ANNUAL REPORT

First year - PhD Program in Sciences & Medical Biotechnology
University of Piemonte Orientale "Amedeo Avogadro", Novara

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PART 1: Scientific activity

PART 2: Didactic and educational activities

PART 1: Scientific activity

Project title:

Neurokinin (NK)-1 receptor expression in monocytes from bipolar disorder patients

Scientific background

Bipolar disorder (BD) is a severe, chronic, recurring mental disorder characterized by episodes of major depression and mania (type1; BDI) or hypomania (type 2; BDII), progressive deterioration and cognitive deficits, often associated with co-morbidities(Perugi et al., 2015). Its pathophysiology is really complex, neurobiological bases and how genetic and environmental influences predispose and/or precipitate the symptoms being not fully understood. Genome-wide association and linkage studies have been performed but the overall results are inconclusive (Nurnberg et al., 2014 and Ogden et al., 2004). Activation of the immune system plays a key role in the BD pathogenesis,mononuclear cells and pro-inflammatory cytokines being particularly investigated (Beumer et al., 2012, Drexhage et al., 2010a, Drexhage et al., 2011 and Haarman et al., 2014). Indeed, higher plasma levels of pro-inflammatory cytokines (TNF-α, mainly) were described in BD patients during both manic and depressive episodes (Brietzke and Kapczinski, 2008, Kim et al., 2007 and Munkholm et al., 2013) and mood stabilizers, as well as some anti-depressants, were shown to reduce cytokine level and/or modulate neuro-inflammation (Leonard, 2014 and Rao et al., 2007). Regarding mononuclear cells, the "monocyte/macrophage-T cell theory", initially proposed for schizophrenia (Smith, 1992) and depression (Maes et al., 1995), was recently extended to BD, a specific "signature" for monocytes being identified (Drexhage et al., 2010b, Drexhage et al., 2011, Haarman et al., 2014 and Padmos et al., 2008).

Others and we previously demonstrated that substance P (SP) triggers inflammatory-immune responses in monocytes, macrophages, microglial cells and lymphocytes, acting preferentially on neurokinin-1 receptors (NK-1R) (Amoruso et al., 2008, Lai et al., 1998, Lai et al., 2006 and Rasley et al., 2002). Besides being expressed in immune cells, NK-1R are distributed in different areas of mammalian brain, including the cingulated andfrontal cortex (Burnet and Burnet and Harrison, 2000 and Nagano et al., 2006).

We recently reported that, in monocytes isolated from patients with major depressive disorder, NK-1R expression was reduced as compared to healthy volunteers, whereasNK-2R (the receptor that preferentially binds neurokinin A, NKA) expression was augmented (Bardelli et al., 2013). Moreover, the tachykinin receptor 1 gene (TACR1, which encodes SP and NKA) and other SP-related genes have been associated with BD (Mendlewicz et al., 2005, Ogden et al., 2004 and Sharp et al., 2014).

Rationale of the study

Based on these evidences, we hypothesized that NK-1R expression could be altered in BD. Therefore, this pilot study was aimed to evaluate the constitutive expression of NK-1R protein in monocytes isolated from BDI and BDII patients in comparison to age-matched healthy volunteers. We also evaluated the ability of SP and [Sar9Met(O2)11]SP (selective NK-1R agonist) to modulate NF-κB activation in both BD patients and healthy volunteers.

Subjects and Methods

This observational study enrolled 20 consecutive BD patients (13 females and 7 males; mean age 55.3+2.8 years) at the Outpatient Centre of Borgomanero and 14 healthy volunteers (8 females and 6 males; age-matched to BD patients, with no family history of psychiatric disorders, drug-free at the time of the study). This study was approved by the Ethic Committee of the Novara Hospital; informed written consent was obtained by each participant. Inclusion criteria for BD patients were: stable pharmacological therapy from at least two months, euthymia or mild depression, informed written consent. Diagnosis of BDI or BDII was assessed by two expert trained psychiatrists by a structured clinical interview based on the DSM-IV-TR defined criteria (First et al., 1994). Clinical assessment included the collection of demographic and clinical variables, as well as the score values obtained in the Hamilton Rating Scale for Depression (HAM-D; Hamilton, 1960) and the Young Mania Rating Scale (YMRS; Young et al., 1978). Eight participants (5 females, 3 males) were diagnosed as BDI patients, while 12 (8 females, 4 males) were BDII; 8 BD patients and 7 healthy volunteers were smokers. BD patients had a long-lasting disease (>26 years) and usually employed two drugs: a mood stabilizer (n=20; lithium/valproate/lamotrigine: 10/6/4) and an antidepressant (n=14; SSRI/SNRI: 8/6) or atypical antipsychotic (n=4). The HAM-D score was 6.9+0.8 (range 1–12), suggestive for euthymia; the YMRS score was 3.3+0.4 (range 0–7).

Monocyte isolation from BD patients and healthy volunteers

Monocytes were isolated from venous blood (20 ml, obtained at fasting) by standard technique (dextran sedimentation and Hystopaque gradient centrifugation; 400g, 30 min, room temperature), and obtained by adhesion (90 min, 37 °C, 5% CO2), as described (Amoruso et al., 2008). Monocyte viability (trypan blue dye exclusion) and purity (flow cytometry analysis of CD14) were>96%.

