representing affected and contralateral areas were dissected and weighed.[17] Each sample was 20 to 30 mg in weight. Then I-123-IMP uptake (count/g) was measured, and I-125-iomazenil uptake was counted 3 days later. The mean serial uptake ratios of the affected to contralateral regions for each ROI were then calculated.

Two to 3 mm thick coronal sections at 1 wk post-occlusion were stained with TTC[15] to identify infarct regions. Coronal histology sections, 5- to 10-μm thick, at 1 hr and 1 wk post-occlusion, were stained with HE, and the histopathological changes were evaluated.

RESULTS

1. Sequential changes in iomazenil and IMP

Both iomazenil and IMP uptakes were decreased in all regions except for remote areas (cerebellum) (Figs. 2, 3). The degree of these decreases differed in the three regions as follows (Fig. 3). There were a marked decrease in the ischemic core (temporal region), a moderate decrease in the peri-infarct area (proximal to distal parietal regions), and a mild decrease in the intra-hemispheric remote area (frontal and occipital regions). At 3–4 wk, the iomazenil uptakes showed a moderate decrease in the proximal parietal (10%) and middle parietal (14%) regions (Table 1). Both iomazenil and IMP uptakes increased over time in the three regions except in the ischemic core where iomazenil uptake decreased between 24 hr and 1 wk (Fig. 3).

2. Relationship between iomazenil and IMP

In all three regions, iomazenil uptake was higher than IMP uptake except in the ischemic core between 1 and 3–4 wk during which iomazenil uptake was lower than IMP uptake (Fig. 2, 3, Table 1).

3. Histopathological changes with TTC and HE stains

TTC stain of coronal sections of a rat brain 1 wk post-occlusion demonstrated unstained tissue in the left temporal region corresponding to tissue necrosis and the high intensity region on MRI.

With HE stain, in the temporal region early changes including pallor, cellular pyknosis (ischemic cell change) and microcavitation showing edema (Fig. 4A) at 1 hr post-occlusion preceded the neuronal loss at 1 wk. Widespread neuronal necrosis with occasional intact neurons were found in the proximal parietal region, and isolated necrotic neurons were represented in the distal parietal region showing incomplete infarctions at 1 wk (Fig. 4B). No necrotic neurons were seen in the intra-hemispheric remote areas.

