Evaluation of Phosphoinositide Turnover on Ischemic Human Brain with 1-[1-^{11}C]-Butyryl-2-Palmitoyl-rac-Glycerol Using PET

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It is important to evaluate cerebral function from neural signal transduction in ischemic brain in judging morbid state and prognosis. We synthesized 1-[1-^{11}C]-butyryl-2-palmitoyl-rac-glycerol (DAG) for the purpose of imaging the second messenger on PET and applied it to clinical cases of cerebral infarction. **Methods:** Five patients, who had ischemic stroke, were examined with PET. [^{15}O]-CO_2 and [^{15}O]-O_2 inhalation methods were applied to cerebral blood flow (CBF), oxygen extraction fraction (OEF) and cerebral metabolic rate of oxygen ($CMRO_2$). For the measurement of phosphoinositide turnover after intravenous injection of DAG, dynamic PET data were collected continuously for 46 min. Arterial blood samples were taken to evaluate changes in the serum concentration of DAG. To quantify the metabolic activity of inositol phospholipid, the incorporation constant k*(DAG) was calculated on the basis of the kinetics of DAG. **Results:** The plasma concentration of DAG increased rapidly and peaked 30 s after injection of DAG solution. In the normal cortex, DAG concentration increased gradually and reached a plateau between 15 and 20 min after injection. In the ischemic core (infarction), DAG concentration increased slowly, and its peak concentration was lower than that in normal tissue. In comparison with blood flow and metabolic parameters, k*(DAG) showed the best correlation with $CMRO_2$, suggesting a reflection of neuronal activity. Locally, CBF and $CMRO_2$ gradually decreased from the normal area toward the ischemic center (infarction), whereas k*(DAG) and OEF significantly decreased only in the ischemic center. **Conclusion:** The k*(DAG) of ischemic brain, including that caused by infarction, significantly correlated with $CMRO_2$, suggesting that metabolic activity of inositol phospholipid reflects neural viability. Maintained metabolic activity of inositol phospholipid in the region around the ischemic core indicated preservation of the signal transduction system through the metabotropic receptor.

Key Words: cerebral ischemia; second messenger; phosphoinositide turnover; PET

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In addition to the advancement of imaging techniques, including PET, clarification of the morbid state of circulation and metabolism in cerebral ischemia has also progressed (*1–3*). However, evaluation of neurotransmission in cerebral ischemia has not been possible because of the difficulty in developing a tracer for that purpose. Therefore, we have been trying to develop 1-[1-^{11}C]-butyryl-2-palmitoyl-rac-glycerol (DAG) as a tracer for neural transmission imaging with second messenger measurements (*4,5*). Receptor-mediated membrane processing plays an essential role in the neural function of the synapses, and phosphoinositide (PI)-derived signals can be targeted as a means of assessing in vivo signal transduction (*6*). Recently, two types of signal transduction have been suggested; one is rapid signal transfer mediated by ionotropic receptors, and the other is related to slowly acquired signals to modulate neural activity or plasticity mediated by metabotropic receptors that use PI signals (*7*). We used DAG PET in ischemic stroke patients to evaluate PI-derived signal transduction and to compare metabolic activity of inositol phospholipid with cerebral blood flow (CBF) and oxygen metabolism.

MATERIALS AND METHODS

Patients

Five patients (four men, one woman; age range 58–81 y), who had ischemic stroke diagnosed by neurologic examination and CT, were selected for this study. PET was used to evaluate cerebral blood flow, oxygen metabolism and PI turnover. All patients showed neurologic symptoms related to cerebral infarction. Four patients were in the chronic stage (1.5–2.5 mo after onset) and one patient (patient 3) was in the subacute stage (1 wk after onset) at the time of the examinations (Table 1).

