قسم الفسيولوجيا
كلية الطب البيطري ـ أدفينا
رئيس القسم : أ٠د/ بهيج نعمة الله ٠

نفاذية الحاجز بين الدم والرحم في الفئران السويسرية بعد احداث التقرص المشيمي : أهمية تخليق البروستاجلاندين

بهيج نعمة الله

عند احداث التقرص المشيمي في بطانة الرحم للفئران السويسرية المنزوعة المبايض والمعالجة بالهرمونات المناسبة حدثت زيادة معنوية في أوزان قرون الرحم وفي نفاذية الحاجز بين الدم والرحم لجزيئيات صبغة الاكريفلافين المحقونة تحت الجلد٠

وقد انخفضت هذه التغيرات انخفاضا معنويا بعد معالجة الفئران بمادة الايندوميثاسين التي تمنع تخليق البروستاجلاندين ٠

أظهرت هذه الدراسة أن الحاجز بين الدم والرحم لا يزال يعمل تحت ظروف أحداث التقرص المشيمي في رحم الفئران والى جانب ذلك أظهرت أهمية تخليق البروستاجلاندين في عملية احداث التقرص المشيمي في الرحم ٠

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PERMEABILITY OF THE BLOOD-UTERINE BARRIER IN MICE FOLLOWING ARTIFICIAL DECIDUALIZATION: IMPORTANCE OF PROSTAGLANDIN SYNTHESIS

(With 1 Table & 2 Figs.)

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SUMMARY

Hormonal sensitization of the spayed mice followed by unilateral induction of decidual cell reaction (DCR) in the endometrium resulted in significant increases in both uterine weights and in permeability of the blood-uterine (B-U) barrier of decidualized horns over those with no decidual reaction (P/ 0.001) and (P/ 0.01) respectively. These changes were significantly reduced following indomethacin treatment (1 mg/100 g b.w. injected S./C. at 5:00, 10:00 and 15:00 on the 4th Day following the application of the decidual stimulus).

The results of the present study showed that the B-U barrier is still functioning following the induction of the DCR in mice uteri. Besides, the importance of prostaglandin synthesis in increasing the permeability of the B-U barrier during the DCR process was demonstrated.

INTRODUCTION

The existance of a B-U permeability barrier has been demonstrated in ovariectomized, estrogen treated rats (McRAE and KENNEDY, 1979 & 1981). Stimulation of the endometrium of these rats resulted in extensive decidual cell reaction (DCR). The stimuli used in producing the DCR vary from trauma (YOUCHIM and DeFEO, 1963) to intraluminal injection of chemical stimuli such as sesame oil (LEDFORD, RANKIN, MARKWALD and BAGETT, 1976), prostaglandin F_2 (PGF_2) (TOBERT, 1976) or adenosine monophosphate (FERNANDEZ-NOVAL and LEROY, 1978). The occurance of DCR is accompanied by increasing endometrial capillary permeability (KENNEDY, 1980; MILLIGAN and MIREMBE, 1984). Many factors are responsible for this increase in endometrial capillary permeability; histamine (TACHI, TACHI and LINDNER, 1970) and prostaglandins (EVANS and KENNEDY, 1978; KENNEDY, 1980) have been implicated as mediators of this vascular response. On the other hand, it has been shown that indomethacin, an inhibitor of prostaglandin biosynthesis (FLOWER and VANE, 1974), inhibited implantation in rats (TOBERT, 1976) and inhibited the increase in permeability of endometrial capillaries of pregnant rats (KENNEDY, 1977), hamsters (EVANS and KENNEDY, 1978) and rabbits (SNABES and HARPER, 1984 and JONES, ANDERSON, NORRIS and HARPER, 1986). Accordingly, the present experiment was undertaken to investigate the possible effect of prostaglandin synthesis inhibition by indomethacin, on the permeability of the B-U barrier in mice after induction of DCR in their uteri.

