Osaka University
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Relevance of spinal neuroinflammation to neuropathic pain: an exploratory clinical study evaluated by ^{11}C-DPA-713 PET and TSPO-related biomarkers.

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Introduction and Objective

Some preclinical studies revealed that the translocator protein 18 kDa (TSPO) was upregulated in astrocytes and microglia in the ipsilateral spinal dorsal horn in the model of neuropathic pain[1-2]. A previous study has reported that ^{11}C-PK11195 PET showed increase of uptake in the corresponding spinal cord on days 7 and 14 after partial ligation of rat sciatic nerve[2]. On the other hand, some kinds of peripheral blood biomarkers have been expected as the less-invasive indicators of the glial activation related to the neuroinflammation. Plasma concentrations of oxysterols (24-OHC), which are oxidized derivatives of cholesterol, and brain-derived neuropathic factor (BDNF) are the candidates of these biomarkers[3-4]. In the present study, we evaluated spinal TSPO-PET and TSPO-related plasma biomarkers in the patients with pure neuropathic pain to validate the relevance of spinal neuroinflammation in the clinical state.

Materials and Methods

Subjects:

Healthy controls (HC): 4 males and 1 female (aged from 21 – 37 years, mean age = 24.6 ± 7.0 years).
Neuropathic patients (NP): 4 males and 2 females (aged from 56 – 84 years, mean age = 72.0 ± 10.1 years).

Diagnosis of neuropathic pain:

All patients who were suffering from pain were diagnosed by pain specialists in the pain clinic of the Osaka University Hospital, according to the guidelines established by the International Association for the Study of Pain (IASP)[5]. Figure 1 and Table 1 show the IASP flow chart of grading system for neuropathic pain and the summary of the NP, respectively.

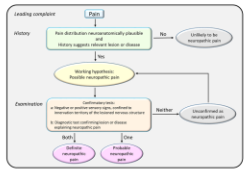

Figure 1 Flow chart of grading system for neuropathic pain[5]

Table 1 Summary of the neuropathic patients (NP)

Pt.	Age/Sex	Clinical diagnosis	Corresponding lesion of the spinal cord	Duration of illness (years)
1	64/M	Diabetic peripheral neuropathy susp.	Blt. L3-L4	7.50
2	75/M	Traumatic peripheral neuropathic pain	Lt. L3	13.92
3	84/F	Postherpetic neuralgia	Lt. L3	2.58
4	77/F	Postherpetic neuralgia	Lt. T3-T4	1.31
5	76/M	Postherpetic neuralgia	Rt. L2	0.14
6	56/M	Diabetic peripheral neuropathy susp.	Blt. L4-S1	2.00 (susp.)

Questionnaires of the pain symptoms before and after TSPO-PET:

Before TSPO-PET (pre-PET): Brief Pain Inventory (BPI), Hospital Anxiety and Depression Scale (HADS), and PainDETECT.
After TSPO-PET (post-PET): Numerical Rating Score (NRS) and PainDETECT.
<Grading of the questionnaires score>

- BPI: with 0 = no pain to 10 = pain as bad as you can image.
- HADS: 0-7 = normal, 8-10 = borderline abnormal, 11-21 = abnormal.
- PainDETECT: 0-12 = unlikely neuropathic pain (nociceptive pain), 13-18 = unclear, 19-38 = likely neuropathic pain.
- NRS: 0 = no pain, 10 = worst pain imaginable.

Measurements of TSPO-PET and TSPO-related biomarkers:

Three-dimensional PET/CT scanner: Eminence BCT/X (Shimadzu Corporation., Kyoto, Japan).
Scan protocol: after intravenous injection of ^{11}C-DPA-713 (529.6 ± 28.0 MBq), a PET examination of the corresponding spinal lesion was conducted from 48 to 60 minutes.
TSPO-related plasma biomarkers: venous blood sampling (3 mL) for the measurement of plasma concentration of 24-OHC, pro BDNF, and mature BDNF.
Genetic polymorphisms (rs6971) : venous blood sampling (2 mL) for the measurement of TSPO Ala147Thr genotype.

Data analysis and Statistics:

VOI analysis: A VOI of the size of 15 mm × 40 mm was located on the reference area of HC (T10-11 level of the spinal cord) and the corresponding spinal cord of NP on ^{11}C-DPA-713 PET image, respectively.
The following analyses were conducted.

