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• LIVER CANCER •

Increased nociceptin/orphanin FQ plasma levels in hepatocellular carcinoma

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Abstract

AIM: The heptadecapeptide nociceptin alias orphanin FQ is the endogenous agonist of opioid receptor-like1 receptor. It is involved in modulation of pain and cognition. High blood level was reported in patients with acute and chronic pain, and in Wilson disease. An accidental observation led us to investigate nociceptin in hepatocellular carcinoma.

METHODS: Plasma nociceptin level was measured by radioimmunoassay, aprotinin was used as protease inhibitor. Hepatocellular carcinoma was diagnosed by laboratory, ultrasound, other imaging, and confirmed by fine needle biopsy. Results were compared to healthy controls and patients with other chronic liver diseases.

RESULTS: Although nociceptin levels were elevated in patients with Wilson disease (14.0±2.7 pg/mL, n=26), primary biliary cirrhosis (12.1±3.2 pg/mL, n=21) and liver cirrhosis (12.8±4.0 pg/mL, n=15) compared to the healthy controls (9.2±1.8 pg/mL, n=29, P<0.001 for each), in patients with hepatocellular carcinoma a ten-fold increase was found (105.9±14.4 pg/mL, n=29, P<0.0001). High plasma levels were found in each hepatocellular carcinoma patient including those with normal alpha fetoprotein and those with pain (104.9±14.9 pg/mL, n=12) and without (107.7±14.5 pg/mL, n=6).

CONCLUSION: A very high nociceptin plasma level seems to be an indicator for hepatocellular carcinoma. Further research is needed to clarify the mechanism and clinical significance of this novel finding.

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INTRODUCTION

The heptadecapeptide nociceptin (N/OFQ), alias orphanin FQ, is the endogenous agonist ligand of a G-protein-coupled, naloxon insensitive opioid receptor-like 1 receptor (ORL1), recently named as NOP[1]. Although N/OFQ is structurally related to opioid peptides, especially to dynorphin A, it does not interact with μ, δ and κ receptors. The nociceptin/NOP system represents a new peptide-based signalling pathway. Nociceptin is involved in a number of pharmacological actions in the central nervous system (CNS), including modulation of pain and cognition. However, numerous studies investigating the functional role of nociceptin in physiology have failed to provide coherent view, and its exact physiological role remains to be determined[2,3]. Although N/OFQ is produced by some brain structure and peripheral neurons, it is present in the liquor and blood[4], and recent data prove that nociceptin transcripts are expressed in human immune cells as well[5]. NOP mRNA is expressed not only in nervous system, but in immune cells and other organs including the liver[6-8]. High nociceptin blood level was shown in patients with acute and chronic pains[9], and Wilson disease[10].

An accidental observation led us to investigate plasma nociceptin level in patients with hepatocellular carcinoma (HCC). While measuring plasma nociceptin in patients with Wilson disease, we noticed that in one patient the nociceptin level was extremely high compared both with the controls and other Wilson patients. This patient had liver cirrhosis and primary hepatocellular carcinoma without any pain. This observation prompted us to study N/OFQ in patients with hepatocellular carcinoma and other liver diseases. Striking differences were found.

MATERIALS AND METHODS

Patients

Plasma nociceptin level was measured in 26 patients with Wilson disease (aged from 14-55 years, 11 with hepatic, and 15 with neurological symptoms, each D-penicillamine treated), 21 patients with primary biliary cirrhosis (age ranged from 36-72 years; each woman with AMA M2 positive, histologically proven and treated with ursodeoxycholic acid; mean disease duration: 9.4 years), 18 patients with chronic hepatitis (14 HCV positive, 1 HBV positive and 3 autoimmune, each proved by liver biopsy), 15 patients with liver cirrhosis (9 alcoholic, 6 HCV positive), and 18 patients with primary hepatocellular carcinoma (8 with alcoholic cirrhosis, 6 HCV cirrhosis, 1 HBV cirrhosis, 1 Wilson disease, 1 PBC, and 1 patient without any underlying liver disease) from the Hepatological Unit, the 1st Department of Medicine, the Semmelweis University, Budapest. The diagnosis of HCC was based on clinical laboratory tests, US, CT, MRI findings and was confirmed by fine needle aspiration cytology, and histology, in which 3 cases underwent surgery, and one case by autopsy. Serum alpha fetoprotein (AFP) was elevated in 11 out of 18 HCC patients. The size of the tumour ranged from 2.5 cm to 12 cm in diameter. It was smaller than 5 cm in 5 patients, and larger than 5 cm in 13 patients. No metastasis was found outside the liver. In the HCC group 12 patients had temporary pain treated with non-opioid analgetics and 6

