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ORIGINAL ARTICLE

A role for the substance P/NK-1 receptor complex in cell proliferation and apoptosis in oral lichen planus

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OBJECTIVES: To determine whether substance P (SP) and NK-1 receptor (NK-1R) are expressed in oral lichen planus (OLP) and are related to cell proliferation and apoptosis in this disease.

MATERIAL AND METHODS: Tissue samples from 50 OLP patients and 26 healthy controls were studied. Immunohistochemistry was performed with anti-SP, anti-NK-1R, anti-ki-67 and anti-caspase-3 monoclonal antibodies and the clinical and pathological data of the OLP patients were evaluated.

RESULTS: With the exception of NK-1R expression in epithelial cell membrane and cytoplasm, all markers were more frequently present in OLP patients than in controls ($P < 0.05$). Higher cytoplasmatic expression of NK-1R was associated with higher epithelial expression of caspase-3 ($P < 0.05$). Higher epithelial expression of NK-1R and SP was associated with higher suprabasal and basal epithelial expression of ki-67 ($P < 0.05$ and $P < 0.005$, respectively).

CONCLUSIONS: Actions of the SP/NK-1R complex may contribute to the immune disorder underlying OLP and trigger stimuli to induce cell proliferation. These results indicate that this complex might play a role in the malignant transformation of OLP.

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Keywords: oral lichen planus; substance P; NK-1 receptor; malignant transformation

Introduction

Malignant transformation of oral lichen planus (OLP) may be related to or dependent on molecular stimuli originating in the inflammatory infiltrate (González-Moles *et al*, 2006; Gonzalez-Moles *et al*, 2008a). Chronic inflammation has been associated with various types of cancer (Coussens and Werb, 2002; Clevers, 2004; Philip *et al*, 2004) and it has been widely reported that inflammatory infiltrate may be a strong risk factor for cancer development in ulcerous colitis, atrophic gastritis and Barret's oesophagus, among other diseases (Balkwill and Mantovani, 2001; O'Byrne and Dalgleish, 2001). It was recently proposed that OLP could be included in this group (González-Moles *et al*, 2006). Some molecules and radicals generated by inflammatory cells can act as mutagenic agents for epithelial cells or affect important cell cycle regulation mechanisms, e.g., apoptosis, cell cycle arrest or cell proliferation. Thus, reactive oxygen species and reactive nitrogen species appear to play key roles in the association between chronic inflammation and cancer (Chaiyarit *et al*, 2005) and inflammatory cells in OLP patients may contribute an excess of nitric oxide (NO) *via* expression of inducible NO synthetase (iNOS) (Chaiyarit *et al*, 2005). The NO generated by iNOS reacts with O_2 to produce ONOO – (Wink and Mitchell, 1998), which induces the formation of both 8-oxo-7.8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-nitroguanine (Yermilov *et al*, 1995) in the nucleus of epithelial cells. Formation of 8-oxodG is a known cause of G–T transversion, which can promote carcinogenesis (Shibutani *et al*, 1991; Normark *et al*, 2003). However, despite the mutagenic effects to which basal cells are exposed in OLP, remarkably few apoptotic phenomena are observed in this cell compartment, as demonstrated by several researchers (Dekker *et al*, 1997; Bloor *et al*, 1999; Neppelberg *et al*, 2001; Tobón-Arroyave *et al*, 2004), including our own group (Bascones-Ilundain *et al*, 2005, 2006, 2007; González-Moles *et al*, 2006), by applying TUNEL technique and analysing the immunohistochemical expression of caspase-3. Moreover, most studies on cell proliferation in OLP have reported a markedly elevated proliferation rate of basal epithelial cells (Schifter *et al*, 1998; Tanda *et al*,

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2000; Valente *et al*, 2001; Taniguchi *et al*, 2002) and some authors have proposed that this might be an important event in the development of cancer on OLP (Taniguchi *et al*, 2002). This increase in the proliferation rate and the low rate of apoptosis are probably produced by stimuli from the inflammatory infiltrate (Mignogna *et al*, 2004).