1) Significant difference between the pre-PET and post-PET : averaged PainDETECT score of NP (paired t-test).
2) Significant differences between HC (n = 5) and NP (n = 6) : spinal SUVmax, SUVmean, and plasma concentration of TSPO-related biomarkers (unpaired t-test).
3) Significances of relationships (n = 11): spinal SUVmax or SUVmean vs plasma concentrations of TSPO-related biomarkers, or vs PainDETECT score of the pre-PET, or vs duration of illness.

Results

Table 2 Results of the questionnaires of the pain symptoms of NP before and after PET examination

NP No.	Pre-PET								Post-PET	
	BPI					HADS		PainDETECT	NRS	PainDETECT
	Worst	Least	Average	Now	Relief (%)*	Depression (D)	Anxiety (A)		Now	
1	3	3	3	3	20	6	8	14	3	10
2	7	3	7	7	50	10	14	24	6	23
3	7	7	7	7	0	6	12	19	3	13
4	8	6	7	8	0	19	16	28	7	29
5	7	4	5	5	20	15	17	11	5	11
6	5	2	5	5	20	17	15	17	5	18
Average	6.2	4.2	5.7	5.8	18.3	12.2	13.7	18.8	4.8	17.3
SD.	1.8	1.9	1.6	1.8	18.3	5.6	3.3	6.3	1.6	7.5

*: The percentage of pain relief which the patient has received by the treatment or medications in the last 24 hours.

Table 3 Values of spinal ^{11}C-DPA-713 uptake and plasma concentrations of TSPO-related biomarkers

Group	No.	^{11}C-DPA-713 PET		Plasma concentration (ng/mL)		
		SUVmax	SUVmean	24-OHC	Pro BDNF	Mature BDNF
HC	1	1.39	1.17	35.9	7.65	19.3
	2	1.64	1.08	33.8	7.98	21.0
	3	1.43	1.07	24.5	6.59	16.8
	4	1.48	1.03	27.0	9.16	17.0
	5	1.26	0.94	34.4	9.62	21.7
	Average	1.44	1.06	31.1	8.20	19.2
	SD.	0.14	0.08	5.0	1.21	2.3
NP	1	1.84	1.45	27.7	9.93	19.2
	2	1.29	1.07	28.9	9.92	22.8
	3	1.29	0.88	33.5	9.19	19.8
	4	1.01	0.82	28.4	8.91	18.0
	5	1.32	1.03	29.0	8.22	21.3
	6	2.10	1.57	24.8	8.04	19.7
	Average	1.47	1.14	28.7	9.04	20.1
	SD.	0.41	0.31	2.8	0.81	1.7

Genetic polymorphisms: One of the HC was diagnosed as a mixed-affinity binder (MAB). The rest 4 HC and all 6 NP were high-affinity binders (HABs).

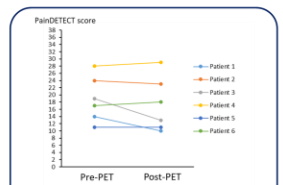

Figure 2 Change of PainDETECT score of NP

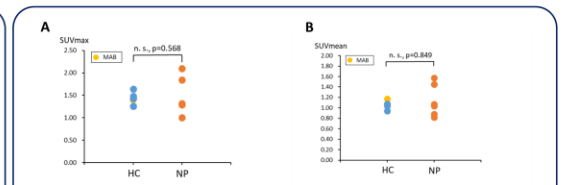

Figure 3 Spinal SUVmax (A) and SUVmean (B) of HC (n=5) and NP (n=6).

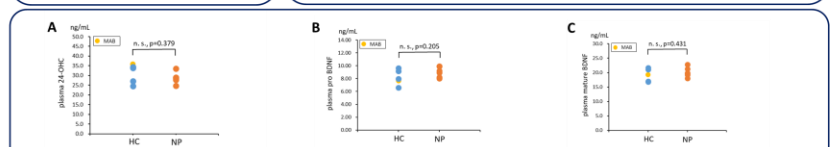

Figure 4 Plasma concentration of 24-OHC (A), pro BDNF (B), and mature BDNF (C) of HC (n=5) and NP (n=6).