patients were without any pain.

Two scoring systems were used for characterisation of patients with HCC. The distribution of patients according to the Barcelona Clinic Liver Cancer (BCLC) classification[11], which includes the performance status, single or multifocal appearance of the tumor, vascular invasion, portal hypertension, Okuda stage and Child-Pugh classification: Stage A1 (n=5), stage A4 (n=6), stage B (n=3) and stage D (n=4). Ranking of patients according to the Cancer of the Liver Italian Program group (CLIP) criteria[12,13], which includes Child-Pugh stage, tumor morphology and extent, presence of portal vein thrombosis and serum level of alpha fetoprotein: CLIP 0 (n=2), CLIP 1 (n=5), CLIP 2 (n=5), CLIP 3 (n=4), CLIP 4 (n=1), CLIP 5 (n=1). Demographics, clinical data and ranking of HCC patients according to the BCLC and CLIP classification are shown in Table 1.

Twenty-nine healthy persons including blood donors and members of the medical staff served as control group. The study was approved by the Local Regional Committee of Science and Research Ethics. Written informed consent was obtained.

Methods

Blood drawn from fasting subjects between 08:00 and 10:00 AM was collected in vacutainer tubes containing K-EDTA as anticoagulant. Aprotinin was added immediately as protease inhibitor. Plasma samples were stored at minus 80 ℃. Nociceptin was measured by radioimmunoassay (^{125}I-Nociceptin kit, Phoenix Pharmaceuticals, Phoenix, CA, USA) with minimum sensitivity of 1 pg/mL, as described before[9]. Comparison of the plasma N/OFQ concentration in groups was made using Mann-Whitney U Test. Correlation between N/OFQ level and liver function test results was evaluated by Spearmann RO correlation.

RESULTS

Results are shown in Figure 1. Although nociceptin levels were elevated in patients with Wilson disease (14.0±2.7 pg/mL), in patients with PBC (12.1±3.2 pg/mL) and liver cirrhosis (12.8±4.0 pg/mL) compared to the healthy controls (9.2±1.8 pg/mL, P<0.001 for each), more than ten-fold higher values were found (105.9±14.4 pg/mL, P<0.0001) in patients with HCC. In patients with chronic hepatitis the N/OFQ level was 10.2±3.6, but the difference was not significant compared to the healthy controls. The clinical data, chemical laboratory findings, AFP and N/OFQ levels of patients with HCC are individually indicated in Table 1.

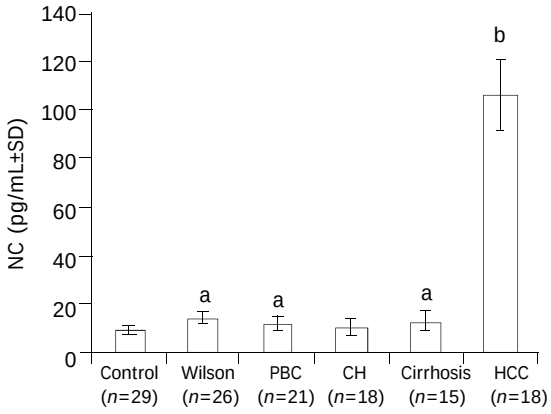

Figure 1 Plasma nociceptin level (pg/mL±SD) in healthy controls (Control) and in patients with Wilson disease (Wilson), primary biliary cirrhosis (PBC), chronic hepatitis (CH), cirrhosis and hepatocellular carcinoma (HCC). [a]P<0.001 *vs* control, [b]P<0.0001 *vs* control and each other group.

