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Hepatitis C virus infection: a risk factor for Parkinson's disease

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SUMMARY. Recent studies found that hepatitis C virus (HCV) may invade the central nervous system, and both HCV and Parkinson's disease (PD) have in common the overexpression of inflammatory biomarkers. We analysed data from a community-based integrated screening programme based on a total of 62 276 subjects. We used logistic regression models to investigate association between HCV infection and PD. The neurotoxicity of HCV was evaluated in the midbrain neuron–glia coculture system in rats. The cytokine/chemokine array was performed to measure the differences of amounts of cytokines released from midbrain in the presence and absence of HCV. The crude odds ratios (ORs) for having PD were 0.62 [95% confidence interval (CI), 0.48–0.81] and 1.91 (95% CI, 1.48–2.47) for hepatitis B virus (HBV) and HCV. After controlling for potential confounders, the association between HCV and PD remained statistically significant (adjusted OR = 1.39; 95% CI, 1.07–1.80), but not significantly different between HBV and PD. The HCV induced 60% dopaminergic neuron death in the midbrain neuron–glia coculture system in rats, similar to that of 1-methyl-4-phenylpyridinium (MPP^+) but not caused by HBV. This link was further supported by the finding that HCV infection may release the inflammatory cytokines, which may play a role in the pathogenesis of PD. In conclusion, our study demonstrated a significantly positive epidemiological association between HCV infection and PD and corroborated the dopaminergic toxicity of HCV similar to that of MPP^+.

Keywords: community, hepatitis C, neurotoxicity, Parkinson's disease, risk factor.

INTRODUCTION

Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic neurons in the substantia nigra, accompanied by the accumulation of α-synuclein aggregates in Lewy bodies [1]. Although the cause of PD remains unclear, it has been shown that numerous viruses are associated with both acute and chronic parkinsonism including influenza, Coxsackie, Japanese encephalitis (JE), western equine encephalitis, herpes and acquired immunodeficiency disorder (HIV). These viruses are neurotropic and they may induce a number of encephalopathies that lead to parkinsonism [2]. Hepatitis C virus (HCV) belongs to the flaviviridae family, which includes well-known neurotropic viruses, such as JE, yellow fever, dengue and tick-borne encephalitis viruses [3]. Recent studies suggest that HCV may invade the central nervous system (CNS). Such neuroinvasive harm shows sign of evidence that patients

Abbreviations: BBB, blood–brain barrier; CI, confidence interval; CNS, central nervous system; HBV, hepatitis B virus; HCV, hepatitis C virus; JE, Japanese encephalitis; KCIS, Keelung community-based integrated screening; LIX, LPS−induced CXC chemokine; MMP, matrix metalloproteinase; OR, odds ratio; PCR, polymerase chain reaction; PD, Parkinson's disease; RANTES, activation normal, T cell expressed and secreted; TH, tyrosine hydroxylase; TIMP-1, tissue inhibitors of metalloproteinases-1; TNF, tumour necrosis factor.

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with mild chronic HCV infection had elevated choline/creatine ratios, a biomarker indicating inflammatory and infective conditions, in the basal ganglia and white matter [4]. Moreover, a viral replicative intermediate of HCV RNA has been found in autopsy brain tissue and activation of macrophages/microglial cells has been found in HCV-positive patients [5,6]. In addition, alteration of striatal dopaminergic neurotransmission has been reported in HCV-infected patients [7]. The evidence that HCV can replicate in the CNS suggests a possible link between PD and HCV.

The association between HCV infection and the pathogenesis of PD is also supported by both HCV infection and PD having in common the overexpression of inflammatory biomarkers that are related to rising concentrations of cytokines in neuronal generation processes, such as abnormal protein handling, oxidative stress, mitochondrial dysfunction, excitotoxicity and apoptotic processes [6].

Despite this speculation, it is rare and difficult to have available information on HCV infection and PD simultaneously in a population- and community-based epidemiological study to assess this hypothesis. Thus, at population and epidemiology level, we attempted to assess whether HCV infection was associated with PD using data from the Keelung community-based integrated screening (KCIS) programme with information on HCV infection, diagnosis of PD and other confounding factors available [8]. At the molecular level, we investigated the dopaminergic toxicity of HCV and compared that of 1-methyl-4-phenylpyridinium (MPP^+), the pathognomonic chemical in experimental parkinsonism study. Furthermore, the differences in the relative amounts of cytokines released from midbrain in the presence and absence of virus were measured by cytokine/chemokine array to investigate the pathogenesis of PD.