Synthesis of DAG

We have described the synthesis of DAG in detail in previous reports (*4,5*). ^{11}C-ethylketene, which was synthesized using a

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TABLE 1
Patient Clinical Data

Patient no.	Age (y)	Sex	Symptom	Location of infarction	Angiogram	Time of study after onset
1	60	M	Lt. hemiparesis	Subcortical, rt. frontal	Rt. ICA occlusion	1.5 mo
2	68	F	Lt. hemiparesis	Cortical, rt. frontal	Not available	2 mo
3	81	M	Rt. hemiparesis, aphasia	Cortical, lt. temporooccipital	Not available	1 wk
4	59	M	Lt. hemiparesis	Cortical, rt. frontal	Rt. ICA occlusion	2 mo
5	58	M	Rt. hemiparesis	Cortical, lt. parietal	Lt. ICA occlusion	2.5 mo

Lt. = left; Rt. = right; ICA = internal carotid artery.

cyclotron, was trapped in a pyridine solution containing 1 μmol 2-palmitoylglycerol. The acylation reaction was performed using ^{11}C-ethylketene under no-carrier-added conditions, and DAG had high specific activity (5 Ci [186 GBq]/μmol, at the end of synthesis). Human application of the DAG PET system and quality control of DAG followed the guidelines established by the PET committee in Nishijin Hospital (Kyoto, Japan) in October 1993.

PET Procedures

PET was performed using a Headtome III (Shimadzu Co., Kyoto, Japan); the spatial resolution of the positron camera was 8.2 mm full width at half maximum in-plane resolution, whereas the average axial resolution was 12.8 mm. [^{15}O]-CO_2 and [^{15}O]-O_2 inhalation methods were applied to CBF, oxygen extraction fraction (OEF) and cerebral metabolic rate of oxygen ($CMRO_2$). For the measurement of PI turnover after intravenous injection of DAG (4 mCi [149 MBq]/10 kg, 40 s), dynamic PET data were collected continuously for one 2-min period and nine to eleven 4-min periods, for a total of 10–12 periods and 38–46 min. Twenty arterial blood samples were taken to evaluate changes in serum concentration of DAG.

Region of Interest

We chose a region of interest (ROI) on the CT slice that shows maximal infarction. As a rule, 20–30 ROIs, which consist of 50–70 pixels, were placed on the PET image. ROIs of the infarction were chosen in the area delineated on the CT image, and ROIs of the peri-infarcted area that were adjacent to the ROIs of infarction were also chosen. ROIs in the contralateral hemisphere were selected as controls and were used to calculate the ratio of the lesion to the normal side. The variation in radioactivity associated with each pixel was less than 18% of the mean value. Using this as a criterion, we limited macroscopic heterogeneity to minimum designating ROIs.

Evaluation of Phosphoinositide Turnover

To evaluate PI turnover in the brain, we measured the accumulation of radioactive metabolites after intravenous DAG injection. Consecutive dynamic scanning revealed an increase in the radioactivity of the brain about 15 min after injection of DAG, which then remained at the same level. This finding supports the idea that membrane trapping of the ^{11}C intermediates in PI turnover is in proportion to the magnitude of the signal transduction in the brain (*4*). Calculations were made according to the following equation:

$$k^*(DAG)\ (mL/s/g) \times 10^4 = Cbr^*(T)/\int_0^T Cpl^*dt.$$

This equation was defined as the incorporation constant of DAG (*8*). Radioactivity in each brain region (Cbr*), which summed up the membrane PI compartments (Cbr,i*), was determined. Because Cbr,i* represents PI components on the same system, we regarded Cbr* to be equal to ΣCbr,i*. Cbr* was divided by plasma non-metabolized 1,2-DAG radioactivity (Cpl*), integrated over the time (T) the experiment lasted. This calculation normalizes brain radioactivity to exposure under the plasma curve and results in an estimate of the unidirectional incorporation rate constant k*(DAG) (mL/s/g) $\times$ 10^4.

Statistical Analysis

Comparison between infarction, peri-infarction and noninfarction of each parameter on PET was performed with one-factor analysis of variance followed by the Scheffé test. The Pearson correlation coefficient was used for the test of correlation between k*(DAG) and other parameters on PET.

RESULTS

Pharmacokinetics of DAG

Representative profiles of DAG pharmacokinetics in plasma, normal cortex and ischemic core (infarction) are shown in Figure 1. The plasma concentration of DAG increased rapidly and peaked 40 s after injection of DAG solution. In the normal cortex, DAG concentration increased gradually and reached a plateau between 15 and 20 min after injection. In the ischemic core (infarction), DAG concentration increased slowly, and its peak concentration was lower than that in normal tissue. According to these observations, we used 20 min as T for the calculation of the incorporation constant of DAG.