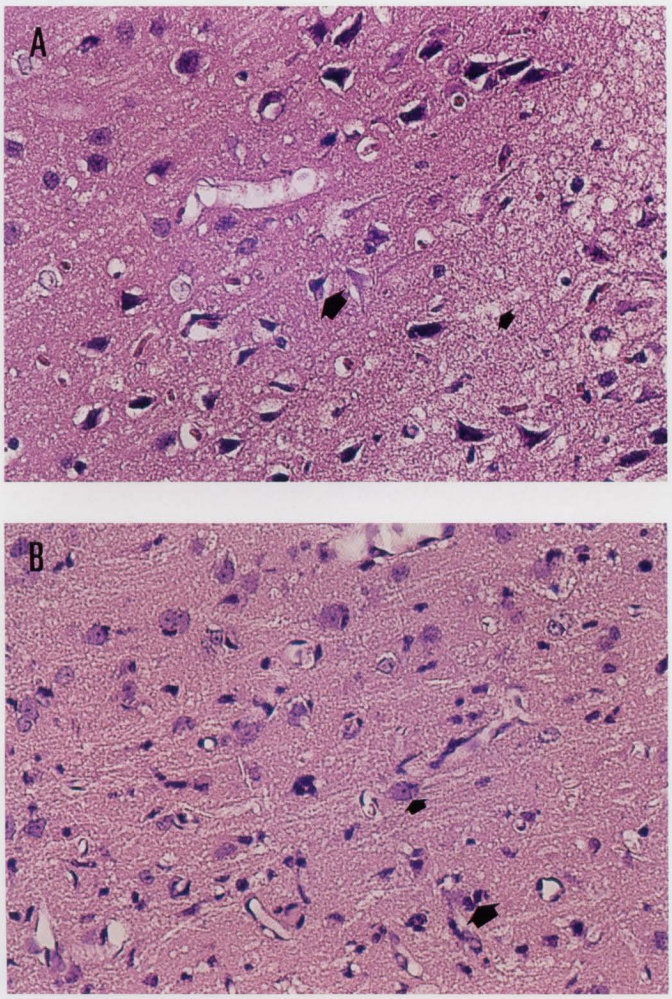

Fig. 4 Photomicrographs on rat brain at the level of the striatum at 1 hr (A), and 1 wk (B) after the occlusion. A (temporal region): Pallor, cellular pyknosis (ischemic cell change) (large arrow), and microcavitation resulting from edema (small arrow) are shown. B (proximal parietal region): Widespread neuronal necrosis (large arrow) with occasional intact neurons (small arrow) are found. (300 ×; hematoxylin-eosin).

DISCUSSION

Our results demonstrated that, in the peri-infarct areas, iomazenil uptake was relatively preserved compared with IMP until the chronic phase was reached. The iomazenil uptakes were moderately reduced when compared with unity in the chronic phase. Hatazawa et al. reported in human subjects with cerebral infarction that I-123-iomazenil in the peri-infarct areas was better preserved than in the infarct area. They reported that iomazenil uptake tended to be higher than that of CBF.[4] Sette et al. also demonstrated, in their baboon experiments, that in the peri-infarct areas, in 7 out of 9 animals C-11-flumazenil was higher than CBF in peri-infarct areas and they suggested that their findings indicate that neurons are relatively well preserved in the same areas.[18] We presume that peri-infarct areas correspond to incomplete infarction,[6,12,13] because widespread neuronal necrosis with occasional intact neurons were observed histopathologically.

In the ischemic core, iomazenil uptake was relatively well preserved in the acute phase, but more decreased than IMP in the chronic phase. Histopathological examination as well as TTC stain evaluation also verified neuronal loss in the ischemic core. In an animal ischemic model of infarct, Matsuda et al.[3] reported that I-125-iomazenil accumulation showed a more noticeable decrease than Tc-99m-HM-PAO, and Odano et al.[19] also demonstrated slight uptake of C-14-2-deoxyglucose but no binding of I-123-iomazenil in the chronic phase. They concluded that iomazenil was a more reliable marker of neuronal viability than CBF or glucose metabolism. The reasons for the relatively well preserved CBF as well as glucose metabolism in the infarct core would be gliotic reactions and infiltration of macrophages.[5,19] Garcia et al. confirmed that neuronal changes induced by MCA occlusion in rats were of two types: acute changes (shrinkage and swelling), prominent during the first 6 hr, and delayed changes (necrosis) which affect large numbers of neurons only from 6 to 12 hr.[20] These histopathological evaluations at different time intervals were consistent with sequential results in our experiments.

In the present study, iomazenil uptake as well as IMP uptake demonstrated a time dependent increase from 1 hr to 3–4 wk in both the infarct core and its borders. Al-

Tikriti et al. reported using a rat model in which I-125-iomazenil uptake was linearly correlated with I-123-IMP uptake in more than 200 regions at 1 hr after MCA occlusion.[21] Our rats were sacrificed at 1 hr post injection of iomazenil in the same way as those of Al-Tikriti et al. The significant reduction in the acute phase and time dependent increase in iomazenil uptake may reflect a sequential CBF increase[3] based on the development of collateral circulation and reperfusion. And an early reduction in iomazenil uptake may be due to an initial irreversible synaptic dismantlement.[14,18]

