Physiol. Res. 43: 27–31, 1994

The Effect of Risperidone and Ritanserin on Human IgG and IgM Synthesis in vitro

E. JAHNOVÁ

Department of Clinical Immunology, Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic

Received February 24, 1993
Accepted November 9, 1993

Summary

We tested risperidone and ritanserin, serotonin-S2 receptor antagonists, for their effects on *in vitro* polyclonal IgG and IgM synthesis by human peripheral blood mononuclear cells (PBMC) stimulated with pokeweed mitogen (PWM). On the basis of the previously reported effect on immune function *in vivo* risperidone in this study was tested in three different groups of PBMC: healthy donors as well as schizophrenic patients before risperidone treatment and schizophrenic patients after the treatment with risperidone. IgG and IgM production after 7 days of culture was measured by ELISA. Risperidone decreased IgG synthesis ($p<0.05$) in PBMC of healthy subjects only at the highest concentration (10^{-6} M) and IgG synthesis enhanced by 5-HT was antagonized by risperidone. This effect, however, was not statistically significant. Neither risperidone nor ritanserin, in the concentration range $10^{-8}-10^{-6}$ M, affected IgM synthesis in this group. Risperidone did not affect the production of IgG and IgM by PBMC of schizophrenic subjects in PWM-stimulated cultures both before and after risperidone therapy. The spontaneous production of IgG in PBMC of schizophrenic subjects before therapy was decreased ($p<0.05$) at concentrations $10^{-6}-10^{-7}$ M of risperidone. We conclude that risperidone and ritanserin did not increase polyclonal IgG and IgM synthesis *in vitro* in contrast to neuroleptics currently used in clinical practice.

Key words

Risperidone – Ritanserin – Serotonin – Immunoglobulin production – Peripheral blood mononuclear cells – Schizophrenia

Introduction

Numerous investigations have dealt with the immunosupressive potential of neuroleptics currently used in clinical practice (Descotes 1986). Chlorpromazine and haloperidol have been the most frequently mentioned derivatives in this respect. It therefore appeared to be of interest to ascertain how the antagonists of serotonin receptors – risperidone and ritanserin as neuroleptics – influence the immune response. Recent clinical studies with serotonin-affecting drugs have provided circumstantial evidence on the role of serotonin (5-hydroxytryptamine, 5-HT), in the pathogenesis of psychiatric disorders (Eriksson and Humble 1990). Serotonin directly modulates the function of immunocompetent cells and participates as a neurotransmitter in central neuroendocrine mechanisms that regulate immunity (Jackson *et al.* 1985, Roszman *et al.* 1985). Several findings indicate that 5-HT has an inhibiting effect on the immune response. Slauson *et al.* (1984) reported that 5-HT suppressed *in vitro* the proliferation of human lymphocytes induced by phytohaemagglutinin (PHA). Jackson *et al.* (1985) observed that 5-HT inhibited the primary antibody response to sheep red blood cells in the mouse. On the contrary, Hellstrand and Hermodsson (1987) observed that 5-HT strongly augmented natural killer (NK) cell cytotoxicity. A few attempts, based on pharmacological observations, have been made to determine the type of receptor corresponding to the inhibitory or stimulatory effect of 5-HT on the immune response. Preliminary findings suggest that 5-HT1 receptor is responsible for the inhibitory effects of serotonin on the immune response. Immune status of subjects treated and the dose are of basic importance for a clinical trial. The aim of the present study was to develop an *in vitro* model of drug-induced impairment of antibody synthesis. The immune

status in our model was evaluated on the basis of the functional activity of B cells in healthy subjects, and patients before and after the therapy. Risperidone was investigated in all three groups, ritanserin only in the group of healthy donors.

Materials and Methods

Risperidone and ritanserin were obtained from Janssen (Belgium), serotonin from Sigma (London) and pokeweed mitogen (PWM) from Serva (Heidelberg). Swine anti-human IgG, IgM horseradish peroxidase-labelled and RPMI-1640 were provided by USOL (Praque).

Preparation of peripheral blood mononuclear cells

Human peripheral blood mononuclear cells (PBMC) were obtained from 29 healthy volunteers and 18 patients with chronic schizophrenia by centrifugation of heparinized blood on Ficoll-Verografin at 600 x *g* for 10 min. The PBMC were washed once in phosphate-buffered saline (PBS, pH 7.2) and three times in the RPMI-1640 medium and finally resuspended in this medium.