We did not find any correlation between nociceptin level and liver chemical laboratory tests including AFP in any group of patients with liver disease. Nociceptin was elevated in each HCC patient including those with normal AFP. The N/OFQ level did not show correlation with the presence and severity of the pain. High levels were found in subgroups of patients with pain (104.9±14.9 pg/mL, n=12) and without pain (107.7±14.5 pg/mL, n=6). There was no difference in nociceptin level between groups with tumor size smaller than 5 cm and larger than 5 cm (109.4±16.5, n=5 and 115.2±12.7, n=13,

Table 1 Profile of patients with HCC and plasma N/OFQ levels

	Age y	Gender	Aetiology	AFP (ng/mL)	Tumor size in UH	Uni-nod.	Multi-nod.	BCLC st.cl.	CLIP cl.	Bi (mg/dL)	ALP (U/L)	GGT (U/L)	Ascites	C-P	N/OFQ (pg/mL)
1	80	M	Alcoholic	937	15×11 cm	+		A 4	3	1.72	306	102	-	A	108.39
2	64	M	Alcoholic	1 385.9	5×7×6 cm	+		A 1	1	1.92	242	107	-	A	95.02
3	60	M	Alcoholic	89	5 -15 mm and 3 cm		+	B	2	1.07	2 169	1 000	-	B	96.05
4	62	M	Alcoholic	91	1-1.5 cm and 3.5 cm		+	B	2	1.12	2 038	995	-	B	104.17
5	80	M	Alcoholic	937	15×11 cm	+		A 1	3	1.71	306	102	-	A	111.59
6	70	M	Alcoholic	459	7×6 cm	+		A 1	2	0.78	192	68	-	A	111.51
7	59	M	Alcoholic	7.7	23 mm	+		D	2	8.32	44.3	97	-	C	128
8	66	M	Alcoholic	2	1.5 cm	+		A 1	0	1.25	192	24	-	A	81.6
9	48	F	HCV	15	6×6 cm	+		A 4	1	5.75	1 766	253	-	B	90.41
10	66	M	HCV	171	two, 2×2 cm lesion		+	A 4	1	2.55	460	232	-	A	89.81
11	76	M	HCV	9	6×7 cm + 5×4 cm		+	D	4	13.91	834	276	+	C	114.65
12	78	M	HCV	350	5×6 cm	+		A 1	0	0.68	270	37	-	A	114.7
13	48	F	HCV	13	21×20 mm and 16×21 mm		+	B	1	1.36	474	1 060	-	A	128
14	81	M	HCV	1 050	8×10 cm	+		A 4	3	3	569	517	-	A	98.31
15	66	M	HBV	6	4×4.5 cm	+		D	2	3.35	442	199	+	C	106.03
16	45	F	WD	5	60×46 mm	+		A 4	1	0.42	910	212	+	B	128
17	68	F	PBC	3 480	11×7.5 cm	+		D	5	4.79	1 856	960	+	C	172.2
18	32	F	Unknown	1 050	100×85×180 mm	+		A 4	3	0.34	233	24	-	-	85.5

AFP=alpha fetoprotein, BCLC st. cl.=Barcelona Clinic Liver Cancer (BCLC) staging classification, CLIP cl.=Cancer of the Liver Italian Program Group (CLIP) scoring system, Bi=Serum total bilirubin, ALP=alkaline phosphatase, GGT=gamma glutamyl transferase, C-P=Child-Pugh score, N/OFQ=nociceptin/orphanin FQ.

respectively) or between single nodular and multinodular HCC groups, and even symptomatic or asymptomatic tumors. No difference was found in the N/OFQ level between any subgroup of patients with different BCLC and CLIP score values.

Repeated nociceptin measurements were done in one patient before and after non-surgical ablation treatment of HCC. The high nociceptin level did not decrease significantly at least within 10 days following ethanol injection treatment of the tumor, since values were 116 pg/mL before, and 110, 108, 112, 103 pg/mL after treatment at 1, 3, 7, and 10 days, respectively.