Substance P (SP), a member of the tachykinin family of neuropeptides, exerts its action by binding to the TACR1 receptor [NK-1 receptor (NK-1R)]. SP has a wide range of functions, including regulation of the intensity of noxious stimuli (DeVane, 2001), regulation of neurogenic inflammation and immune response (Manthy, 1991; Harrison and Geppetti, 2001) and participation in psychological stress pathways (Culman and Unger, 1995; de Felipe *et al*, 1998). By binding to NK-1R, SP activates members of the mitogen-activated protein kinase (MAPK) cascade, including extracellular signal-regulated kinases 1 and 2 (ERK 1/2), which translocate to the nucleus to induce cell proliferation and protect the cell from apoptosis (Esteban *et al*, 2006). SP can also stimulate cell proliferation *via* transactivation of the epidermal growth factor receptor (EGFR) (Koon *et al*, 2004). SP is also found in affected tissue in multiple autoimmune diseases, such as Crohn's disease, rheumatoid arthritis and diabetes (Sakai *et al*, 1998; Goode *et al*, 2000; Persson-Sjögren *et al*, 2005). Besides the injured tissue, SP and NK-1R are expressed in infiltrating leucocytes in these diseases, indicating that leucocyte expression of these molecules may also be critical for their development (Persson-Sjögren *et al*, 2005).

There are few available data on the origin of the signals that induce proliferation and inhibit apoptosis in OLP. Given the role of SP as a potent activator of cell proliferation and protector against apoptosis *via* its NK-1R and its frequent expression in autoimmune diseases, this study was designed to determine whether the expression of SP and NK-1R is related to the high cell proliferation and low apoptosis rate observed in OLP, which may both be implicated in the malignant transformation of this disease.

Material and methods

Patients diagnosed with OLP at the Oral Medicine Clinic of the School of Dentistry of Granada University from January 2001 to December 2004 were included in this case–control study, which was approved by the institutional human ethics committee of Granada University (No. FOD/GR/03/2001).

Data were gathered from clinical records completed at the first visit, which systematically gathered information on demographic data, age, gender, family history of interest, tobacco and alcohol consumption, emotional state, presence of hepatitis B or C infection, pharmaceutical drug intake, months with OLP and symptoms of OLP (pain or burning sensation). A complete examination of the oral cavity was performed to determine the clinical type of OLP and the intraoral sites involved, taking clinical photographs of lesions. Involvement of other mucosae, skin, nails, or scalp was also documented. Biopsies of the most representative lesions of the disease were performed in all patients. In erosive lesions, the biopsy always included both erosive and preserved epithelial areas.

After the review of clinical records and histopathological study, the following inclusion criteria were applied: presence of approximately symmetrical bilateral reticular lesions with or without erythematous and/or erosive lesions; presence of liquefaction degeneration of basal epithelial layer and presence of subepithelial band-like inflammatory infiltrate. Exclusion criteria were presence of epithelial dysplasia, being in receipt of drug treatment (including specific OLP treatment) at the first visit and presence of lesions that were asymmetrical or close to silver amalgam restorations. Cases had to meet all inclusion criteria and no exclusion criteria. After applying these criteria, a final sample of 50 patients were selected.

Controls were selected from among patients without OLP undergoing dental treatment at the same clinic, applying the same exclusion criteria. The control group comprised 26 patients who were clearly informed of the study objectives and gave their consent to performance of a biopsy of oral mucosa with healthy, non-inflamed appearance close to the area anaesthetized for the scheduled dental treatment. Control samples were always obtained from gingival mucosa.

Sections of 3–5 μm thickness were used for histopathology and immunohistochemistry studies. The histopathology study of haematoxylin and eosin-stained sections documented the presence of keratosis, acanthosis, granulosis, spongiosis, basal epithelial layer liquefaction degeneration, civatte bodies, lymphocytic exocytosis, epithelial detachment, subepithelial band-like inflammatory infiltrate and morphology of epithelium-connective tissue interface (papillary or flat). Keratosis was recorded as absent, presence of parakeratosis or presence of orthokeratosis. The intensity of remaining variables was semi-quantitatively graded as absent, mild, moderate or intense.

