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Evidence of neurotrophic events due to peritoneal endometriotic lesions

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ABSTRACT

To investigate the neurotrophic properties of endometriosis, as well as the involvement of neurotrophic factors in the development of chronic pelvic pain in patients with endometriosis, we performed a prospective clinical study. The presence of neurotrophins was investigated in the peritoneal fluid (PF) of patients with peritoneal endometriotic lesions or adenomyosis, as well as from women with non-endometriotic adhesions and from women without endometriosis/adenomyosis/adhesions. The PF from patients with peritoneal endometriotic lesions was divided in three groups: asymptomatic endometriosis, minimal pain and severe pain. PF from patients with adenomyosis or with non-endometriotic adhesions and the control group were divided in patients without pain and with pain. Neurotrophin expression in PF was analyzed using Elisa and the neuronal growth assay with cultured chicken sensory ganglia (dorsal-root-ganglia, DRG) and sympathetic ganglia. PF from women with peritoneal endometriotic lesions overexpress nerve growth factor (NGF) and neurotrophin-3 (NT-3), but not brain derived neurotrophic factor (BDNF), whereas the PF of women with adenomyosis or adhesions seems to express normal amounts of these factors. Neurotrophin expression did not differ among the pain groups. Furthermore, the PF from patients with peritoneal endometriotic lesions induced a strong sensory and a marginal sympathetic neurite outgrowth, while the PF from women with adenomyosis and non-endometriotic adhesions induced an outgrowth similar to the control group. The induced neurite outgrowth could only be inhibited in DRG incubated with peritoneal endometriotic lesions. Interestingly, the outgrowth of sympathetic ganglia was inhibited in all studied groups.

The present study suggests that only peritoneal endometriotic lesions lead to an increased release of NGF and NT-3 into the PF and that NGF modulates the nerve fiber growth in endometriosis.

1. Introduction

Endometriosis is a benign gynaecological estrogen-dependent disease, which induces chronic inflammation. This disease affects 10–15% of women in the premenopausal age [15,18]. The presence of epithelial, stromal and smooth muscle-like cells outside the uterine cavity characterizes endometriosis. As it is a disease of the uterine tissue, endometrial tissue is supposed to reach the peritoneal cavity through retrograde menstruation [33] or through an infiltration of the basal layer of the uterus into the myometrium, which leads to an ectopic manifestation [27]. Endometriosis can be classified as endometriosis genitalis externa, endometriosis genitalis interna, endometriosis extragenitalis and deep infiltrating endometriosis [37]. The endometriosis genitalis externa refers to ectopic endometriotic tissue in the peritoneum, ovaries (endometriomas), uterine ligaments, Douglas pouch, vagina or vulva [37]. The endometriosis genitalis interna is located within the myometrium (adenomyosis) or the fallopian tubes. Furthermore, endometriosis extragenitalis can appear in the recto vaginal septum, pelvic cavity, intestine and ureter [14,37]. Deep infiltrating endometriosis which can occur as deep infiltrating nodules (<5 mm) in the Douglas pouch, the sacrouterine ligaments or the recto vaginal septum, is also called adenomyosis externa [34,37]. Dysmenorrhoea, chronic pelvic pain, dyspareunia, dyschezia, dysuria and sub-fertility/infertility are the main symptoms of endometriosis [15]. The prominent symptoms of adenomyosis uteri are predominantly caused by the uterine dysfunction and results in abnormal uterine bleeding and dysmenorrhoea, but furthermore, adenomyosis is associated with pelvic pain [31]. The pain pathogenesis of peritoneal endometriosis as well as adenomyosis is not well studied and needs further investigations.

The presence of nerve fibers in direct contact to peritoneal, ovarian and deep infiltrating endometriosis has recently been confirmed [11,29,38], as well as the de novo innervation of peritoneal

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lesions in a rodent endometriosis model [10]. Furthermore, an imbalance between sensory and sympathetic nerve fibers in peritoneal endometriotic lesions has recently been demonstrated [4]. Current studies support the incidence of neurotrophic factors, like the nerve growth factor (NGF) and neurotrophin-3 (NT-3) in endometriotic implants [2,29], and brain-derived neurotrophic factor (BDNF) in ovarian endometriotic cysts [12], as well as the presence of NGF in the peritoneal fluid of patients with endometriosis [6], suggesting that endometriosis may be related to neurotrophic events. These data gives evidence of a neurotrophin participation in the endometriosis-associated innervation which is related to pain generation in endometriosis-associated symptoms [7].