Regional Analysis of DAG Accumulation and Other Metabolic Parameters in Focal Ischemia

Results of regional analysis of DAG are shown in Figure 2. Generally, DAG increased homogeneously in the cerebral cortex and showed a low concentration only in the core of focal ischemia (infarction). In the infarction area, k*(DAG) was 84% ± 10% of the concentration observed in the contralateral side. CBF, $CMRO_2$ and OEF were 63% ± 17%, 38% ± 14% and 69% ± 35%, respectively, of the values attained in the contralateral sides. In the peri-infarcted area, k*(DAG), CBF, $CMRO_2$ and OEF were 97% ± 11%, 78% ± 18%, 71% ± 19% and 89% ± 18%, respectively, of the values observed in the contralateral side. There were significant

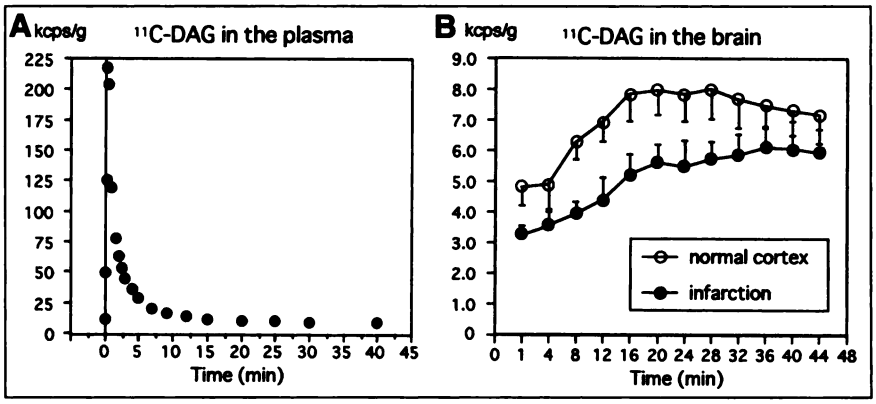


FIGURE 1. Representative patterns of radioactivities (patient 3) in plasma (A), normal cortex and infarction (B) after intravenous injection of 1-[1-^{11}C]-butyryl-2-palmitoyl-racglycerol (DAG). Regarding time-activity curve in brain, one region of interest, of approximately 50 pixels, was chosen in normal cortex and infarction. Each point was shown on mid time point during each collecting period with PET. In plasma, DAG increased and decreased quickly. In brain, radioactivities increased gradually and reached plateau at approximately 15–20 min. Bars represent mean ± SD.

differences between the peri-infarcted area and the noninfarcted area in CBF and $CMRO_2$, but there were no significant differences between k*(DAG) and OEF. In summary, k*(DAG) and OEF were significantly reduced only in the core of the ischemic area (infarction), whereas CBF and $CMRO_2$ showed a stepwise decrease from the noninfarction area to the peri-infarction and infarction areas.

Correlations Between the Incorporation Constant of DAG and Other Metabolic Parameters

Overall comparisons between k*(DAG) and other parameters on the infarcted slice are shown in Figure 3. Between k*(DAG) and CBF, there was a positive correlation (r = 0.681–0.777, $P < 0.001$) in the chronic stage but a poor correlation in the subacute stage (r = 0.031; P was not significant). Regarding oxygen metabolism, there were significant positive correlations between k*(DAG) and $CMRO_2$ (r = 0.633–0.859, $P < 0.001$) in both stages and variable correlations between k*(DAG) and OEF (r = 0.363–0.784). In illustrative cases (Fig. 4), there was matched hypoperfusion and hypometabolism in the ischemic region in the chronic stage (patient 1). However, in the subacute stage (patient 3), regarded as a luxury perfusion period, there were significant dissociations between CBF and other parameters including k*(DAG).

DISCUSSION

Mapping of intracellular signal transduction visualizing the second messenger activity in the brain in vivo has not materialized because of difficulties in developing a tracer. Because PI turnover is affected by many transmitter sys-

