Methylene blue dyeing of cellular nuclei during salpingoscopy, a new in-vivo method to evaluate vitality of tubal epithelium

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The Fallopian tube can be damaged by different noxious substances that may change cellular ultrastructure and function. Alteration of the cell membrane allows the passage of certain aniline dyes, which can stain the nucleus. A total of 310 Fallopian tubes from 163 patients who underwent a surgical or diagnostic laparoscopy during fertility studies was analysed by salpingoscopy. Cellular nuclei were stained by injection of 20 ml of a 10% solution of methylene blue in saline solution (NaCl 10%) through the cervical cannula prior to salpingoscopy. Evaluation of nuclear staining with methylene blue, adhesions, vascular alterations, and the flattening of folds in relation to pregnancy outcome was undertaken. Quantification of salpingoscopic findings was carried out according to a score. Flattening of folds and vascular alterations showed no difference in the pregnant and non-pregnant groups. On the other hand, adhesions and nuclear dyeing were significantly greater in the non-pregnant group (adhesions 13.6 versus 26.8%, P < 0.004, and nuclear dyeing: 25 versus 41.7%, P < 0.009, pregnant versus non-pregnant). Methylene blue dye is a new tool to evaluate in vivo cyto-histological tubal damage, and is a useful and simple method to provide a prognosis of salpingean function.

Key words: cell viability/dyed nuclei/Fallopian tube/methylene blue/salpingoscopy

Introduction

The Fallopian tube can be damaged by different noxious substances, and infections represent the most aggressive of these, leaving behind sequelae with a poor prognosis. Toxins attack the functions of cells (Vazquez et al., 1983; Hershlag et al., 1991; Cotran et al., 1994), acting on four ultrastructural systems which are fundamental for cell viability: (i) cell membrane, which regulates ionic homeostasis and osmotic equilibrium; (ii) aerobic respiration, fundamental for oxidative phosphorylation and production of ATP; (iii) protein synthesis; and (iv) integrity of genetic information and translation.

If alterations are irreversible, they produce cell death (necrosis or apoptosis) and the prognosis will depend on the percentage of dead cells (Vazquez et al., 1983).

Ultrastructural lesions in tissues can only be recognized by histology, and there are practically no in-vivo studies to demonstrate such damage (Vazquez et al., 1983; Hershlag et al., 1991).

Historically, from the beginning of hysterosalpingography (Sicard et al., 1922), clinical evaluation of the Fallopian tube concentrated on patency. However, considering a tube normal because it is permeable is a simplistic approach to the pathophysiology of the organ.

Salpingoscopy, which assesses the morphological component of the salpingean mucosa, improves the evaluation of the organ (De Bruyne et al., 1989; Kerin et al., 1990; Marconi et al., 1992), even though it does not reveal its physiological state.

Taking into consideration the degree and type of alteration of the components of the endosalpinx, different classifications have been suggested in order to quantify and interpret lesions from an anatomical point of view. Unfortunately, none of these evaluate tubal physiology (Brosens et al., 1987; Kerin et al., 1992; Maguiness and Djananbakhch, 1992; Scudamore et al., 1994b).

During salpingoscopy, tubal cells with stained nuclei after chromotubation with methylene blue have been described (Marconi et al., 1992). Such staining could reflect damage to the viability and functionality of the cytoplasmic membrane. Alterations to cell membranes allow the passage of certain aniline dyes such as methylene blue, which then stain the cell nuclei.

The objective of the present study was to show that staining tubal cell nuclei with methylene blue provides a simple cytological parameter for the direct indication of cellular damage.

Materials and methods

A total of 310 Fallopian tubes from 163 patients who underwent a surgical or diagnostic laparoscopy during fertility studies was evaluated by salpingoscopy. The rigid optic of the colpomicrohysteroscope (R.Wolff, GmbH, Knittlingen, Germany) was used. It was inserted into the abdominal cavity through a second incision. The methodology as well as the morphological results have been described previously (Marconi et al., 1992).

Prior to salpingoscopy, 20 ml of a 10% solution of methylene blue in saline solution (NaCl 10%) was injected through the cervical cannula. Through the same cannula, 50 ml of saline solution was subsequently injected in order to wash away excessive dye for better visibility.

When introducing the optic in the tube through the fimbrial ostium, in some patients intense blue dotting could be observed. By increasing the magnifying power to ×80, it could be seen that the dotting corresponded to staining of cell nuclei. Dyed nuclei were evenly aligned along the edge of the endosalpingean major folds, probably indicating staining of tubal cells (Figure 1A, C). Dispersed inflammatory cells with high affinity for the vital dye could also be observed.

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In order to quantify salpingoscopic findings, a classification was designed. A score of 1–4 was used to quantify the intensity of each pathology: flattening of folds, adhesions, nuclear staining and vascular alterations. Score 1 corresponded to absence of lesion and 4 to maximal intensity. Therefore a normal tube would have a score of 4, and a severely damaged one would total 16.
Figure 2. Distribution of salpingoscopic alterations in the pregnant and non-pregnant group. *$P < 0.05$ versus pregnant within each alteration. Vasc.alt. = vascular alterations, N.dye = nuclear dyeing.

Statistical analysis was performed by the $\chi^2$ test, and Student’s $t$-test.

Results

Of the 163 patients, 67 (41.1%) became pregnant.