In order to identify the role of neurotrophic factors in peritoneal fluid of patients with endometriosis, we investigated the NGF, BDNF and NT-3 expression in the peritoneal fluid of women with pelvic peritoneal endometriotic lesions, with adenomyosis with different pain severity and in controls (women with neither endometriosis nor adenomyosis or non-endometriotic adhesions) and the relevance of these neurotrophins in a sensory and sympathetic neurite outgrowth model.

2. Material and methods

2.1. Tissue samples

Peritoneal fluid samples from 93 patients with surgical and histological proven peritoneal endometriotic lesions or adenomyosis, and with non-endometriotic adhesions but without endometriosis or adenomyosis were obtained at the beginning of the laparoscopy and hysterectomy, respectively. Patients with other forms of endometriosis (ovarian endometriotic cysts or deep infiltrating endometriosis) were not included in this study. The samples were selected blindly and randomly. Patients with chronic inflammatory diseases or with cancer were excluded at the beginning of the study and their peritoneal fluids were not collected. Only 2 ml of clear peritoneal fluid was collected. In case of a dilution of the fluids, the fluid was discarded. The patient data was analyzed, an the patients were divided in peritoneal endometriosis, adenomyosis, non-endometriotic associated adhesions or control group as well as in different groups depending on the pain symptomatology and severity, than the samples were randomized. Peritoneal endometriosis was staged during the surgery according to the revised American Society of Reproductive Medicine (rASRM) [5] (rASRM I = 36, II = 29, III = 0, and IV = 0). All women with peritoneal endometriotic lesions included in this study ($n = 65$) were premenopausal with an age-range of 19 to 45 (mean age ± standard deviation (SD): 31.71 ± 7.65). At the time of the surgery, 19 patients received hormonal treatment (combined oral contraceptives or progesterone-only pill). The menstrual cycle phase was estimated using the first day of the last menstrual period (secretory phase $n = 27$, proliferative phase $n = 19$).

The preoperative pelvic pain score for the patients with endometriosis was determined via a standardized questionnaire with visual analogue scale (VAS) from 1 to 10 [7], where $\leqslant 3$ = minimal pelvic pain, 4–6 = mild pelvic pain, $\geqslant 7$ = severe pelvic pain (Table 1). Peritoneal fluids of patients with peritoneal endometriosis were divided into three groups: (a) women with asymptomatic endometriosis ($n = 11$), (b) women with minimal pelvic pain ($n = 27$) and (c) women with severe pelvic pain ($n = 27$).

All women with adenomyosis ($n = 17$) or adhesions ($n = 11$) were premenopausal with a mean age of 39.63 ± 11.42 or 36.33 ± 6.67, respectively (range: 19–52). At the time of surgery 24% with adenomyosis and 36% with adhesions were on hormonal treatment (combined oral contraceptives or progesterone-only pill). 8 women with adenomyosis and 3 women with adhesions without hormonal therapy were in the secretory phase and 5 women with adenomyosis and 4 women with adhesions in the proliferative phase.

Peritoneal fluid from women, who underwent laparoscopy for other benign gynaecological diseases (uterine fibroids), was used as control ($n = 30$), if endometriosis could be excluded. All control women were premenopausal between 23 and 42 (mean age ± SD: 32.75 ± 5.01). 20% of the women were under hormonal treatment (combined oral contraceptives/progesterone-only pill). 9 women without hormonal treatment were in the secretory and 15 women were in the proliferative cycle phase. The adenomyosis (AM), adhesions (AD) and control group (CG) were divided into patients with pelvic pain (AM: $n = 12$; AD: $n = 8$ and CG: $n = 18$) and without pelvic pain (AM: $n = 5$; AD: $n = 3$ and CG: $n = 12$).

All symptomatic patients suffered from pelvic pain. But only for the endometriosis group we could collect the severity of pain; regarding the other groups, we could only discover whether they

Table 1
Clinical data on the patient groups.

Patient information	pEL	AM	AD	Control
N	65	17	11	30
Age	31.71 ± 7.65	39.63 ± 11.42	36.33 ± 6.67	32.75 ± 5.01
Hormonal therapy	19	4	4	6
No hormonal therapy	46	13	7	24
Menstrual phase	0	0	0	0
Secretory phase	27	8	3	9
Proliferative phase	19	5	4	15
Uterine fibroids	0	0	0	30
rASRM				
I	36			
II	29			
III	0			
IV	0			
Pain form				
Pelvic pain	54	12	8	18
Dysmenorrhoea	45	11	0	10
Pain severity				
Asymptomatic	11	5	3	12
Minimal pain	27	n.a.	n.a.	n.a.
Severe pain	27	n.a.	n.a.	n.a.