However, progressive and significant elevation in plasma nociceptin level parallel with the increase of AFP was observed in a PBC patient with HCC during the follow up. This 67 year old patient was monitored and treated with ursodeoxycholic acid throughout 18 years when her liver tumor was detected by yearly regular ultrasound check up. Fine needle aspiration cytology proved HCC, and she died within two years. Nociceptin was measured in blood samples collected from other reason. The AFP level was normal throughout the years, but elevated at the time of the diagnosis of HCC (426 ng/mL) and rose up to 3 480 ng/ml. The plasma nociceptin (10.6 pg/mL) was within the normal range (9.2±1.8 pg/mL) in the tumor free stage, ten-fold higher (103.7 pg/mL) when the tumor was diagnosed and reached 172.2 pg/mL before the death. Sixteen-fold higher nociceptin content was measured in the tumor tissue (0.16 pg/mg) compared to the tumor-free liver tissue sample (0.01 pg/mg) taken during the autopsy. Detailed presentation of this case is published in this issue of World Journal of Gastroenterology (Horvath *et al* World J Gastroenterol 2004; 10: 152-154)

DISCUSSION

Shortly after cloning of the three known opioid receptors a fourth member of the subfamily of G protein-coupled receptors was identified. In a search for additional opioid receptor subtypes, a sequence of an opioid-like receptor, termed ORL1 was found, which, however, did not bind any of the known natural opioid ligands. Therefore ORL1 represented an "orphan" receptor. Later two working groups simultaneously reported the isolation of the natural ligand for ORL1, a 17 aminoacid polypeptide and named it orphanin FQ or nociceptin[14,15].

Anatomic studies have revealed high levels of expression of N/OFQ messenger RNS in brain structures involved in sensory, emotional and cognitive processing. Like all other neuropeptides, the heptadecapeptid N/OFQ is synthesized as a part of a larger precursor named prepro-nociceptin, a 176 aminoacid polypeptide in human, from which N/OFQ and nocistatin are cleaved in brain cells and peripheral neurons[16,17]. Although nociceptin is present in the brain, the liquor and blood, human immune cells and polymorphonuclear cells, the gastrointestinal tract and other organs, there are no data on N/OFQ or its receptor in malignant diseases with exception of human neuroblastoma cell lines[18,19]. This is the first report focusing on nociceptin in hepatocellular carcinoma and other liver diseases.

The novel finding of this study is the striking elevation in plasma N/OFQ level in patients with hepatocellular carcinoma. Although nociceptin was elevated in patients with Wilson disease, primary biliary cirrhosis and liver cirrhosis, very high levels over ten-fold increase compared with healthy controls was found in HCC. Since nociceptin level was extremely high in each patient with HCC, and the difference among HCC patients and groups of patients with other liver diseases was highly significant ($P<0.0001$), the very high plasma level of nociceptin seems to be an indicator for HCC. It is remarkable that nociceptin level was very high in even those patients with normal level of alpha-fetoprotein[20]. The size of HCC is an important element of prognostic assessment[21]. However, nociceptin level was equally very high in patients with tumor size smaller and larger than 5 cm in diameter.

Further research is needed to clarify the mechanism and clinical significance of the highly elevated nociceptin level in HCC. Increased production or decreased catabolism of nociceptin may be the cause of elevation in plasma level. It is to be determined whether the tumor produces nociceptin or it gives signal for brain cells or peripheral neurons to increase the secretion of N/OFQ. We had the opportunity to determine the tissue nociceptin content in one PBC patient with HCC. Although more than 15-fold higher nociceptin content was measured in the carcinoma tissue than in the tumor-free liver tissue samples taken during autopsy, it did not necessarily mean that nociceptin was produced by the tumor. It is also possible that the tumor simply accumulates nociceptin via binding it by NOP receptors. This possibility could be supported by the fact that ORL1/NOP receptor mRNA has been detected in the liver[22], although no study is yet available on nociceptin or its receptor mRNA expression in hepatocellular carcinoma tissue.