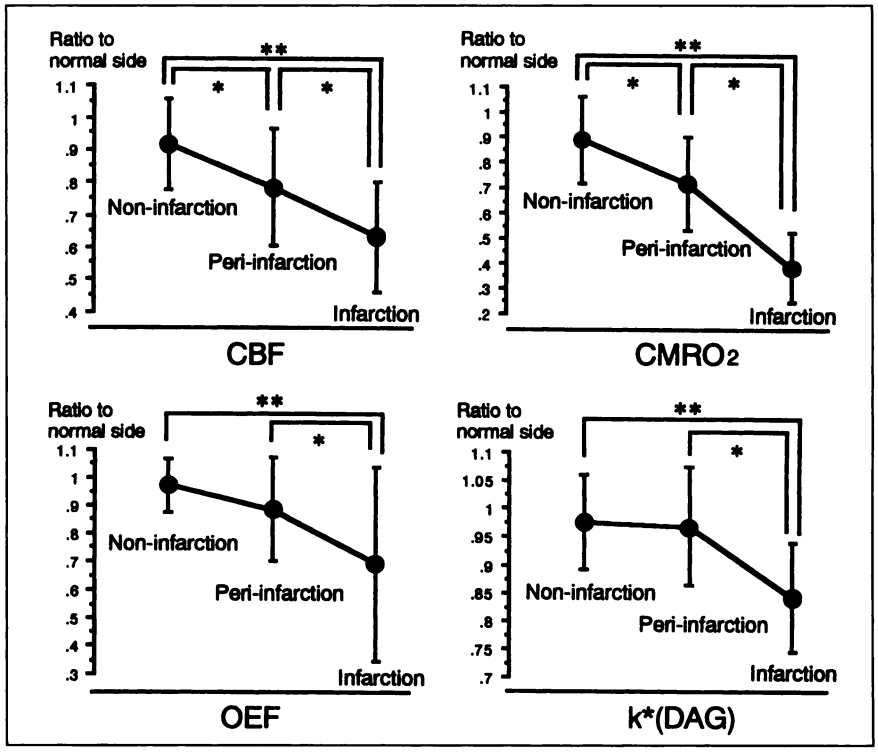
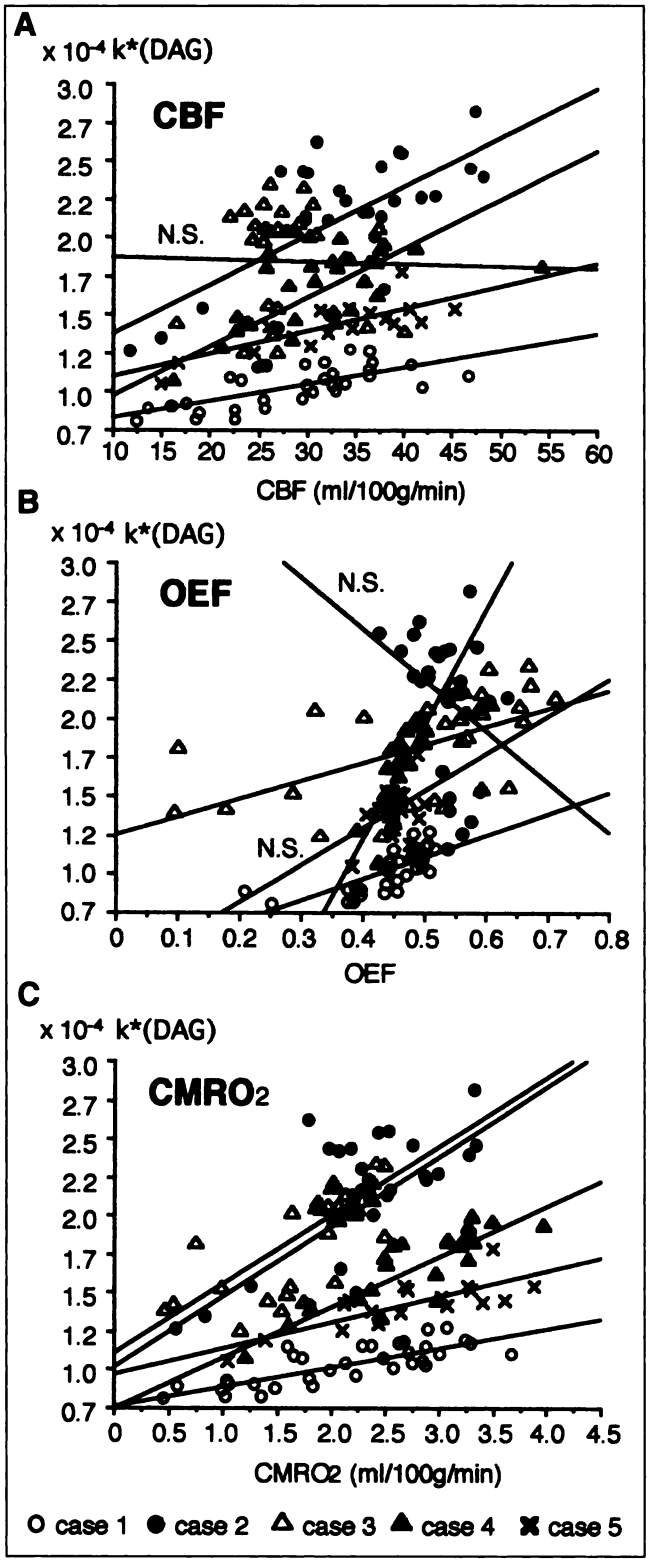