MATERIALS AND METHODS

Study population and community-based model for ascertained PD

The study population was derived from a community-based integrated screening programme in Keelung (KCIS), the northernmost area in Taiwan [8]. A total of 63 163 subjects aged 40 or older were enrolled between 2000 and 2004. The ascertainment of PD was either through active community-based survey or hospital-based clinical series cases. The medical record consisted of information on medical visits, such as ICD codes, date of visit and prescriptions. The three major diagnostic codes were kept for financial reimbursement for all treatments, therapies and prescriptions. The major diagnostic code 332.0 was used to identify patients with PD and excluded parkinsonism caused by other reasons, such as vascular disease-related parkinsonism, drug-induced parkinsonism, multiple system atrophy and parkinsonism secondary to brain insults. Finally, 887 PD cases were found.

Serum viral markers and biochemical variables

In the KCIS programme, HBsAg was detected using radioimmunoassay kits (Abbott Laboratories, Chicago, IL, USA), and anti-HCV was detected using a third-generation enzyme immunoassay (Abbott Laboratories). Venous blood samples were taken after the subjects had fasted at least 12 h for the measurement of plasma glucose, triacylglycerol, total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol. The definition of metabolic syndrome was based on the modified National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria, that is when ≥3 of the following criteria were satisfied: (i) central obesity (waist circumference ≥90 cm for men and ≥80 cm for women), (ii) hypertriacylglycerolemia (≥150 mg/dL), (iii) an abnormally low HDL-cholesterol concentration (<40 mg/dL for men and <50 mg/dL for women), (iv) raised blood pressure (≥130 mmHg systolic or ≥85 mmHg diastolic) or (v) a raised fasting glucose concentration (≥100 mg/dL).

Harvest of HCV or HBV viral particles from serum

To prepare the HCV or hepatitis B virus (HBV) viral particles, 1 mL of serum from the chronic hepatitis B or C patients, and 0.5 mL of 30% polyethylene glycol 6000 in 1.5 M NaCl was added and left overnight at 4 °C. The sample was then centrifuged for 30 min at 3300 g, and the pellet was resuspended in culture medium for the coculture experiments. The viral titre of the medium was quantified by real-time PCR using an ABI 5700 sequence detection system (PE Applied Biosystems, Warrington, UK).

Midbrain neuron–glia coculture

Neuron–glia cocultures from E-14 Wistar embryonic rat midbrain were obtained. Midbrain neuro-glia coculture has been used to study neuroinflammation in vitro, such as the potential neuroprotective effect of anti-inflammatory compounds. For the details of the test refer to Hung's paper [9]. The cells were seeded in Dulbecco's Modified Eagle's Medium (DMEM) with 10% foetal bovine serum at 5×10^5 in 24-well plates previously coated with poly-D-lysine. The culture was kept in a humidified chamber at 37 °C in a 5% CO_2 atmosphere. Twenty-four hours after plating, the culture was changed to Minimum Essential Medium (MEM) with 2% FBS and 2% horse serum. After 1 week, the primary midbrain neuron–glia cocultures were treated with 100 nM MPP^+ in the presence or absence of testing compounds for 48 h. Dopaminergic neurons were characterized by immunostaining with a rabbit antityrosine hydroxylase (anti-TH) antibody (1:5000; Calbiochem, Darmstadt, Germany). A biotinylated goat anti-rabbit IgG was used for staining revealed by the ABC method (Vector Laboratories, Burlingame, CA, USA) and developed using

0.04% (w/v) diaminobenzidine to produce a brown reaction product. The number of TH (+) cell was counted under a microscope.

Cytokine/Chemokine arrays

Commercially available cytokine antibody arrays from R&D Systems (Minneapolis, MN, USA, Proteome Profiler Rat Cytokine Array Panel A; ARY008) were used to measure differences in the relative amounts of cytokines released from midbrain in the presence and absence of virus. The array consists of a nitrocellulose membrane dotted with antibodies that recognize 29 distinct cytokines, chemokines and growth factors. Following a 48 h incubation of midbrain cultures with or without HBV or HCV, the culture media were collected for analysis. A detection antibody cocktail consisting of 29 biotinylated antibodies, each targeted to a specific cytokine, was added to the treated media samples. The media were then incubated overnight at 4 °C with the cytokine antibody arrays. Following this, the media was washed from the array and a Streptavidin–HRP solution was added to the membrane for 30 min at room temperature. The cytokine array was again washed, and a chemiluminescent HRP substrate (EMD Millipore, Darmstadt, Germany) was added to the membrane. The membranes were then exposed to X-ray film for 1–5 min, and the relative pixel density of each spot on the array was quantified using Image J (National Institutes of Health, Bethesda, MD, USA) software.