Hatazawa et al. reported that CBF was significantly decreased in the superior frontal gyrus, a territory of the anterior cerebral artery and the cerebellum in patients with cerebral infarction in the MCA territory. CBF uptake was significantly lower than iomazenil uptake in those regions.[4] They speculated that the discrepancy between iomazenil uptake and that of CBF in these areas indicated that the alteration in iomazenil uptake is not due to altered delivery but may predominantly reflect a receptor-mediated phenomenon. In the present study, CBF was significantly lower than iomazenil uptake in the intra-hemispheric remote areas. The iomazenil uptake showed no marked reduction between 1 and 3–4 wk. Neither iomazenil uptake nor CBF in the cerebellum demonstrated any significant reduction throughout the entire experimental period. Crossed cerebellar diaschisis has been difficult to observe in rats presumably because the cerebro-cerebellar loop is not well developed in rats.[22] Preservation of iomazenil uptake in the intrahemispheric remote areas was consistent with the normal histopathological findings.

In the present study, there are several methodological limitations to the use of BZR techniques in evaluating neuronal damage. We used a direct counting technique as opposed to autoradiography. The former allows immediate counting but the latter procedure is more time consuming than the former. The major disadvantage of the counting technique with arbitrary sampling is that it is more difficult to do anatomical localization than with the autoradiography technique. To overcome this disadvantage, we used MRI for defining the morphological template, but combined experiments on histology sections and autoradiography should be designed for much more detailed histopathological comparisons in the future. Based on the previously reported rat and gerbil experiments[3,19] and human SPECT studies,[4] I-123-iomazenil uptake ratios of the lesion to the contra-lateral region were evaluated. The relative qualitative analysis technique used in this study should be applicable to *in vivo* autoradiography or SPECT. Bilateral decreased CBF[11] as well as iomazenil uptake could be underestimated with this relative quantification. Absolute quantifications of BZR should be applied for much more detailed examinations. We measured iomazenil and IMP uptakes normalized by weight, so the early reduction in uptakes at 1 and 24 hr could be overestimated due to ischemic brain edema.[23] Absolute quantification of CBF by using a diffusible tracer such as C-14-iodo-antipyrine should be employed for much more detailed comparisons. Another fundamental limitation is that BZR binding or iomazenil uptake may not be assessed in striatal or thalamic infarctions because BZR binding in these areas is normally low.[2,4,24]

Garcia et al. reported with a rat ischemia/reperfusion model that significant neurological improvement was able to be obtained by re-establishing flow, and incomplete histological ischemic injury was observed.[25] Nakagawara et al. evaluated the binding of I-123-iomazenil in patients with embolic stroke, and concluded that the reduction in the BZR concentration in reperfused cortex that remained structurally intact is likely to be the result of injury involving only a limited number of neurons.[6] The I-123-iomazenil could become a much more accurate indicator of sublethal injury than CBF after therapy with thrombolytic and neuroprotective agents.[26] Further combination studies of functional prognosis and iomazenil with animal experiments and human SPECT are warranted in the future.

CONCLUSION

Our study on an experimental rat model for cerebral ischemia showed that iomazenil uptake, which correlated with histological neuron distribution, was better preserved in peri-infarct areas than in the ischemic core. On the other hand, CBF was decreased to a similar degree in both ischemic core and peri-infarct areas. This discrepancy between iomazenil uptake and CBF may be a useful marker in characterizing neuronal damage. A comparison of iomazenil and flow tracer uptakes would be applicable to human SPECT studies estimating BZR binding for a practical method.

ACKNOWLEDGMENTS

This study was supported by grants in aid for scientific research from the Ministry of Education, Science and Culture of Japan, and Fujita-Gakuen Research Foundation.

The authors gratefully acknowledge Mr. Miki Kurami, Ba.Sc., and his colleagues, Nihon Medi-Physics, Co., Ltd. for valuable cooperation in basic animal experiments.

This paper was presented, in part, at the 43rd Annual Meeting of the Society of Nuclear Medicine on June 3–5, 1996 in Denver, Colorado, and 44th Annual Meeting of the Society of Nuclear Medicine on June 1–5, 1997 in San Antonio, Texas.

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