Immunohistochemistry

The peroxidase–antiperoxidase technique was used for the immunohistochemical staining, performing immunohistochemical analysis by means of the avidin–biotin method. Slides were deparaffinized in xylene, hydrated and incubated with 0.5% (v/v) H_2O_2 in methanol for 20 min to block endogenous peroxidase activity. Slides were then washed with Tris-buffered saline (TBS) and heated for 15 min at 100°C in 10 mM sodium citrate buffer (pH 6.0) for antigen retrieval. Non-specific binding was blocked by incubation with 1% BSA for 1 h. Sections were incubated with primary antibodies overnight at 4°C. The colour was developed using diaminobenzidine as chromogen. Slides were extensively washed with TBS after each step. Finally, they were counter-stained with Mayer's hematoxylin and mounted with DPX mountant. For the negative control, the primary antibody was replaced with normal serum (ref. MAD-004082R; Master Diagnostica, Granada, Spain).

For the positive control, tissue was used from a squamous cell carcinoma known to express intensively the proteins under study. The following primary antibodies were used: anti-caspase-3 (Pharmagen, San Diego, CA, USA; prediluted), anti-ki-67 (Mib-1 clone; Dako, Carpinteria, CA, USA; prediluted), anti-SP (Sigma, St Louis, MO, USA) and anti-NK-1R (Sigma-Aldrich, Madrid, Spain).

Expression of the proteins was studied by cell count in four high-magnification fields (40×). Counts were made of the total cells and the marked cells in each field and the mean percentages of expression were calculated for each case. Brown-stained cells were considered positive, taking no account of the intensity of the staining. Separate counts were made in basal layer, suprabasal layers and inflammatory infiltrate for caspase-3 and ki-67 expression. The immunohistochemical expression of SP was detected by observation of brown staining in the corium and in the membrane, cytoplasm and nucleus of epithelial cells and also in infiltrating lymphocytes. Immunohistochemical expression of NK-1R was detected by brown staining in the membrane and cytoplasm of epithelial cells, in the inflammatory infiltrate and in subepithelial blood vessels.

Cases were assigned to one of the following categories: 0% positive cells (−), < 10% positive cells (+), 10–25% positive cells (++), 26–50% positive cells (+++) or > 50% positive cells (++++). SP expression in the corium was considered positive when brown staining appeared in at least two of the four high-power fields studied. Some markers were recorded as 'not assessed' in the few cases when there was some doubt about the immunohistochemistry result despite repetition of the test, sometimes as a result of fixation and tissue preservation problems.

Statistical analysis

SPSS-Windows v 12.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses, applying Fisher's exact test, ANOVA and *t*-test as appropriate.

Results

The group of patients with OLP comprised 32 females (64%) and 18 males (36%), with a mean age of 55.1 years (range, 31–79 years). The control group comprised 13 females (50%) and 13 males (50%) with ages within the age range of cases. There were four patients (8%) with hepatitis C, two (4%) with hepatitis B and 12 (24%) with a history of emotional disorders; 10 patients (20%) were tobacco users and 12 (24%) consumed alcohol.

Erosive forms of OLP were observed in 29 (58%) of the patients. No isolated atrophic lesions were detected in any patient. The disease involved multiple intraoral sites in 26 patients (52%), and lesions beyond the oral cavity were found in six patients (12%) (five lesions on the skin, one on genitals and one on nails). Symptoms associated with the disease (burning, pain) were reported by 30 patients (60%).

In the histopathology study, the intensity of liquefaction degeneration was moderate in 17 patients (33.3%) and intense in 5 (9.8%). Inflammatory infiltrate was mild in five patients (10%), moderate in 20 (40%) and intense in 25 (50%).