FIGURE 2. Comparison of ratio of lesion to normal side between noninfarction, peri-infarction and infarction areas. Cerebral blood flow (CBF) and cerebral metabolic rate of oxygen ($CMRO_2$) showed significant stepwise decreases from noninfarction to infarction areas, whereas incorporation constant of ^{11}C-diacylglycerol [k*(DAG)] and oxygen extraction fraction (OEF) decreased significantly only in infarction area compared with noninfarction or peri-infarction ones (*$P < 0.05$, **$P < 0.01$). Bars represent mean ± SD.

tems, this is an adequate system for evaluating synaptic activity (*6*). In PI-coupled receptor systems, phosphatidylinositol 4,5-bisphosphate is hydrolyzed by PI-specific phospholipase C, generating the two second messengers inositol trisphosphate and endogenous DAG (*7*). At steady state, both of these second messengers are actively recycled to maintain effective intracellular levels of agonist-sensitive PI (*9*). Thus, in our labeling technique using DAG, the incorporation of DAG is solely dependent on DAG kinase activity in the steady state of PI turnover (*10*), and the tracer pool can be regarded as representing integration of PI downstream from phosphatidic acid produced by DAG kinase (*4*). After developing DAG as a tracer of PI turnover, we have demonstrated the reflection of the postsynaptic activity using this tracer in many experimental systems such as spreading depression and stimulation of muscarinic receptor (*4,5,8*). In cerebral infarction, DAG accumulation was a stable parameter different from rapidly changing ones that follow circulatory changes such as CBF and OEF, and it showed the best correlation with $CMRO_2$ as a whole. These results are logical when one considers that high-energy activity is required for maintaining cerebral synaptic activity and $CMRO_2$ reflects postsynaptic activity (*11*). Therefore, k*(DAG) reflects the neuronal activity or neuronal viability from the macroscopic and global viewpoints. When these parameters were examined locally in the brain, CBF and $CMRO_2$ gradually and significantly decreased from the normal area toward the margin and the center of the infarcted area, whereas k*(DAG) significantly decreased only in the ischemic center. The constant showed almost the same level in the area surrounding infarction as in the normal brain, although a slight but not significant decrease was found. These data indicate that the signal transduction system through metabotropic receptors activating PI turnover was maintained in the brain around ischemia.



CONCLUSION

The results show that DAG PET combined with CBF and oxygen metabolism study can be a useful tool for characterizing neural activity in damaged brain after cerebral ischemia or infarction.

FIGURE 3. Correlations between incorporation constant of 1-[1-^{11}C]-butyryl-2-palmitoyl-rac-glycerol [k*(DAG)] and cerebral blood flow (CBF) (A), oxygen extraction fraction (OEF) (B) and cerebral metabolism rate of oxygen ($CMRO_2$) (C). There was significant correlation between k*(DAG) and CBF except in patient 3 (patient 1: $r = 0.681$, $P < 0.001$; patient 2: $r = 0.682$, $P < 0.001$; patient 3: $r = 0.031$, $P = 0.863$; patient 4: $r = 0.775$, $P < 0.001$; patient 5: $r = 0.777$, $P < 0.001$). There was significant correlation between k*(DAG) and $CMRO_2$ in all patients (patient 1: $r = 0.790$, $P < 0.001$; patient 2: $r = 0.707$, $P < 0.001$; patient 3: $r = 0.755$, $P < 0.001$; patient 4: $r = 0.859$, $P < 0.001$; patient 5: $r = 0.796$, $P < 0.001$). There was variable correlation between k*(DAG) and OEF (patient 1: $r = 0.730$, $P < 0.001$; patient 2: $r = 0.363$, $P = 0.058$; patient 3: $r = 0.554$, $P < 0.001$; patient 4: $r = 0.784$, $P < 0.001$; patient 5: $r = 0.429$, $P = 0.076$). Of five patients, patient 3 was examined in subacute stage and others were examined in chronic stage. N.S. = no significant correlation between parameters.