Effect of drugs and 5-HT on IgG and IgM synthesis

PBMC (10^6 cells/ml in 0.25 ml) were incubated in the complete medium with 1 μg/ml PWM and drugs or 5-HT at concentrations $10^{-4}-10^{-8}$ M in sterile microtitre plates for 7 days at 37 °C and 5 % CO_2. The supernatants were recovered after centrifugation (300 x *g*, 10 min), stored at −20 °C and subsequently assayed by ELISA for the IgG and IgM content.

ELISA for measurement of IgG and IgM

The microtitre plates were coated overnight at 4 °C with swine anti-human IgG or IgM in 0.05 M carbonate/bicarbonate buffer (pH=9.6). The plates were washed thre times in PBS-Tween. Normal human serum (standardized for IgG and IgM content) and culture supernatant test samples of each ELISA plate were serially diluted. After 1 h incubation at room temperature the plates were washed again in PBS-Tween and horseradish peroxidase-labelled anti-human IgG and IgM was added for 1 h. The plates were then washed 3 times and a substrate solution (0.4 mg/ml o-phenylenediamine dihydrochloride and 0.1 % hydrogen peroxide in 0.15 M citrate-phosphate buffer, pH 5.0) was added and the reaction was stopped after 15 min by adding 50 μl of 2 M sulphuric acid. Optical density was determined at 490 nm on the DYNATECH MR-5000 plate reader.

Table 1
Effects of serotonin, risperidone and ritanserin on IgG and IgM synthesis by PBMC of healthy subjects *in vitro*

	Concentration (M)			
Drugs	0	10^{-8}	10^{-7}	10^{-6}
5-HT	0	10^{-6}	10^{-5}	10^{-4}
	IgG (μg/ml)			
5-HT	1.48±0.21	2.42±0.25	2.54±0.55	2.21±0.65
Risperidone	1.59±0.41	1.28±0.41	1.23±0.32	1.04±0.37*
Ritanserin	1.69±0.41	1.60±0.29	1.55±0.31	2.23±0.41
5-HT+Risperidone	1.60±0.23	1.68±0.44	1.70±0.60	2.09±0.57
5-HT+Ritanserin	1.58±0.31	2.00±0.10	2.49±0.35	2.18±0.28
	IgM (μg/ml)			
5-HT	0.90±0.12	0.09±0.09	0.75±0.18	0.60±0.13
Risperidone	0.95±0.26	0.70±0.17	1.00±0.33	0.75±0.20
Ritanserin	0.78±0.13	0.87±0.11	0.75±0.09	0.11±0.07
5-HT+Risperidone	0.92±0.25	0.71±0.20	0.84±0.40	0.90±0.44
5-HT+Ritanserin	0.76±0.12	0.94±0.10	0.91±0.11	0.90±0.08

*Results are means±S.E.M. (n=29). Statistical significance was calculated using the Wilcoxon test. * ($p<0.05$)*

Results

Effects of serotonin, risperidone and ritanserin on the synthesis of IgG and IgM by PBMC of healthy subjects in vitro.

Serotonin had a non-significant enhancing effect on IgG synthesis and no effect on IgM synthesis in the concentration range $10^{-6}-10^{-4}$ M (Table 1). Risperidone, a 5-HT2 antagonist with concomitant binding properties to dopamine, decreased IgG synthesis in the highest concentration 10^{-6} M only ($p<0.05$). The enhanced IgG synthesis by the 5-HT was antagonized by 5-HT2 receptor antagonist risperidone. This effect, however, was not statistically significant (Table 1). IgM synthesis was not potentiated or inhibited by either serotonin or risperidone. Ritanserin a more selective antagonist than risperidone, within the concentration range $10^{-8}-10^{-6}$ M, had no significant effect on PWM-stimulated IgG and IgM synthesis. This antagonist did not block increased IgG synthesis by 5-HT (Table 1).

Effects of risperidone on PWM-stimulated and spontaneous synthesis of IgG and IgM in vitro by PBMC of schizophrenic subjects before and after treatment with risperidone.

Neither IgG nor IgM synthesis in PWM-stimulated PBMC of schizophrenic patients *in vitro* before treatment was changed by risperidone in concentrations $10^{-6}-10^{-8}$ M (Table 2). The spontaneous IgG production was decreased after adding risperidone in concentrations 10^{-7} and 10^{-6} M.