Table 1 displays the immunohistochemical results for SP, NK-1R, ki-67 and caspase-3 in the OLP and control groups (Figures 1 and 2). With the exception of NK-1R expression in epithelial cell membrane and cytoplasm, all markers were expressed more frequently at all tissue locations in the OLP group than in the control group. Table 2 shows associations that were significant or very close-to-significant between NK-1R expression and expressions of caspase-3 and ki-67. Among data not included in the table, we highlight the significant association in OLP patients between a higher NK-1R expression in T lymphocytes and a more intense inflammatory infiltrate ($P < 0.05$). Table 3 depicts the significant associations between SP and Ki-67 expression in the epithelium basal layer. Tables 4 and 5 show significant and close-to-significant associations between SP and NK-1R expressions at different tissue levels. Among data not included in these tables, we mention the significant associations between NK-1R and SP expressions of lymphocytes from inflammatory infiltrate ($P < 0.005$), between NK-1R expression in epithelial cell membrane and cytoplasm ($P < 0.05$) and between NK-1R expression in epithelial cell membrane and lymphocytes from inflammatory infiltrate ($P < 0.05$).

Discussion

To our knowledge, this is the first study on the expression of SP and NK-1R in OLP. Expressions of SP, ki-67 and caspase-3 were significantly higher in OLP patients compared with controls in all tissue compartments analysed. NK-1R expression was also significantly higher in infiltrating lymphocytes and chorionic vessels of OLP patients compared with controls. Some studies have demonstrated that the local release of SP may contribute to the immune disorder that underlies some chronic inflammatory diseases (Cook *et al*, 1994). Thus, an increase in SP expression was reported during intestinal inflammation (Keränen *et al*, 1996a) and a highly elevated number of SP binding sites in small arterioles was observed in colon surgery specimens from patients with intestinal inflammatory disease (Manthy, 1991). In our view, the abundant expression of SP and its receptor in some autoimmune diseases and in OLP suggests that the local release of this neurokinin may contribute to the immune disorder underlying these diseases.

Substance P can be produced by B and T cells in an autocrine or paracrine manner, stimulating or enhancing the effects of lymphocyte proliferation and differentiation, lymphokine secretion and immunoglobulin production (Esteban *et al*, 2006). It has been shown that NK-1R expression and SP secretion by macrophages are induced by INF-γ (Marriott and Bost, 2000). In addition, macrophage production of IL-12 in response to INF-γ is enhanced by SP (Kincy-Cain and

Table 1 Immunohistochemistry findings in oral lichen planus (OLP) patients ($n = 50$) and controls ($n = 26$)

Variable	*OLP,* n (%)	*Controls,* n (%)	P	*Variable*	*OLP,* n (%)	*Controls,* n (%)	P
SP corium				SP TL			
−	1 (2.0)	20 (76.9)	< 0.005	−	0	15 (57.6)	< 0.005
+	49 (98.0)	6 (23.0)		+	9 (18.0)	9 (34.6)	
				+ +	17 (34.0)	2 (7.6)	
				+ + +	20 (40.0)	0	
				+ + + +	4 (8)	0	
SP mem				SP cyt			
−	2 (4.0)	25 (96.0)	< 0.005	−	1 (2.0)	26 (100.0)	< 0.005
+	27 (54.0)	1 (3.85)		+	29 (58.0)	0	
+ +	12 (24.0)	0		+ +	19 (38.0)	0	
+ + +	9 (18.0)	0		+ + +	1 (2.0)	0	
SP nucleus							
−	1 (2.0)	12 (46.5)	< 0.005				
+	10 (20.0)	13 (50.0)					
+ +	17 (34.0)	1 (3.85)					
+ + +	10 (20.0)	0					
+ + + +	12 (24.0)	0					
NK1R-TL				NK1R cyt			
−	12 (24.0)	19 (73.0)	< 0.005	−	43 (86.0)	26 (100.0)	0.1
+	18 (36.0)	6 (23.0)		+	4 (8.0)	0	
+ +	5 (10.0)	1 (3.8)		+ +	3 (6.0)	0	
+ + +	15 (30.0)	0					
NK1R mem				NK1R vess[a]			
−	45 (90.0)	26 (100.0)	0.3	+	12 (24.0)	6 (23.8)	< 0.005
+	1 (2.0)	0		+ +	7 (14.0)	1 (3.85)	
+ +	4 (8.0)	0		+ + +	20 (40.0)	0	
				+ + + +	1 (2.0)	0	
Ki 67 basal				Ki 67 suprab			
−	2 (4.0)	13 (50.0)	< 0.005	−	29 (58.0)	26 (100.0)	< 0.001
+	11 (22.0)	12 (46.2)		+	13 (26.0)	0	
+ +	14 (28.0)	1 (3.8)		+ +	4 (8.0)	0	
+ + +	19 (38.0)			+ + +	0	0	
NA	4			NA	4		
Caspase-3 basal				Caspase-3 suprabasal			
−	21 (42.0)	26 (100.0)	< 0.001	−	32 (64.0)	26 (100.0)	0.001
+	22 (44.0)	0		+	14 (28.0)	0	
+ +	2 (4.0)	0		+ +	0	0	
+ + +	1 (2.0)	0		+ + +	0	0	
NA	4			NA	4		