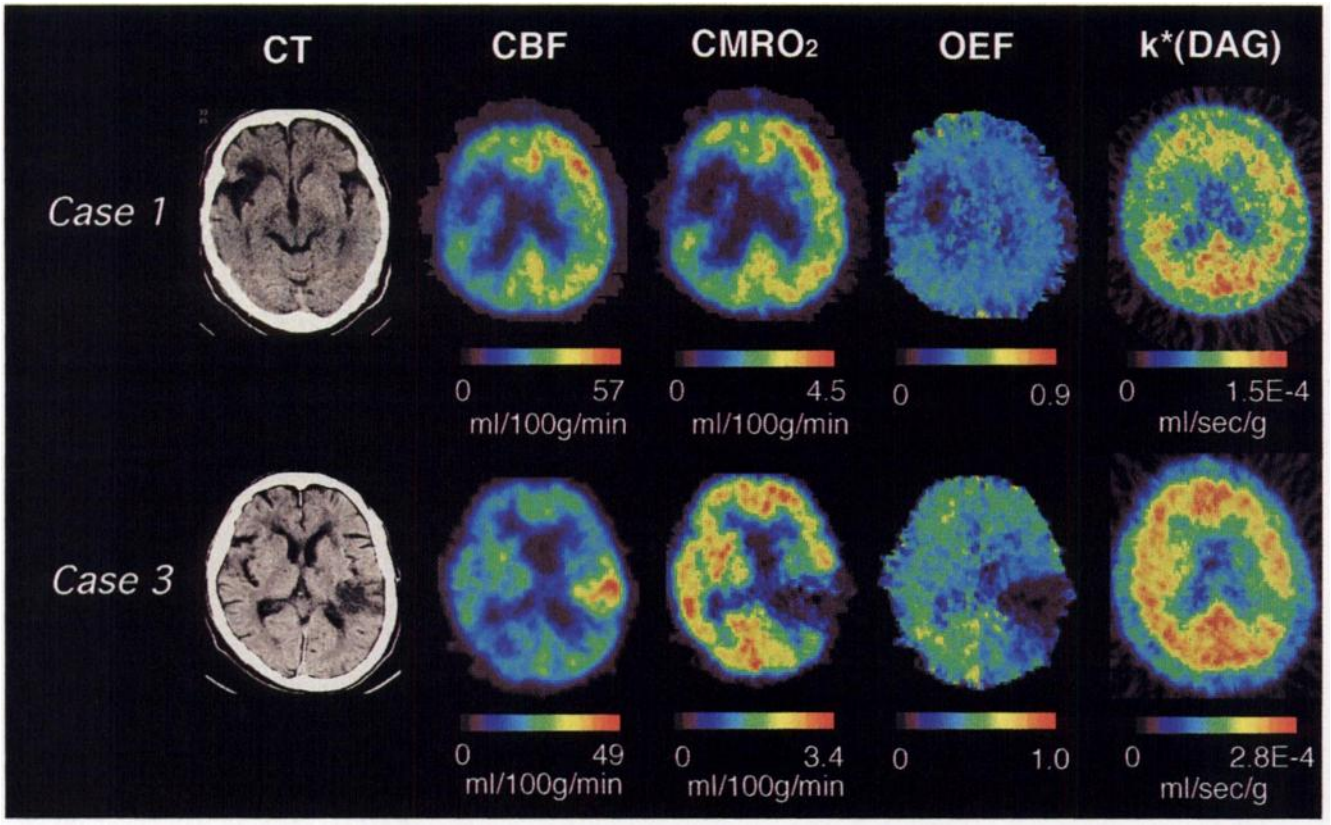


FIGURE 4. Representative PET images obtained in chronic (patient 1, top) and subacute (patient 3, bottom) stages. In chronic stage, incorporation constant of 1-[1-^{11}C]-butyryl-2-palmitoyl-rac-glycerol [k*(DAG)] decreased in ischemic core concomitant with cerebral blood flow (CBF) and oxygen metabolism. In subacute stage, there were some dissociations among circulatory and metabolic parameters; k*(DAG), cerebral metabolism of oxygen ($CMRO_2$) and oxygen extraction fraction (OEF) decreased in ischemic area, whereas CBF increased in peri-infarct area.

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