Nociceptin is involved in the processing of pain signals, and it modulates the pain perception[23,24]. However, we did not find difference in N/OFQ level between patients with pain and without. There was no correlation between the plasma N/OFQ level and the severity of pain.

Whether a very high plasma N/OFQ level is a specific marker of HCC should be investigated and subjected to further study. Since nociceptin transcripts are expressed in immune cells[5], it has also been shown that polymorphonuclear cells express nociceptin receptors and N/OFQ stimulates neutrophil chemotaxis and recruitment[25]. The high nociceptin level could also be an indicator of altered reaction of the body including immunological, cytokine and other mechanisms. The bacterial endotoxin (LPS) and proinflammatory cytokines including TNF-alpha, commonly increased in malignancies, induce N/OFQ mRNS in astrocytes. It may suggest a role for nociceptin in neural-glial communication and in inflammatory responses[26].

In humans, the N/OFQ gene has been mapped to the chromosomal location 8p21[27]. Transcription of this gene was shown to be enhanced by cytokines, neurotrophic factors[26], also by estrogen[28]. This cAMP-dependent transcription could be blocked by glucocorticoids[19]. Our patients in this study have not received glucocorticoid treatment, and only one PBC patient used transdermal estrogen hormone replacement for osteoporosis prevention.

Elevated N/OFQ level might partly represent a compensatory mechanism in the nociceptin/NOP system to modulate pain perception in the central nervous system. That mechanism might explain why some patients with a very high plasma N/OFQ level did not have any pain despite advanced stage of the malignant liver tumor. It is remarkable that nociceptin in HCC patients was 3-fold higher than the highest values reported in patients with chronic pain without malignant diseases[9]. We believe that our results could stimulate further investigation.

REFERENCES

1 **Calo' G**, Rizzi A, Bigoni R, Guerrini R, Salvadori S, Regoli D. Pharmacological profile of nociceptin / orphanin FQ receptors. *Clin Exp Pharmacol Physiol* 2002; **29**: 223-228

2 **Terenius L**, Sandin J, Sakurada T. Nociceptin / orphanin FQ metabolism and bioactive metabolites. *Peptides* 2000; **21**: 919

3 **Heinricher MM**. Orphanin FQ / nociceptin: from neural circuity to behavior. *Life Sci* 2003; **73**: 813-822

4 **Brooks H**, Elton CD, Smart D, Rowbotham DJ, McKnight AT, Lambrt DG. Identification of nociceptin in human cerebrospinal fluid: comparison of level in pain and non-pain states. *Pain* 1998; **78**: 71-73

5 **Arjomand J**, Cole S, Evans C. Novel orphanin FQ / nociceptin transcripts are expressed in human immune cells. *J Neuroimmunol* 2002; **130**: 100

6 **Peluso J**, Gaveriaux-Ruff C, Matthes HW, Filliol D, Kieffer BL. Orphanin FQ / nociceptin binds functionally coupled ORL1 receptors on human immune cell lines and alters peripheral blood mononuclear cell proliferation. *Brain Res Bull* 2001; **54**: 655-660

7 **Peluso J**, LaForge KS, Matthes HW, Kreek MJ, Kieffer BL, Gaveriaux-Ruff C. Distribution of nociceptin / orphanin FQ receptor transcript in human central nervous system and immune cells. *J Neuroimmunol* 1998; **81**: 184-192

8 **Reinscheid RK**, Nothacker HP, Civelli O. The orphanin FQ / nociceptine gene: structure, tissue distribution of expression and functional implications obtained from knockout mice. *Peptides* 2000; **21**: 901-906

9 **Ko MH**, Kim YH, Woo RS, Kim KW. Quantitative analysis of nociceptin in blood of patients with acute and chronic pain. *Neuroreport* 2002; **13**: 1631-1633

10 **Hantos MB**, Szalay F, Lakatos PL, Hegedûs D, Firneisz G, Reiczigel J, Torok T, Tekes K. Elevated plasma nociceptin level in patients with Wilson disease. *Brain Research Bulletin* 2002; **58**:311-313