TL, T lymphocytes; mem, membrane; cyt, cytoplasm; NA, not assessed; SP, substance P; NK-1R, NK-1 receptor.
[a]Blood vessels in corium underlying epithelium were observed in only 40 cases and 7 controls.

Bost, 1997). T cells are reported to up-regulate NK-1R in response to antigen and IL-12 (Blum *et al*, 2001) and to increase INF-γ production in response to SP (Blum *et al*, 2003). The present results also support the proposition that actions mediated by the SP/NK-1R complex may contribute to the immune disorder that underlies OLP, as an association was found between NK-1R expression in T lymphocytes and the intensity of OLP inflammatory infiltrate. Furthermore, we believe that the significant association observed between SP expression in epithelial cell membranes and NK-1R expression in chorionic blood vessels and epithelial cell membrane and cytoplasm suggests that pathogenic mechanisms related to SP overexpression are complemented by an increase in the number of NK-1 receptors that are able to receive orders mediated by this neurokinin, as demonstrated in other experimental models (Esteban *et al*, 2006). Our results also demonstrate a significant association in SP expression among different tissue levels (Tables 4 and 5), supporting the proposal that a diffusion pathway for SP of lymphocyte origin is established *via* the corium and epithelium, favouring its cellular action (Cook *et al*, 1994; Weinstock *et al*, 1998). Our findings may also suggest the possibility that SP originates from the epithelial cells themselves, since it is known to be produced by various cell lines (neurons, B-lymphocytes, T-lymphocytes, eosinophils). This hypothesis needs to be tested in future studies, for example by using ISH for SP.

Another important aspect of SP overregulation in OLP is related to the well-documented participation of this neurokinin in pathogenic pathways implicated in psychological stress (DeVane, 2001). SP is known to be the most abundant neurokinin in the mammalian central nervous system, and mapping studies have indicated that the NK-1 receptor is highly expressed in regions that are critical for the regulation of affective behaviour and neurochemical response to stress (de Felipe *et al*, 1998). These data suggest that local SP release may form a nexus between abnormal emotional states, very common in OLP (Rojo-Moreno *et al*, 1998) and the immune disorder developed in the disease.