MATERIAL and METHODS

1- Hormonal sensitization and induction of DCR:

Mature female mice weighing approximately 21 g were ovariectomized under ether anaethesia. One week after ovariectomy, animals were started on a hormone regimen developed by FINN (1971) for maximum sensitization of the uterus to the decidual stimulus. Figure 1 outlines the schedule of this procedure. Artificial decidualization was induced by traumatization of one uterine horn in each female as described elsewhere (DUNGAN, WYNGARDEN and GORNETTE, 1968). Uterine responsiveness to trauma was maintained by subcutaneous injection of 400 ug progesterone plus 40 ng oestradiol-17B in olive oil/day.

2- Treatment with indomethacin:

On day 4, animals were divided into two equal groups each of six mice. One group was injected with indomethacin (img/100g body weight) in 0.2 ml olive oil administered subcutaneously at 05.00, 10:00 and 15:00h (EVANS and KENNEDY, 1978). The second group of mice received an equal volume of oil injected subcutaneously and was considered as a control group.

3- Permeability studies:

On the same day (Day 4) mice in the indomethacin and control groups were given a single subcutaneous dose of 2% acriflavin dye in saline (40 mg/Kg body weight) injected at 16:00h, then killed by decapitation 5h later (21:00h that day) as described elsewhere (NEMETALLAH and ELLIS, 1985). Blood samples were collected in heparinized tubes then centrifuged to separate plasma. The uteri were exposed through a midline incision. The cervical end of each horn was tightly ligated with a thread then dissected out and placed on a filter paper. Each horn was injected with 0.2 ml of 0.8% saline solution using a 30 gauge needle connected to a tuberculin syringe. The uterine horn was then massaged gently and the fluid was withdrawn into the syringe as completely as possible. The weight of each horn was recorded to the nearest mg after removing any piece of thread attached to the uterine horn. The concentration of acriflavin in samples of plasma and uterine perfusate was determined spectrophotofloremetrically uisng an Aminco Bowman spectrophotofluormeter. Fluorescence was read at 340 nm excitation wave and 680 nm emission wave. A standered graph was prepared using solutions of known concentrations of acriflavin. The concentration of acriflavin dye in the perfusate of each uterine horn was divided by the concentration of the dye in the same unit volume of blood plasma. This quotient, multiplied by 100, reflected that percentage of acriflavin molecules in the plasma which was able to cross the B-U barrier. Comparisons were made by Student's t test.

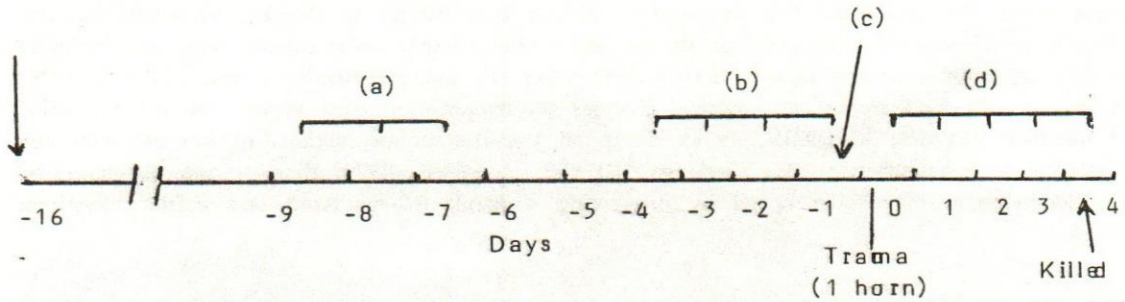

Fig. (1): Hormonal treatment of spayed mice to provide maximal sensitivity to traumatic decidulization. (a) 0.1 ug estradiol-17B (b) 1mg progesterone (c) 10ng estradiol-17B (d) 400 ug progesterone plus 40 ng estradiol-17B/day.