Table 2
Effect of risperidone on unstimulated (−PWM) and PWM-stimulated (+PWM) synthesis of IgG and IgM *in vitro* by PBMC of schizophrenic subjects before and after treatment with risperidone

	Concentration (M)	IgG (μg/ml)		IgM (μg/ml)	
		− PWM	+ PWM	− PWM	+ PWM
		Before treatment			
Risperidone	0	2.61±0.41	1.90±0.13	0.17±0.05	1.11±0.11
	10^{-8}	1.41±0.32	1.65±0.42	0.15±0.03	1.20±0.12
	10^{-7}	1.69±0.16*	1.72±0.18	0.20±0.08	1.16±0.12
	10^{-6}	1.99±0.21*	1.59±0.31	0.18±0.09	0.93±0.09
		After treatment			
Risperidone	0	0.79±0.09	1.16±0.09	0.10±0.02	0.55±0.06
	10^{-8}	1.01±0.06	1.23±0.11	0.14±0.01	0.64±0.08
	10^{-7}	1.17±0.08	0.91±0.12	0.16±0.03	0.52±0.06
	10^{-6}	0.80±0.03	1.10±0.11	0.13±0.02	0.50±0.07

*Results are means ± S.E.M. ($n=18$). Statistical significancy was calculated using the Wilcoxon test. * ($p<0.05$).*

Discussion

In our preliminary clinical trials (Jahnová *et al.* 1993) we investigated the humoral immunity of schizophrenic patients and the effect of risperidone on this immunity. We found increased spontaneous production of IgG compared with the PWM-stimulated IgG production in these schizophrenic patients. After risperidone treatment, both the spontaneous and stimulated IgG production decreased and at the same time the ratio between the spontaneous and PWM-stimulated IgG production returned to normal.

In an *in vitro* model, risperidone was added to cultures of PWM-stimulated as well as unstimulated lymphocytes from healthy donors and to lymphocyte cultures from schizophrenic patients before and after risperidone treatment. Risperidone decreased stimulated IgG production only at the highest concentration in the group of healthy donors.

Routinely used neuroleptics such as chlorpromazin and haloperidol are known to affect the immune response *in vitro* (Martinez and Coleman 1990) and *in vivo* (Sanders and Mucmmore 1964, Levy

and Munson 1976). In some individuals chlorpromazin increases the production of autoantibodies (Canoso and Sise 1982). Martinez and Coleman (1990) found that chlorpromazin also enhances *in vitro* synthesis of IgG in PWM-stimulated lymphocytes from healthy donors and assumed that the increase of polyclonal IgG synthesis could provide the mechanism by which the drug influences production of antinuclear autoantibodies.

To find out whether the decrease of antibody production caused by risperidone was 5-HT receptor-mediated, we studied the influence of serotonin on IgG and IgM synthesis. Though serotonin *in vivo* reduces the antibody response in experiments (Jackson *et al.* 1985), our *in vitro* study on human lymphocyte cultures showed a tendency to increased IgG synthesis. Serotonin is known to have an inhibitory effect on the immune response. It decreases the proliferative response of lymphocytes to various mitogens including PWM. The trend observed in our study towards the increase of IgG production, although not consistent with the results concerning the inhibitory action of 5-HT, is in agreement with the immunomodulatory action of serotonin and its possible involvement in autoimmune disorders (Mašek *et al.* 1989). In our study, serotonin was confirmed to influence the increase of IgG production by human lymphocytes from healthy donors. The IgM synthesis remained unchanged. Risperidone added to a culture together with serotonin blocked the increased serotonin-induced IgG production and serotonin blocked the decrease of IgG synthesis caused by risperidone. The receptor involved in this mechanism was not just the 5-HT2 receptor alone because ritanserin, which is a more selective 5-HT2 antagonist, did not have the same effects.

The immune status of the subject and the drug dose are crucial for observing the drug effect on the immune response. The physiological situation was modelled *in vitro* using lymphocytes of healthy donors. The altered immune function was modelled *in vitro* with lymphocytes from patients with schizophrenia obtained before and after treatment with risperidone. The effect of risperidone on IgG synthesis *in vitro* was observed in the spontaneous as well as PWM-stimulated production. A statistically significant decrease in the spontaneous IgG production was found in the lymphocytes of schizophrenic patients at higher concentrations (10^{-7}, 10^{-6} M). The spontaneous production of IgG by the lymphocytes of healthy donors remained unchanged when risperidone had been added to the culture (unpublished results). In all other groups studied, risperidone did not influence IgG and IgM production. We found in the *in vitro* models that risperidone decreased IgG production in a concentration-dependent manner under physiological conditions (healthy volunteers) in PWM-stimulated lymphocyte cultures and under conditions of altered immunity (schizophrenic patients) in the spontaneous IgG production. Lymphocytes obtained from the patients after the treatment with risperidone showed no change in their IgG and IgM synthesis following risperidon addition *in vitro*.