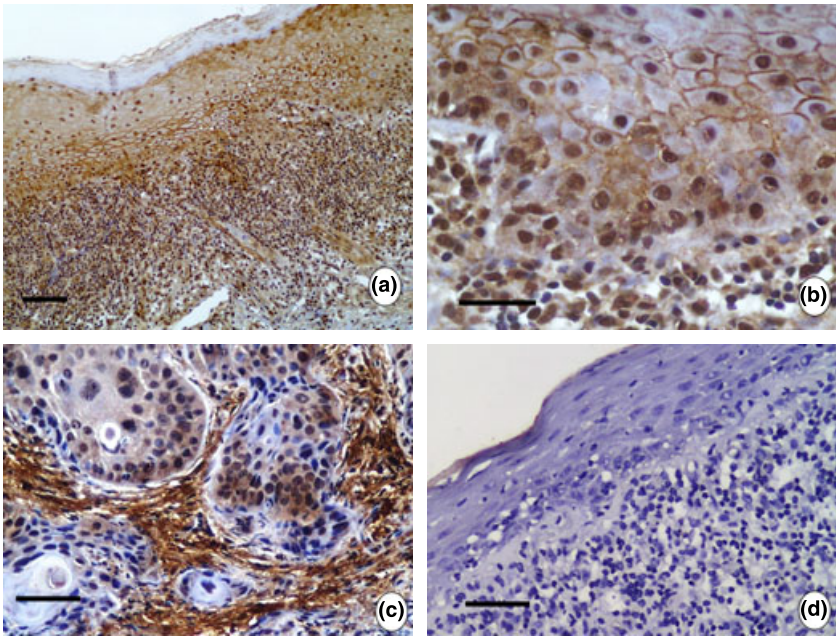

Figure 1 (**a**) Substance P expression in nucleus, cytoplasm and membrane of epithelial cells in OLP and in lymphocytes of the inflammatory infiltrate (immunohistochemical technique; 10×). (**b**) Higher magnification view of SP expression in OLP (immunohistochemical technique; 40×). (**c**) SP expression in squamous carcinoma used as positive control (immunohistochemical technique; 20×). (**d**) Image of tissue section of OLP used as negative control (immunohistochemical technique; 20×). Scale bar = 100 μm

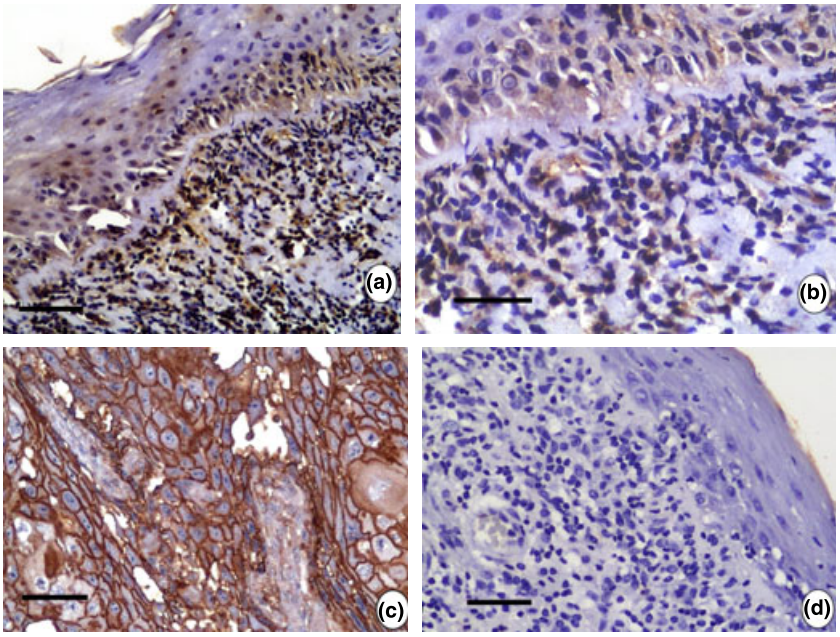

Figure 2 (**a**) NK-1R expression in cytoplasm of epithelial cells and lymphocytes in oral lichen planus (OLP) (immunohistochemical technique; 20×). (**b**) Higher magnification view of NK-1R expression in OLP (immunohistochemical technique; 40×). (**c**) NK-1R expression in squamous carcinoma used as positive control (immunohistochemical technique; 20×). (**d**) image of tissue section of OLP used as negative control (immunohistochemical technique; 20×). Scale bar = 100 μm