RESULTS

In the present experiment, hormonal sensitization of the spayed mice followed by unilateral induction of decidual reaction in the endometrium resulted in a significant increase inthe weights of the decidualized horns over those with no decidual reaction either in control or indomethacin treated mice (P/ 0.001). However, indomethacin (1mg/100g body weight injected S/C at 05:00, 10:00 and 15:00 on the 4th Day after decidual induction) caused a significant reduction in the % gain of weight of the decidualized horns as compared with corresponding values inthe decidualized horns of control animals (P/ 0.01) (Table 1). The changes in permeability of the blood uterine barrier to the macromolecules of acriflavin were closely parallel to the changes in uterine weights. The percentage of acriflavin dye molecules crossing the B-U barrier was highly increased in the decidualized horns than in the non decidualized horns in both control and indomethacin treated mice (P/ 0.01). Similarly, a significantly lower (P/ 0.05) percentage of acriflavin dye concentrations appeared in uterine lumens of decidualized horns of the indomethacin treated mice as compared to the dye concentrations in decidualized horns of control mice group (P/ 0.05) (Fig. 2).

The permeability of the B-U barrier to acriflavin molecules in the non decidualized horns of indomethacin treated mice was not significantly different from that in control mice.

DISCUSSION

The existance of a blood uterine permeability barrier (B-U barrier) has been previously demonstrated in ovariectomized, estrogen-treated rats (Mc RAE and KENNEDY, 1979 & 1981). The present study indicated that the B-U barrier is still existing and functioning under the condition (s) of artificially stimulated decidual cell reaction (DCR) in mice uteri. The DCR model has been used extensively inthe investigation of uterine responses to implantation in the rodent (GLASSER and CLARK, 1975 and LEDFORD, et al. 1976). The increase in weight of the decidualized horns observed in the present study is consistant with similar findings in mice (LODFORD, et al. 1976), rat (KENNEDY, 1977) and hamster (EVANS and KENNEDY, 1978) uteri. The weight gain in the decidualized uteine horns was attribute to increased cellular differentiation (LEDFORD, et al. 1976 and LUNDKVIST and NILSSON, 1982), increased endometrial vascular permeability (PSYCHOYOS, 1973; KENNEDY and LUKASH, 1982 and MILLIGAN and MIREMBE, 1984) and to the resulting oedema (FINN, 1977). The causes of the changes in vascular permeability are still unknown, although prostaglandins (PGs) have been strongly implicated (KENNEDY, 1980 and KENNEDY and LUKASH, 1982). The present results adds further support to the hypothesis that PGs are mediators of the cellular and vascular changes which preceeds and accompanies the DCR (TOBERT, 1976). Indomethacin treatment did reduce the mean weights of the decidualized uterine horns and decreased the permeability of the B-U barrier to the injectd acriflavin. This finding is in accordance with previous studies which showed that indomethacin treatment inhibited the DCR in pseudopregnant rats (CASTRACANE; SAKSENA and SHAIKH, 1974 and TOBERT, 1976) and delayed the appearance of vascular changes at implantation sites in rats (KENNEDY, 1977) and hamsters (EVANS; KENNEDY, 1978). Since all the therapeutic actions of indomethacin can be attributed to inhibition of PGs synthesis (FLOWER and VANE, 1974) then, it may be suggested that indomethacin may have acted by preventing a local, PG-mediated endometrial reactions.

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Table (1)

Effect of indomethacin (1mg/100 g body wt. at 05:00, 10:00 and 15:00h on Day 4) on body and uterine weights in mice 4 days after the induction of unilateral traumatic decidualization

	Body wt. (g)	Uterine wt. (mg/100g B.w.)		% gain in uterine wt.
		Non-decidualized horns.	Decidualized horns.	
Control	27.5±0.9	116.3±14.9^{a}	318.9±36.4^{a,b}	191.6±39.6^{c}
Indomethacin	24.9±0.4	103.4± 5.7^{a}	176.2±11.9^{a,b}	70.7± 8.0^{c}

Values are mean ± s.e.m. for 6 animals/group.

- Significantly different from each other in the same horizontal raw a) P/ 0.001.
- Significantly differnet from each other in the same vertical raw b) P/ 0.005 & c) P/ 0.01.