Statistical analysis

The relationships between hepatitis virus infection status, demographic factors, smoking habits, metabolic syndrome and PD were expressed as odds ratios (OR) and 95% confidence intervals (CI) using a univariate logistic regression model. Interactions between all pairs of factors were tested in the model. Afterwards, the adjusted ORs of hepatitis virus, smoking and metabolic syndrome were estimated after controlling for age, gender, education level and also possible interactions between the two variables. To compute adjusted odds ratios for the association between HCV and PD, smoking and metabolic syndrome together with age, gender and education levels were retained in the final model.

RESULTS

Table 1 shows the frequencies of different characteristics in the study population. Of 61 363 participants, subjects aged over 60 years accounted for 57%. In total, 7137 (11.71%) individuals were HBsAg(+) and 2729 (4.48%) were anti-HCV(+). The carrier rate of HBsAg in males was higher than in females (13.21% *vs* 10.73%), but the carrier rate of anti-HCV was lower in males than females (3.59% *vs* 5.06%). The prevalence of metabolic syndrome was 32.21% and 30.10% in males and females, respectively.

The crude ORs for having PD were 1.11 (95% CI, 1.11–1.12) and 1.43 (95% CI, 1.25–1.64) for age and gender, respectively. The risk of PD was lower among high (more than 13 years of education) or intermediate education levels (10–12 years) (OR = 0.29; 95% CI, 0.23–0.37, and OR = 0.35; 95% CI, 0.26–0.47, respectively) as compared with those with low education level (<10 years). After testing for the interaction between each two independent variables of interest, only the interaction between gender and levels of education was statistically significant.

In the univariate analysis, the crude ORs for having PD for HBV and HCV infection were 0.62 (95% CI, 0.48–0.81) and 1.91 (95% CI, 1.48–2.47) (Table 2). After controlling for age, gender, education level and the interaction between gender and education level, the association between HCV and PD still remained statistically significant (adjusted OR = 1.39; 95% CI, 1.07–1.80). However, the association between HBV infection and PD was not statistically significant in the multivariable regression analysis (adjusted OR = 1.01; 95% CI, 0.77–1.32). The interactions between gender and hepatitis virus infection were not significant. Furthermore, the positive association still remained between HCV and PD (adjusted OR = 1.40; 95% CI, 1.08–1.82) after adjusting for age, gender, education level, the interaction between gender and education level, smoking and metabolic syndrome.

MPP^+ and HCV-induced dopaminergic neuronal death in neuron–glia cocultures

MPP^+ (100 nM) induced loss of TH (+) neurons by 29.5 ± 2.8% in midbrain neuron–glia cocultures (Fig. 1b). In the presence of HCV but not HBV, the toxic effect of MPP^+ was significantly increased in a dose-dependent manner (Fig. 1g). The toxicity of MPP^+ was increased up to 50% by co-incubation with 10^4 HCV viral particles/mL. Application of HCV (10^5 viral particles/mL) induced neurotoxicity in TH^+ neurons directly by 26.1 ± 3.2% in midbrain neuron–glia cocultures (Fig. 1f) which was similar to that of MPP^+ (100 nM) ($P < 0.05$),whereas HBV (10^5 viral particles/mL) did not cause neurotoxicity in TH (+) neurons in the midbrain neuron–glia cocultures (Fig. 1e).

Figure 2 shows the released cytokines from midbrain with HCV or HBV infection or control. Of the 29 distinct cytokine, chemokines and growth factors detected by the array (Fig. 2a), the soluble intercellular adhesion molecule-1 (sICAM-1), LPS−induced CXC chemokine (LIX), regulated on activation normal, T cell expressed and secreted (RANTES) increased in the HCV- and HBV-infected samples (Fig. 2b). Moreover, the levels of cytokines in the HCV-infected samples were higher compared with those of HBV-infected samples (Fig. 2c). The most prominent increase was found in the level of LIX (eight fold). Interestingly, the down-regulation of the tissue inhibitors of metalloproteinases-1 (TIMP-1) was only obvious in the HCV-infected samples.