We highlight the direct association observed between the SP expression in membrane, cytoplasm and nucleus and the proliferative activity measured by ki-67 expression. It was previously demonstrated that apoptosis is infrequent and proliferative activity is high in OLP (Dekker *et al*, 1997; Bloor *et al*, 1999; Neppelberg *et al*, 2001; Tobón-Arroyaye *et al*, 2004; Bascones-Ilundain *et al*, 2005, 2006, 2007; González-Moles *et al*, 2006), which may represent a preservation mechanism for epithelium under attack in order to prevent ulceration (González-Moles *et al*, 2006). There is increasing evidence that these phenomena may be triggered by stimuli from the inflammatory infiltrate itself *via* the action of Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES) chemokine and cyclooxygenase-2 (COX-2). We believe that proliferative stimuli in OLP may also be triggered by the action of SP. Thus, SP/NK-1R complex is known to induce cell proliferation *via* activation of members of the MAPK cascade, including ERK 1/2 (22 of CM) and p38MAPK (Xia *et al*, 1995). Once activated, ERK1/2 translocates into the nucleus to induce proliferation (DeFea *et al*, 2000). SP also stimulates cell proliferation by transactivation of EGFR (Koon *et al*, 2004). This proliferative pathway acquires special relevance if it is taken into account that actions of SP can be specifically blocked by NK-1R

Table 2 Association of expression of NK1-R with expression of caspase-3 and Ki-67

	Variables					
	Caspase-3 basal		*Caspase-3 suprabasal*		*Ki-67 suprabasal*	
	–	+	–	+	–	+
NK1R in cytoplasm, *n* (%)						
–	21 (100.0)	19 (76.0)	30 (93.7)	10 (71.4)	26 (89.6)	13 (76.4)
+	0	3 (12.0)	2 (6.2)	1 (7.1)	3 (10.3)	1 (5.8)
++	0	3 (12.0)	0	3 (21.4)	0	3 (17.6)
P	< 0.05		< 0.05		0.06	
NK1R in T lymphocytes, *n* (%)						
–			3 (9.38)	6 (42.8)	10 (34.4)	0
+			14 (43.7)	3 (21.4)	11 (37.9)	6 (35.2)
++			3 (9.3)	2 (14.2)	4 (13.7)	1 (5.8)
+++			12 (37.5)	3 (21.4)	4 (13.7)	10 (58.8)
P			< 0.05		< 0.005	
NK1R in membrane, *n* (%)						
–					28 (96.5)	13 (76.4)
+					1 (3.4)	0
++					0	4 (23.5)
P					< 0.005	

Blank spaces indicate absence of significance.

Table 3 Association of SP expressions with Ki-67 basal layer expression

	SP in membrane					*SP in cytoplasm*					*SP in nucleus*					
	–	+	++	+++	P	–	+	++	+++	P	–	+	++	+++	++++	P
Ki-67 basal																
–	0	1 (50.0)	1 (50.0)	0	< 0.005	0	2 (100.0)	0	0	< 0.05	1 (50.0)	1 (50.0)	0	0	0	< 0.005
+	0	10 (90.9)	1 (9.09)	0		1 (1.09)	7 (63.6)	3 (27.2)	0		0	6 (54.5)	5 (45.4)	0	0	
++	1 (7.14)	12 (85.7)	1 (7.1)	0		0	12 (85.7)	1 (7.1)	1 (7.1)		0	3 (21.4)	10 (71.4)	0	1 (7.1)	
+++	1 (5.26)	2 (10.5)	7 (36.8)	9 (47.3)		0	28 (60.8)	16 (34.7)	1 (2.1)		0	0	0	9 (47.3)	10 (52.6)	

SP, substance P.

Table 4 Associations of substance P (SP) and NK-1R expression between different tissue levels