We conclude that serotonin *in vitro* tends to increase IgG production, which points to its possible role in autoimmune diseases. Under physiological conditions, risperidone does not increase IgG synthesis but decreases it (within the reference range) and blocks the serotonin-induced polyclonal synthesis. It can therefore be assumed that it will not influence the antinuclear phenomena and drug-induced autoimmunity. The mechanism of the effect of serotonin through the 5-HT2 receptor is not yet fully clear because ritanserin, a specific 5-HT2 receptor antagonist, does not have similar effects. The risperidone-caused decrease of IgG production in the stimulated lymphocyte culture under physiological conditions and the spontaneous IgG production during altered immunity seem to support the conclusion that the immune status of the subject is important for investigating the possible ability of a drug to either stimulate or inhibit various immune processes.

Acknowledgements

I would like to thank Dr. A. Mayer (Psychiatric Clinic, Medical Faculty Hospital, Bratislava) for providing peripheral blood of schizophrenic patients from his clinical study, and Miss M. Vargová for technical assistance.

References

CANOSO R.T., SISE H.S.: Chloropromazine-induced lupus anticoagulant and associated immunologic abnormalities. *Am. J. Hemat.* **13:** 121–129, 1982.

DESCOTES J.: Immunotoxicity of drugs acting on the nervous system. In: *Immunotoxicity of Drugs and Chemicals.* Elsevier, Amsterdam, 1986, pp. 125–160.

ERIKSSON E., HUMBLE M.: Serotonin in psychiatric pathophysiology. In: *The Biological Basis of Psychiatric Treatment.* R. POHL, S. GERSHON (eds), Karger, Basel, 1990, pp. 66–119.

HELLSTRAND K., HERMODSSON S.: Role of serotonin in the regulation of human natural killer cell cytotoxicity. *J. Immunol.* **139:** 869–875, 1987.

JACKSON J.C., CROSS R.J., WALKER R.F., MARKESBURY W.R., BROOKS W.H., ROSZMAN T.L.: Influence of serotonin on the immune response. *Immunology* **54:** 505–512, 1985.

JAHNOVÁ E., KELEOVÁ A., ARPÁŠOVÁ D., MAYER A.: Humoral immune response in schizophrenic patients: effect of risperidone. *Homeostasis* **34:** 59–64, 1993.

LEVY J.A., MUNSON A.E.: Supression of antibody mediated primary haemolytic plaque-forming cells by haloperidol. *Fed. Proc.* **35:** 333, 1976.

MAŠEK K., HORÁK P., KADLEC O., FLEGEL M.: The interactions between neuroendocrine and immune systems at the receptors level. The possible role ot serotonergic system. In: *Interactions among CNS, Neuroendocrine and Immune Systems* J.W. HADDEN, K. MAŠEK, NISTICO (eds), Pythagoras Press, Rome, Milan, 1989, pp. 226–234.

MARTINEZ F., COLEMAN J.W.: A comparison of the effects of chloropromazine and more selective histamine and 5-hydroxytryptamine antagonists on human IgG synthesis in vitro. *Int. J. Immunopharmacol.* **12:** 185–191, 1990.

ROSZMAN T.L., JACKSON J.C., CROSS R.J., TITUS M.J., MARKESBERY W.R., BROOKS W.H.: Neuroanatomic and neurotransmitter influences on immune function. *J. Immunol.* **135:** 769–772, 1985.

SANDERS J.C., MUCMMORE E.: Phenothiazine effect on human antibody synthesis. *Br. J. Psychiat.* **110:** 84, 1964.

SLAUSON D.O., WALKER C., KRISTENSEN F., WANG Y., DE VECK A.L.: Mechanism of serotonin-induced lymphocyte proliferation inhibition. *Cell. Immunol.* **84:** 240–252, 1984.

Reprint Requests

Dr. E. Jahnová, Department of Clinical Immunology, Institute of Preventive and Clinical Medicine, Limbová 14, 833 01 Bratislava, Slovak Republic.