Table 1 Characteristics of study cohort

	Study population				Males				Females			
	Participants without PD		PD Cases		Participants without PD		PD Cases		Participants without PD		PD Cases	
Variables	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Total	62 276		887		24 762		431		37 514		456	
Age at entry												
40–49	23 827	38.26	26	2.93	8462	34.17	8	1.86	15 365	40.96	18	3.95
50–59	15 255	24.50	58	6.54	5515	22.27	18	4.18	9740	25.96	40	8.77
60–69	13 012	20.89	215	24.24	5407	21.84	86	19.95	7605	20.27	129	28.29
70–79	8317	13.36	427	48.14	4364	17.62	225	52.20	3953	10.54	202	44.30
≥80	1865	2.99	161	18.15	1014	4.09	94	21.81	851	2.27	67	14.70
Years of education												
<9	39 427	63.46	748	84.81	13 673	55.28	322	75.06	25 754	68.87	426	94.04
10–12	15 286	24.60	85	9.64	6869	27.77	66	15.38	8417	22.51	19	4.19
13–16	6950	11.19	48	5.44	3868	15.64	41	9.56	3082	8.24	7	1.55
>16	467	0.75	1	0.11	323	1.31	0	0.00	144	0.39	1	0.22
Missing	146		5		29		2		117		3	
HBsAg												
Negative	53 057	88.23	738	92.37	20 564	86.69	354	92.67	32 493	89.23	384	92.09
Positive	7076	11.77	61	7.63	3156	13.31	28	7.33	3920	10.77	33	7.91
Missing	2143		88		1042		49		1101		39	
Anti-HCV												
Negative	57 468	95.57	734	91.86	22 875	96.45	359	93.98	34 593	95.00	375	89.93
Positive	2664	4.43	65	8.14	843	3.55	23	6.02	1821	5.00	42	10.07
Missing	2144		88		1044		49		1100		39	
Smoking												
Never	43 818	71.08	630	71.43	9657	39.40	218	51.05	34 161	92.00	412	90.55
Ever	4279	6.94	83	9.41	3823	15.60	72	16.86	456	1.23	11	2.42
Current	13 545	21.97	169	19.16	11 030	45.00	137	32.08	2515	6.77	32	7.03
Missing	634		5		252		4		382		1	
Metabolic syndrome												
No	41 572	66.89	519	58.71	16 182	65.55	280	65.42	25 390	67.78	239	52.41
Yes	20 575	33.11	365	41.29	8505	34.45	148	34.58	12 070	32.22	217	47.59
Missing	129		3		75		3		54		0	

HCV, hepatitis C virus; PD, Parkinson's disease.

DISCUSSION

Epidemiologically, we found that anti-HCV(+) patients had statistically significant increased risk of developing PD in the population-based study. This finding was further supported by HCV-induced dopaminergic neuronal toxicity in vitro. The dopaminergic neuronal toxicity induced by HCV was similar to that of MPP^+. The levels of chemokines such as sICAM-1, LIX and RANTES were increased, and TIMP-1 was down-regulated in the HCV-infected midbrain culture.

Hepatitis C virus is a positive-strand RNA virus of the flaviviridae family that primarily infects hepatocytes, causing acute and chronic liver disease, but it is also associated with a variety of CNS abnormalities, such as cognitive dysfunction, fatigue and depression [10,11]. Although the evidence for extrahepatic HCV replication is still controversial [12], a recent study showed that the essential HCV receptors (including CD81, claudin-1, occluding, LDLR and scavenger receptor-B1) are expressed on brain microvascular endothelial cells, a major component of the blood–brain barrier (BBB), that may provide a gate for HCV to infect the CNS [13]. This is supported by the detection of negative-strand HCV RNA in postmortem brain tissue [5].