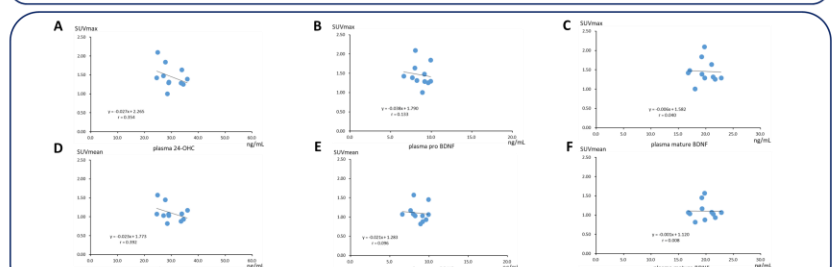

Figure 5 Relationships between plasma concentration of TSPO-related biomarkers and spinal SUVmax (A-C), and between SUVmean (D-F) (n=11).

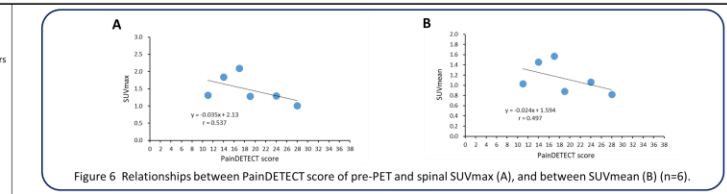

Figure 6 Relationships between PainDETECT score of pre-PET and spinal SUVmax (A), and between SUVmean (B) (n=6).

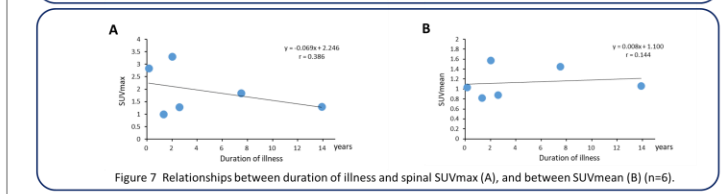

Figure 7 Relationships between duration of illness and spinal SUVmax (A), and between SUVmean (B) (n=6).

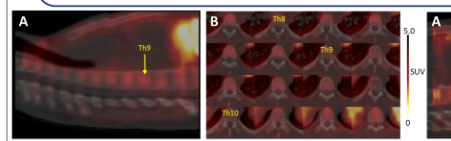

Figure 8 Sagittal (A) and axial (B) ^{11}C-DPA-713 PET image in a healthy control (Subject 2, spinal T10-11 level).

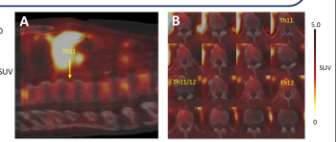

Figure 9 Sagittal (A) and axial (B) ^{11}C-DPA-713 PET image in a patient with postherpetic neuralgia (Patient 5, spinal L2 level).

Summary of results

1) No definite abnormal uptakes were observed in the spinal cord of NP (Figure 9).
2) No significant difference between the pre-PET and post-PET in the averaged PainDETECT score of NP.
3) No significant differences between HC and NP in the spinal ^{11}C-DPA-713 uptake, and plasma 24-OHC and BDNF.
4) No significances of relationships between the spinal SUV vs plasma 24-OHC and BDNF (r = 0.008-0.392, n= 11), or vs PainDETECT score of the pre-PET (r = 0.497-0.537, n= 6), or vs duration of illness (r = 0.144-0.386, n=6).

Discussion and Conclusion

There are several discussions and limitations in the present study as follows: limited spatial resolution of the PET scanner, limited sensitivity of the PET scanner/plasma biomarkers against a weak spinal glial activation, study populations without age matching, and differences between preclinical and clinical pathophysiology as for the duration of the illness. The present patients, however, shows refractory neuropathic pain throughout their disease. Other pathophysiological background except neuroinflammation, therefore, should be considered to explain their persistent pain symptoms. The present clinical exploratory study revealed that no definite evidence of spinal neuroinflammation was observed by TSPO-PET in the pure neuropathic pain. Therapeutic intervention for inhibition of spinal microglia and astrocyte activation may not be effective to relieve the symptom from neuropathic pain.

Acknowledgements

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