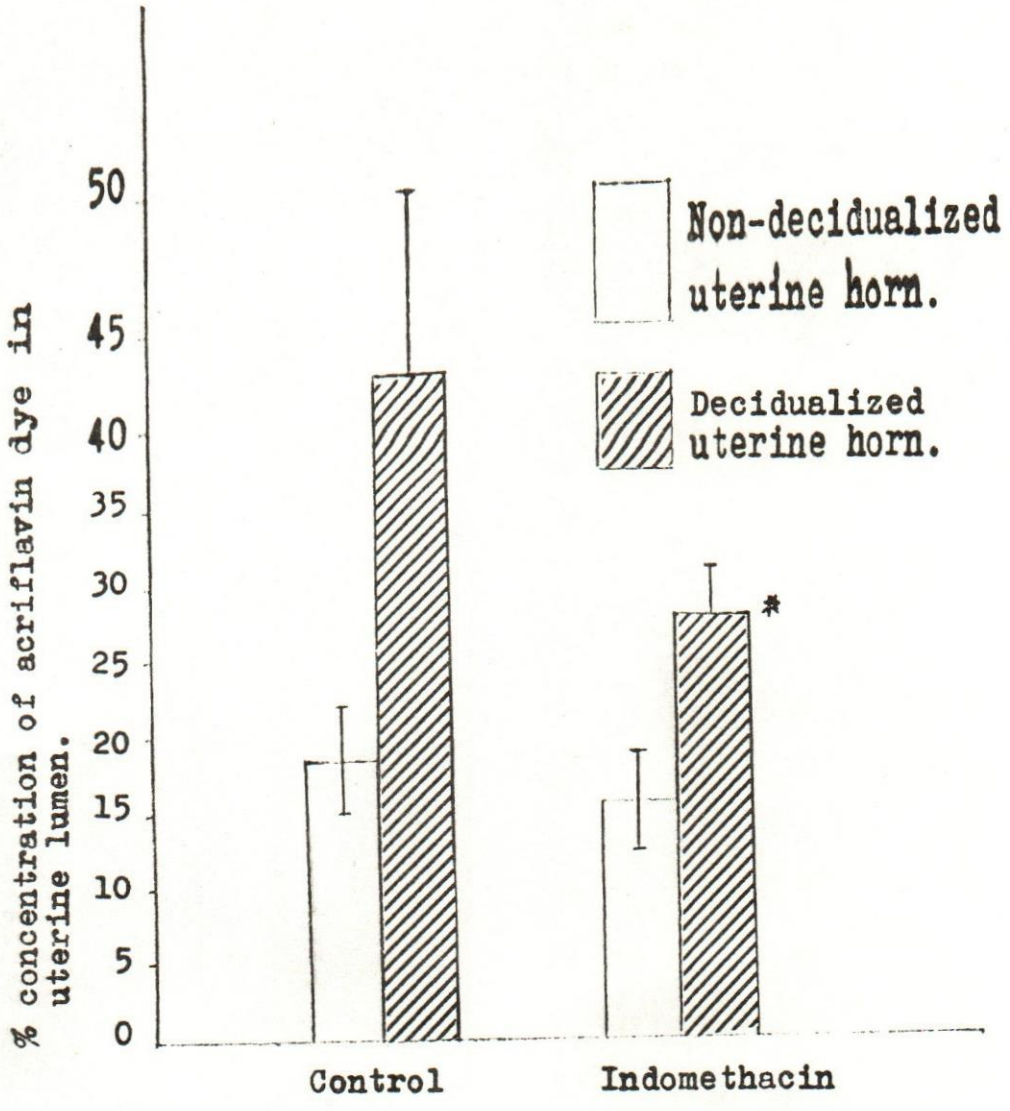

Fig. (2)

Percentage concentration of acriflavin molecules in the uterine lumen of control and indomethacin treated mice.

Unilateral decidualization was induced by traumatization of one uterine horn after proper hormonal sensitization. Values are means±s.e.m. for 6 animals. Significantly different from control * $P \leq 0.05$.