Hepatitis C virus-infected patients have been demonstrated to have impairment of midbrain dopaminergic function [7]. Our study found that HCV can induce dopaminergic neuronal toxicity similar to that of MPP^+. Viruses may directly injure neurons by viral replication or through activation of both innate and adaptive immune responses resulting in neuronal damage through inflammation [1]. The emerging evidence has shown that inflammation makes a significant contribution to neuronal death in

Table 2 The association between Parkinson's disease with stratification by hepatitis B virus (HBV), HCV and metabolic syndrome

Variables	Crude OR (95% CI)	Adjusted OR*(95% CI)	Adjusted OR† (95% CI)
HBsAg			
Negative	1.00	1.00	NA
Positive	**0.62 (0.48, 0.81)**	1.01 (0.77, 1.32)	**NA**
Anti-HCV			
Negative	1.00	1.00	**1.00**
Positive	**1.91 (1.48, 2.47)**	**1.39 (1.07, 1.80)**	**1.40 (1.08, 1.82)**
Smoking			
Never	1.00	1.00	1.00
Ever/current	0.98 (0.85, 1.14)	0.88 (0.75, 1.05)	0.88 (0.73, 1.05)
Metabolic syndrome			
No	1.00	1.00	1.00
Yes	**1.42 (1.24, 1.63)**	1.01 (0.88, 1.16)	1.05 (0.91, 1.21)

*Each odds ratio (OR) was adjusted by age (as a continuous variable), gender, education level and the interaction between gender and education. †Odds ratio (OR) has been adjusted by age (as a continuous variable), gender, education, anti-hepatitis C virus (HCV), smoking, metabolic syndrome and the interaction between gender and education.

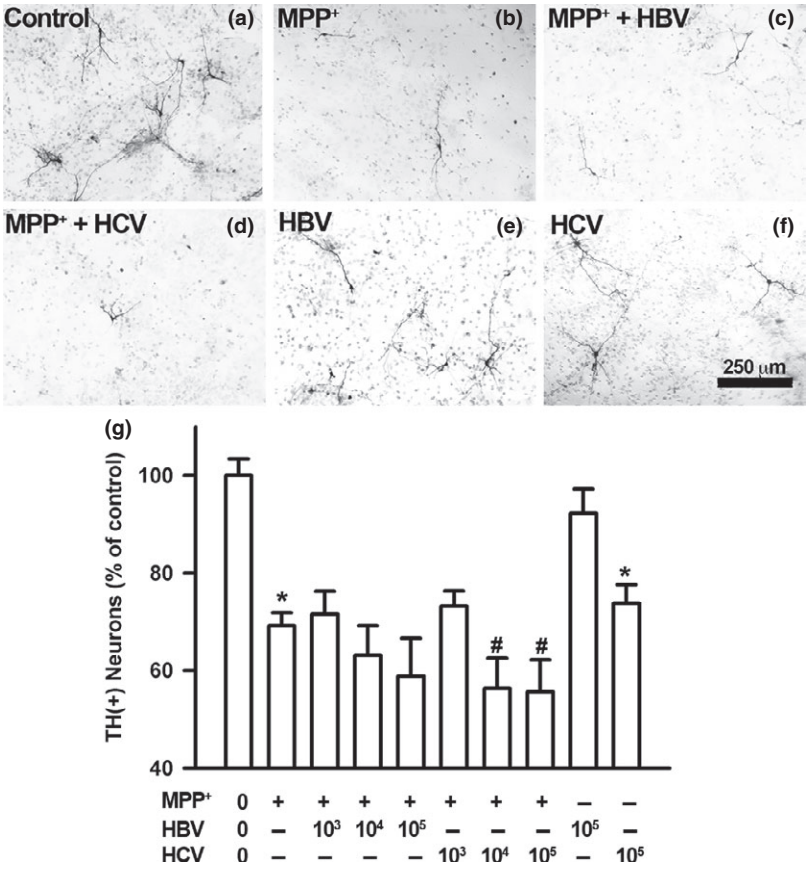

Fig. 1 The toxicity in vitro of HBV, hepatitis C virus (HCV) and MPP^+. Midbrain neuron–glia cocultures were obtained from E14 Wistar rats. (**a**) Midbrain cells of the control group. (**b–d**) The MPP^+ (100 nM) induced loss of TH+ neurons in midbrain neuron–glia cocultures and that with MPP^+ combined with HBV (10^5 viral particles/mL) or MPP^+ combined with HCV (10^5 viral particles/mL). (**e**) Failure of HBV (10^5 viral particles/mL) to cause neurotoxicity of TH+ neurons. (**f**) HCV induced (10^5 viral particles/mL) loss of TH+ neurons similar to that of MPP^+ (100 nM). (**g**) Quantitative analysis of survival of TH+ neurons in midbrain neuron–glia cocultures at 48 h. The MPP^+ (100 nM) induced loss of TH+ neurons by 29.5 ± 2.8%, similar to that of HCV (26.1 ± 3.2%). *$P < 0.05$ compared with control. #$P < 0.05$ compared with HCV (10^3 viral particles/mL). Bar represents 150 μm.