2.3. Western blotting for NK-1R in monocytes

Monocytes (3×106) were scraped in ice-cold phosphate buffered saline containing protease inhibitors and centrifuged (14,000g; 30 s, 4 °C). The pellet was re-suspended in 10 mM Tris–HCl (containing EDTA and protease inhibitors), subjected to freezing and thawing and centrifuged again (Amoruso et al., 2008); the pellet so obtained was used for the experiments. Na+/K+-ATPase was selected as reference house-keeping membrane enzyme. Immunoblots were performed as described (Bardelli et al., 2013), using a polyclonal NK-1R antibody (ab466; Abcam) and a monoclonal anti-Na+/K+-ATPase (α subunit) antibody (clone M7-PB-E9; Sigma). Western blots were quantified by densitometry (Bio-Rad software) and expressed as the ratio between NK-1R and Na+/K+-ATPase protein expression (Bardelli et al., 2013).

2.4. Monocyte stimulation and evaluation of NF-κB p50 subunit

NF-κB activation was evaluated by measuring the nuclear content of p50 subunit. Monocytes were challenged for 2 h with 10−6 M phorbol 12-mirystate 13-acetate (PMA, used as standard stimulus), SP or the selective agonist [Sar9Met(O2)11]SP: these conditions were previously demonstrated as the optimal ones (Bardelli et al., 2005). In some cases, monocytes were pre-treated for 30 min with the antagonist GR 71251 ([D-Pro9(spiro-gamma-lactam)Leu10,Trp11]SP) and then challenged with SP. Nuclear extracts were evaluated using Trans AMTM NF-κB p50 Chemi Transcription Factor Assay kit (Active Motif Europe), according to Bardelli et al. (2013). The p50 subunit activity was measured by a luminometer and results are presented as RLU (Relative Luminescence Unit).

2.5. Statistical analysis

Statistical analyses were performed using Graph Pad Prism 5. Data are presented as mean+SEM of 'n' independent experiments on monocytes from different BD patients or healthy donors. Differences between groups were analyzed using non-parametric Mann Whitney test. $P<0.05$ was considered statistically significant.

Preliminary results

NK-1R expression in monocytes from BD patients and healthy subjects

NK-1R expression was significantly lower ($P<0.001$) in monocytes from BD patients as compared to healthy subjects with no major differences between BDI and BDII in spite of a higher NK-1R/Na+/K+-ATPase ratio (not reaching statistical significance) in BDII patients. Tobacco smoke significantly increased NK-1R in monocytes from healthy volunteers, smokers presenting a 3-fold higher constitutive expression, while NK-1R expression in monocytes isolated from BD patients was unaffected by tobacco smoke

Nuclear translocation of NF-κB p50 subunit in BD patients and healthy subjects

To ensure a quantitative evaluation of NF-κB activation, we measured nuclear translocation of the p50 subunit in monocytes from 5 individuals (2 non-smokers and 3 smokers) for each group. Data evaluation is in progress.

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Pharmacol. Res., 68 (2013), pp. 24–30

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Handb. Exp. Pharmacol., 213 (2012), pp. 401–417

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Bipolar Disord., 16 (2014), pp. 137–150

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Science, 281 (1998), pp. 1640–1645

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Proc. Natl. Acad. Sci., 103 (2006), pp. 7771–7776

PART 2

CONGRESSES AND TRAVEL GRANTS

No grants have been received for congress participation

Congresses attended:

- LA GESTIONE PRATICA DELLA FARMACOTERAPIA NEL PAZIENTE BIPOLARE, Lucca, April 2015
- Centres of Excellence for Relapse Prevention (CERP) in Psychotic Disorders Annual Congress – Barcelona ESP, April 2015
- ADHD, CLINICA E TRATTAMENTO; Brescia, May 2015

ORAL COMMUNICATIONS

- Italian Society of Psychiatry National Congress, October 2015 – Taormina (CT)
  Oral communication in 2 different thematic symposia
- Meet Brains – Join Minds – October 2015 – Novara - Scientific director
- Psychopharmacology of behavioural disorder in dementia – November 2015 – Istituto Scientifico Maugeri - Pavia

PUBLICATIONS (2015)

Acta Psychiatr Scand. 2015 Aug 7. doi: 10.1111/acps.12468.

Metabolic syndrome and drug discontinuation in schizophrenia: a randomized trial comparing aripiprazole olanzapine and haloperidol.

Parabiaghi A1, Tettamanti M1, D'Avanzo B1, Barbato A1; **Cattaneo CI** as part of GISAS Group

PART 2: Didactic and educational activities

- Seminars and lessons of the PhD Program at the University of Eastern Piedmont, Novara (see followings)
- Seminars at the Department of Psychiatry, ASL Novara
- Lecture at Swiss Psychiatric Association: "Bipolar Disorder, affective temperaments and personality disorder", April 23rd
- Several Lessons about mood disorder during ECM courses - ASL Novara 2015

Attended seminars

16th December "From the legend of Prometheus to regenerative medicine" Prof. Antonio Musarò

17th December 2014
"Microglia microvesicles: messengers from the diseased brain"
Prof.Furlan

27th January 2015 ,

Myeloid cells as therapeutic target in cancer Prof. Antonio Sica

19th January

Prof. Dr Yong-Sang Song, MD, PhD

Anticancer strategy Targeting cancer cell metabolism in ovarian cancer

20th January

Regulation of hepatocytes differentiation during the transitions between epithelial and mesenchymal states

Dr Tonino Alonzi, PhD

21st January 2015

Targeting the liver to cure myocarditis: a lesson from a model of STAT3-dependent auto-immune myocarditis Prof Valeria Poli

-April 9th at 12.00

Prof Zhong"Signal control in iNKT cell development and function" Dr. Xiaoping Zhong, MD, PhD

-April Tuesday 21st, 2015 at 14.00

"Actin-based mechanisms in the control of gene expression and cell fate" Prof. Percipalle

-May 14th

Conflicting interests and scientific communication. What ethical standards to apply? How effective are these standards in practice? K. Ruff

-May 25th

"Ribosomopathies" Prof. Steve Ellis

September the 4th, 2015

Carlo Ignazio Cattaneo