Analysis of the frequency of altered patterns of salpingoscopic pathology revealed that flattening of folds and vascular alterations were not different between groups (31.3% and 20% in the pregnant group and 37.5% and 21.7% in the non-pregnant group) (Figure 2). On the other hand, the incidence of adhesions and nuclear staining was significantly greater in the pregnant group and 37.5% and 21.7% in the non-pregnant group, respectively. The lower concentration of nuclear colouring varied from isolated to moderate and to highly concentrated in the pregnant versus non-pregnant (Figure 2).

In peritoneal factors and tubal obstructions, scores were 3.08 and 2.21, respectively ($P = 0.23$), whereas scores in normal laparoscopies presented endosalpingean pathology and an increase in basophilia (Cotran et al., 1994).

Methylene blue is a vital dye which can stain neuronal axes and nerve terminals. This dye can enter the cell when there are structural alterations in the cell membrane, which can be induced by reversible or irreversible lesions. Nuclear basophilia is the result of picnosis in the nucleus.

Hershlag et al. (1991) compared salpingoscopic and histological findings in Fallopian tubes and found a significant correlation between studies. In histological electron microscopy studies, tubes with normal or mildly altered mucosa showed a homogeneous cytoplasm, abundant mitochondria, normal nuclei and a conserved ciliatory apparatus. By contrast, in lesioned tubes, there was a rupture of the plasma membrane, digested mitochondria, and vacuolization of the cytoplasm.

These are signs of irreversible cellular injury, which allow the passage of methylene blue. Therefore we can suggest that nuclear staining with methylene blue is the expression of damage to the tubal cells, which would not be detected using a simple salpingoscopic observation. The severity and

Discussion

The use of salpingoscopy as a complementary tool in evaluating the Fallopian tube has been extensively discussed, and most authors agree that it should be used systematically (Brosens et al., 1987; Shapiro et al., 1988; De Bruyne et al., 1989, 1997; Marconi et al., 1992; Marana et al., 1995b). The external appearance of the tube does not necessarily reflect the state of the lumen. De Bruyne et al. (1997) found that 49% of patients with normal laparoscopies presented endosalpingean pathology. A similar discordance (35.2%) was found in our studies (Marconi et al., 1992).

The most frequently described endosalpingean alterations are related to the folds of the mucosa, to the presence of adhesions which occlude the lumen of the tube or folds, or to alterations in salpingeal vascularization (Shapiro, 1988; Mershlag et al., 1991; Kerin et al., 1992; Marconi et al., 1992; Scudamore et al., 1994a). The presence of salpingoscopic adhesions which are sequelae of an inflammatory process will affect the prognosis of the tube (Brosens et al., 1987; De Bruyne et al., 1989, 1997; Marana et al., 1991, 1995a; Marconi et al., 1992; Scudamore et al., 1994a; Surrey and Surrey, 1996). From animal experiments, Vazquez et al. (1995a) suggest that flattening of the mucosa can be reversed, but emphasize the importance of the occurrence of adhesions as a pathological index.

Brosens et al. (1987) and De Bruyne et al. (1997) reported that 60% of pregnancies occurred in patients with hydrosalpinx without adhesions in their lumen, as assessed by salpingoscopy. This percentage decreased markedly in patients with adhesions, while the percentage of patients with ectopic pregnancies increased.

In the present study, the frequency of mucosal flattening and vascular alterations was not different between pregnant and non-pregnant patients. However, the presence of adhesions and of nuclear dyeing correlated negatively with the occurrence of pregnancies.

Noxious substances can alter cell function reversibly or irreversibly. Reversible changes affect the plasma membrane, the mitochondria, the endoplasmic reticulum or the nucleus. Irreversible changes, such as necrosis or apoptosis, increase cytoplasmic eosinophilia, which is reflected in electron microscope studies by a discontinuity in the cellular membrane and in the organelles, with intense alteration of the mitochondria. In the nucleus, pycnosis is evident, with nuclear constriction and an increase in basophilia (Cotran et al., 1994).

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prognosis of the organ will depend on the percentage of epithelium that is altered, and on the intensity of the phenomenon.

Vazquez (1995) have reported that there is a poor prognosis when inflammatory cells are present in the wall of the hydrosalpinx. The presence of inflammatory cells in the submucosa or the muscularis gives a worse prognosis. These cells, which are generally lymphocytes, have nuclei with a high affinity for methylene blue due to their high chromatin content.

We have previously described that in salpingoscopy after chromotubation, not only lymphocytes but also cells of the salpingeal mucosa were stained by methylene blue. This phenomenon was evident especially in patients who had suffered inflammatory processes (Marconi et al., 1992). When comparing the presence of adhesions and dyed nuclei relative to the pathology described by laparoscopy, we found that normal or endometriotic laparoscopies presented a practically normal score, whereas in laparoscopies with peritoneal factors or tubal obstructions, scores were significantly higher. Such data show the impact that infections have on the tubal epithelium. Irreversible cellular damage was detected which would probably influence the future of the organ.

In the present study, nuclear staining was present in almost 42% of the tubes from patients who did not get pregnant, with a score of 3 + 0.7, while nuclear staining was observed in only 25% of patients who got pregnant, with a score of 2.5 + 0.6, the differences being significant.

We can conclude that staining with methylene blue is an additional tool to evaluate in vivo the cyto-histological damage produced by toxins which have altered the integrity of the cell. It is a helpful method to provide a prognosis of salpingeal function. It is easy to use, representative of the pathology and can be quantified simply.

Salpingoscopy questions the reconstruction of the tube in patients with severe alterations of the organ, as the possibilities can be quantified simply. Laparoscopy or a simple salpingoscopy use in diagnostic laparoscopy.


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