PD [6]. Levels of proinflammatory mediators, including TNF-α, IL-6 and IL-1β, are elevated in the brains and peripheral blood mononuclear cells of patients with PD [14]. HCV infection also stimulates macrophages or monocytes to release proinflammatory mediators [15,16]. In our study, we found that sICAM-1 and RANTES were significantly up-regulated by HCV in rat midbrain neuron–glia coculture. Serum levels of circulating ICAM-1 rise in

(a)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Line 1	Pos	Pos	CINC-1	CINC-1	CINC-2α/β	CINC-2α/β	CINC-3	CINC-3	CNTF	CNTF	CX3CL1	CX3CL1	GM-CSF	GM-CSF	sICAM-1	sICAM-1	IFN-γ	IFN-γ	Pos	Pos
Line 2			IL-1α	IL-1α	IL-1β	IL-1β	IL-1ra	IL-1ra	IL-2	IL-2	IL-3	IL-3	IL-4	IL-4	IL-6	IL-6	IL-10	IL-10		
Line 3			IL-13	IL-13	IL-17	IL-17	IP-10	IP-10	LIX	LIX	L-selectin	L-selectin	MIG	MIG	MIP-1α	MIP-1α	MIP-3α	MIP-3α		
Line 4	Pos	Pos	RANTES	RANTES	CXCL7	CXCL7	TIMP-1	TIMP-1	TNF-α	TNF-α	VEGF	VEGF					Neg	Neg		

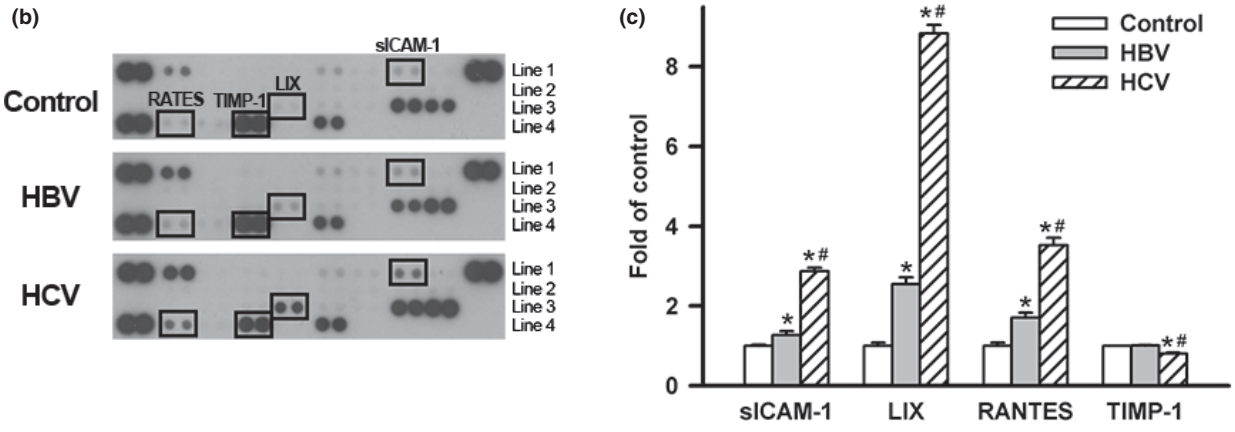

Fig. 2 The cytokine/chemokine results in the presence and absence of viruses. (**a**) The positions of cytokines on the membrane are shown in the array map provided by the manufacturer (Proteome Profiler Rat Cytokine Array Panel A; ARY008). (**b**) Medium collected from rat midbrain neuron–glia coculture exposed to Control, HBV or hepatitis C virus (HCV) (10^5 viruses/mL) for 48 h. The levels of several chemokines in medium from midbrain culture were affected by HBV or HCV including RANTES, TIMP-1, LIX and sICAM-1. (**c**) Bar graph illustrates the relative expression levels of cytokines/chemokines based on densitometry of signal intensities. $^*P < 0.05$ compared with control. $^{\#}P < 0.05$ compared with HBV.

inflammatory diseases of the CNS [17]. In autopsy brains of PD, the activated microglia with overexpression of ICAM-1 were up-regulated in the SN and putamen [18]. ICAM-1-positive reactive astrocytes in PD are indicative of a sustained inflammatory process [19]. Astrocytes can be induced to produce sICAM-1, which can induce a TNF-α-dependent inflammation and damage the dopaminergic neurons in PD [20].