	SP in corium		*SP T lymphocytes*				*SP membrane*			
	–	+	+	++	+++	++++	–	+	++	+++
SP in cytoplasm										
–	1 (100.0)	0	0	0	1 (5.0)	0	0	1 (3.7)	0	0
+	0	29 (59.1)	7 (77.7)	11 (64.7)	10 (50.0)	1 (25.0)	2 (100.0)	20 (74.0)	6 (50.0)	1 (11.1)
++	0	19 (38.7)	2 (22.2)	6 (35.2)	9 (45.0)	2 (50.0)	0	5 (18.5)	6 (50.0)	8 (88.8)
+++	0	1 (2.04)	0	0	0	1 (25.0)	0	1 (3.7)	0	0
P	< 0.005		0.07				< 0.05			
SP in nucleus										
–			0	1 (5.8)	0	0	0	0	1 (8.3)	0
+			4 (44.4)	2 (11.7)	4 (20.0)	0	0	10 (37.0)	0	0
++			4 (44.4)	10 (58.8)	3 (15.0)	0	1 (50.0)	14 (51.8)	2 (16.6)	0
+++			0	2 (11.7)	8 (40.0)	0	0	2 (7.4)	3 (25.0)	5 (55.6)
++++			1 (11.1)	2 (11.7)	5 (25.0)	4 (100.0)	1 (50.0)	1 (3.7)	6 (50.0)	4 (44.4)
P			< 0.005				< 0.005			

antagonists. Thus, NK-1R antagonist L-773,060 has been used as an anti-inflammatory or analgesic (Bang *et al*, 2003) in some inflammatory diseases, including arthritis, intestinal inflammatory disease and cystitis (Quartara and Maggi, 1998), suggesting that L-773,060 may have therapeutic value in OLP. This drug, one of

Table 5 Associations between substance P (SP) and NK1-R expressions at different tissue levels

	SP membrane			
	–	+	++	+++
SP in nucleus				
–	0	0	1 (8.3)	0
+	0	10 (37.0)	0	0
++	1 (50.0)	14 (51.8)	2 (16.6)	0
+++	0	2 (7.4)	3 (25.0)	5 (55.6)
++++	1 (50.0)	1 (3.7)	6 (50.0)	4 (44.4)
P		< 0.005		
NK1R cytoplasm				
–	2 (100.0)	25 (92.5)	10 (83.3)	6 (66.6)
+	0	2 (7.4)	2 (16.6)	0
++	0	0	0	3 (33.3)
P		< 0.05		
NK1R membrane				
–	2 (100.0)	26 (96.3)	11 (91.6)	6 (66.6)
+	0	1 (3.7)	0	0
++	0	0	1 (8.3)	3 (33.3)
P		< 0.05		
NK1R vessels				
–	0	8 (29.6)	1 (8.3)	1 (11.1)
+	2 (100.0)	5 (18.5)	0	5 (55.5)
++	0	2 (7.4)	5 (41.6)	0
+++	0	12 (44.4)	5 (41.6)	3 (33.3)
++++	0	0	1 (8.3)	0
P		< 0.05		

the best known NK-1R antagonists, is approved by the FDA and no side effects have been reported, even at high doses (Esteban *et al*, 2006). Nevertheless, despite the above, the anti-tumour activity linked to NK-1R antagonists may be of greater interest (Esteban *et al*, 2006). Our group recently demonstrated that SP promotes tumour growth and L-773,060 has been reported to exert anti-tumour activity against human SKN-BE(2) neuroblastoma, GAMG glioma, COLO 679, COLO858, MELHO melanoma, WERI-Rb-1 and Y-79 retinoblastoma and HEp-2 laryngeal carcinoma cell lines (Keränen *et al*, 1996b; Muñoz *et al*, 2004, 2005a,b, 2007, 2008). This anti-tumour activity is specifically related to the capacity of antagonists to block the NK-1 receptors expressed by these cell lines (Muñoz *et al*, 2005a,b).

In relation to OLP, our group has proposed that high epithelial proliferation and low apoptosis may underlie the malignant transformation observed in some of these lesions (González-Moles *et al*, 2006; González-Moles *et al*, 2008b; Gonzalez-Moles *et al*, 2008a). The role of SP in OLP malignant transformation *via* its tumour growth stimulating actions is yet to be elucidated. However, the abundant expression of this neurokinin at all tissue levels in OLP lesions suggests its possible implication. The hypothetical protective role of treatment with the NK-1R antagonist L-773,060 against malignant transformation of OLP is of major interest and further research is warranted to explore this possibility.

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