11 **Llovet JM**, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999; **19**: 329-337

12 **The Cancer of the Liver Italian Program (CLIP) Investigators**. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients. *Hepatology* 1998; **28**: 751-755

13 **Zhao WH**, Ma ZM, Zhou XR, Feng YZ, Fang BS. Prediction of recurrence and prognosis in patients with hepatocellular carcinoma after resection by use of CLIP score. *World J Gastroenterol* 2002; **8**: 237-242

14 **Reinscheid RK**, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ Jr, Civelli O. Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. *Science* 1995; **270**: 792-794

15 **Meunier JC**, Mollereau C, Toll L, Suandeau C, Moisand C, Alvineire P, Butour JL, Guillemot JC, Ferrara P, Monserrat B. Isolation and structure of the endogenous agonist of opioid receptor-like ORL-1 receptor. *Nature* 1995; **377**: 532-535

16 **Okuda-Ashitaka E**, Ito S. Nocistatin: a novel neuropeptide encoded by the gene for the nociceptin / orphanin FQ precursor. *Peptides* 2000; **21**: 1101-1109

17 **Okuda-Ashitaka E**, Minami T, Tachibana S, Yoshihara Y, Nishiuchi Y, Kimura T, Ito S. Nocistatin, a peptide that blocks nociceptin action in pain transmission. *Nature* 1998; **392**: 286-289

18 **Spampinato S**, Di Toro R, Qusem AR. Nociceptin-induced internalization of the ORL1 receptor in human neuroblastoma cells. *Neuroreport* 2001; **12**: 3159-3163

19 **Sirianni MJ**, Fujimoto KI, Nelson CS, Pellegrino MJ, Allen RG. Cyclic AMP analogs induce synthesis, processing and secretion of prepro nociceptin / orphanin FQ-derived peptides by NS20Y neuroblastoma cells. *DNA Cell Biol* 1999; **18**: 51-58

20 **Qin LX**, Tang ZY. The prognostic significance of clinical and pathological features in hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 193-199

21 **Bruix J**, Llovet JM. Prognostic assessment and evaluation of the benefits of treatment. *J Clin Gastroenterol* 2002; **35**(Suppl 2): S138-142

22 **Wang JB**, Johnson PS, Imai Y, Persico AM, Ozenberger BA, Eppler CM, Uhl GR. cDNA cloning of an orphan opiate receptor gene family member and its splice variant. *FEBS Lett* 1994; **348**: 75-79

23 **Inoue M**, Kawashima T, Takeshima H, Calo G, Inoue A, Nakata Y, Ueda H. *In vivo* pain-inhibitory role of nociceptin / orphanin FQ in spinal cord. *J Pharmacol Exp Ther* 2003; **305**: 495-501

24 **Meunier JC**. Utilizing functional genomics to identify new pain treatments: the example of nociceptin. *Am J Pharmacog* 2003; **3**: 117-130

25 **Serhan CN**, Fierro IM, Chiang N, Pouliot M. Cutting edge: Nociceptin stimulates neutrophil chemotaxis and recruitment: inhibition by aspirin-triggered-15-epi-lipoxin A_4. *J Immunol* 2001; **166**: 3650-3654

26 **Buzas B**, Symes AJ, Cox BM. Regulation of nociceptin / orphanin FQ gene expression by neuropoietic cytokines and neurotrophic factors in neurons and astrocytes. *J Neurochem* 1999; **72**: 556-563

27 **Mollereau C**, Simons MJ, Soularue P, Liners F, Vassart G, Meunier JC, Parmentier M. Structure, tissue distribution and chromosomal localization of the prepronociceptin gene. *Proc Natl Acad Sci U S A* 1996; **93**: 8666-8670

28 **Xie GX**, Ito E, Maruyama K, Suzuki Y, Sugano S, Sharma M, Pietruck C, Palmer PP. The promoter region of the human prepronociceptin gene and its regulation by cyclic AMP and steroid hormones. *Gene* 1999; **238**: 427-436

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