RANTES signalling upstream of caspase activation plays a key role in neuronal apoptosis [21]. Human microglia synthesize RANTES in response to pro-inflammatory stimuli, and anti-inflammatory cytokines regulate the production of RANTES by activated microglia [22,23]. $CD4^+$ T cells have a pivotal role in accelerating CNS inflammation and demyelination within infected mice, by regulating RANTES expression, which in turn coordinates the trafficking of macrophages into the CNS, leading to myelin destruction [24]. The overexpression of sICAM-1 and RANTES might result in neuroinflammation and facilitation of PD progression.

It has been reported that LIX was up-regulated in primary astrocytes after exposing to neurotoxin [25]. More recently, using a cerebellar slice culture system, it has been reported that lysolecithin promoted the release of LIX, which results in demyelination [26]. We found that LIX was significantly up-regulated by HCV in the midbrain culture and may indicate the potential dopaminergic neuronal damage.

In addition, we found that TIMP-1 was down-regulated by HCV. TIMP-1 is an important marker for neuroinflammatory and neurodegenerative diseases [27]. TIMP-1 is known to aid cell survival. The TIMP family inhibits the activity of matrix metalloproteinases (MMPs), a large family of zinc-dependent proteases [28]. TIMP-1 is currently studied with particular interest in CNS disease progression because of its robust overexpression in response to inflammatory myelin injury [29]. TIMP-1 is predominantly expressed in astrocytes and likely acts as an endogenous factor to rescue cells from the toxic effects of MMP activities during neuroinflammation [30]. Cerebrospinal fluid (CSF) and brain tissue samples from HIV-1-associated patients with dementia showed reduced TIMP-1 levels compared to control patients [31]. Furthermore, TIMP-1/MMP expression in neuroinflammation can impact neuronal function and survival in disease conditions. A recent study also shows the direct role of TIMP-1 in neuroprotection, indicating that its expression serves as a neuroprotective response of astrocytes [32]. Here, we found that TIMP-1 was down-regulated by HCV, suggesting that one of the neuroprotectants derived from astrocytes was inhibited by HCV infection.

Chronic infection with HCV induces insulin resistance, leading to metabolic syndrome and the underlying pathway through the expression of cytokines, such as TNF-α and IL-6 [14]. Thus, the influence of HCV infection on PD may be confounded by metabolic syndrome. However, after adjustment for metabolic syndrome, the positive association between HCV and PD still remained. This result also supported the fact that HCV can pass through the blood–brain barrier to induce neuroinflammation and lead to PD.

We failed to find a significant association between HBV infection and PD in the community-based integrated screening programme. The lack of association between HBV infection and PD was also consistent with Forton *et al.*'s [4] findings that an elevated choline/creatine ratio was not seen in patients with HBV infection. In addition, our results revealed that HBV does not induce dopaminergic neuronal toxicity. Furthermore, there is no neuroinvasive evidence of HBV, neither of the Hepadnaviridae family in general, to which HBV belongs.

The positive association between HCV infection and PD has clinical implications for high endemic HCV areas. The WHO has estimated that the prevalence of HCV infection is 2.2–3% worldwide, representing 130–170 million people [33–35]. Taiwan is an endemic area for hepatitis viruses and chronic liver disease [36]. The seroprevalence of anti-HCV in Taiwan reaches 4.4% in the general population [37]. As therapy for chronic hepatitis or liver disease has improved, older-onset diseases, such as PD, have been gradually noticed more often [38]. Because of the positive association between HCV and PD which is independent of metabolic syndrome, neurological tests may be considered in anti-HCV(+) groups to detect the earlier stages of PD.

In summary, our study not only demonstrated a significantly positive association between HCV infection and PD from a large population-based epidemiological study but also proved the dopaminergic neuronal toxicity by HCV in vitro at the molecular level through an increase in cytokines induced by HCV.

DISCLOSURES

The authors declare no conflict of interest.

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