Platelet-activating factor antagonists and implantation in rabbits


Department of Obstetrics and Gynecology, University of Texas Health Science Center, San Antonio, TX 78284–7836 USA

In an initial experiment, rabbits were injected i.v. with a platelet-activating factor (PAF) antagonist CV-3988 twice a day on days 5 and 6 of pregnancy. Some inhibition of implantation was observed. This effect could not be reproduced in subsequent experiments at the same or at larger or smaller doses. The non-metabolized analogue of PAF, N-carbamyl-PAF (C-PAF) had an inhibitory effect on implantation only when given at toxic concentrations. When CV-3988 and C-PAF were given together on days 5 and 6, there was no effect on implantation. None of the other PAF antagonists tested – BN52021, SRI63,441, WEB2086 or TCV-309 – at various doses could inhibit implantation when given on the same days of pregnancy. TCV-309, at 0.1 mg kg\(^{-1}\) i.v. given on days 2–4 of pregnancy, was also ineffective. These results provide no clear support for a role of PAF in implantation in rabbits.

Introduction

Platelet-activating factor (PAF: 1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) is a potent lipid mediator, which is synthesized in many cells in response to inflammatory stimuli (Braquet et al., 1987; Hanahan and Kumar, 1987; Pinckard et al., 1988). It has been suggested that PAF is involved in many reproductive processes (Harper, 1989). PAF is secreted from the zygotes of a variety of species, including mice, humans and sheep (O'Neill et al., 1985; Angle et al., 1988a; Collier et al., 1988, 1990; Kodama et al., 1989; Adamson et al., 1991; Battye et al., 1991), although other workers have reported that PAF is not secreted by zygotes (Amiel et al., 1989; Smal et al., 1990). PAF is thought not to be secreted by unfertilized ova (O'Neill, 1987), and secretion of PAF is a good indicator of the probability that a zygote will give rise to a pregnancy (O'Neill et al., 1985), although this correlation is not absolute (Collier et al., 1990). Both mouse and human embryos exposed to PAF in vitro give rise to a better implantation rate after transfer to a recipient than do those in the absence of PAF (O'Neill et al., 1989; Ryan et al., 1990), and result in normal offspring (O'Neill et al., 1992). PAF receptors have been detected in rabbit oviduct membrane preparations (Yang et al., 1992), and secreted embryonic PAF could exert biological actions through such receptors.

PAF has also been detected in uterine tissue of rats (Yasuda et al., 1986, 1988; Nakayama et al., 1987), rabbits (Angle et al., 1988b) and humans (Alecozay et al., 1989, 1991). PAF appears to be located mainly in the endometrium in rabbits, and to increase rapidly during the first few days of pregnancy and pseudopregnancy (Angle et al., 1988b). In pregnant animals, uterine PAF concentrations fall sharply, especially at the site of implanting membranes, between days 6 and 7 (Angle et al., 1988b). PAF is a very potent inducer of vascular permeability, and the decrease of PAF at the implantation site could therefore imply involvement of PAF in the increased vascular permeability at the implantation site which occurs at the same time. Taken together, the available evidence suggests that PAF is linked to the implantation process, either as a cause or effect.

Attempts have been made to block the establishment of pregnancy by the use of PAF antagonists. In mice, PAF antagonists inhibit pregnancy, if given i.p. on days 1–4, and this effect is reversible by concomitant administration of PAF (Spinks and O'Neill, 1988). However, Milligan and Finn (1990) found that PAF antagonists given i.p. but every hour for 24 h after induction of implantation by oestradiol administration in mice with delayed implantation, or once daily for 4 days to intact mice, did not inhibit implantation. Furthermore, PAF itself administered into the uterine lumen failed to induce a decidual reaction. In contrast, Ando et al. (1990a, b) showed that two PAF antagonists, ONO-6240 and CV-6209, given during early pregnancy, decreased litter sizes in mice. CV-6209 was found to be most effective when given on days 4 and 5 of pregnancy, and this was apparently a maternal effect, as implantation was suppressed when day 4 embryos from saline-treated donor mice were transferred to CV-6209-treated recipient mice. Once implanted, however, embryonic growth and development were normal (Ando et al., 1990b). In addition, a PAF antagonist administered into the uterine lumen of rats inhibited implantation most effectively on day 4 (the day before implantation) (Acker et al., 1988), and PAF itself administered into the lumen on day 5 of pseudopregnancy induced a decidual reaction, which could be inhibited by concomitant administration of a PAF antagonist (Acker et al., 1989). To clarify the putative relationship of PAF to the implantation process, we conducted studies attempting to block implantation, in rabbits.
Table 1. Effects of platelet-activating factor (PAF) antagonists on implantation in rabbits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg kg⁻¹)</th>
<th>Days</th>
<th>Number of animals</th>
<th>Total number</th>
<th>Implantation rate (%)</th>
<th>Number per pregnant animala</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated</td>
<td>Pregnant</td>
<td>Corpora lutea</td>
<td>Implantations</td>
</tr>
<tr>
<td>None</td>
<td>Vehicle</td>
<td>5, 6</td>
<td>22</td>
<td>19</td>
<td>252</td>
<td>176</td>
</tr>
<tr>
<td>CV-3988</td>
<td>2.0</td>
<td>5, 6</td>
<td>27</td>
<td>11</td>
<td>345</td>
<td>91</td>
</tr>
<tr>
<td>None</td>
<td>Vehicle</td>
<td>5, 6</td>
<td>34</td>
<td>32</td>
<td>333</td>
<td>280</td>
</tr>
<tr>
<td>CV-3988</td>
<td>0.2</td>
<td>5, 6</td>
<td>6</td>
<td>6</td>
<td>61</td>
<td>49</td>
</tr>
<tr>
<td>CV-3988</td>
<td>2.0</td>
<td>5, 6</td>
<td>6</td>
<td>6</td>
<td>71</td>
<td>63</td>
</tr>
<tr>
<td>CV-3988</td>
<td>2.0</td>
<td>5, 6</td>
<td>6</td>
<td>6</td>
<td>64</td>
<td>55</td>
</tr>
<tr>
<td>CV-3988</td>
<td>4.0</td>
<td>5, 6</td>
<td>6</td>
<td>6</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>CV-3988</td>
<td>2.0</td>
<td>5, 6</td>
<td>6</td>
<td>4</td>
<td>53</td>
<td>30</td>
</tr>
<tr>
<td>+ C-PAF</td>
<td>1.0 µgkg⁻¹</td>
<td>5, 6</td>
<td>6</td>
<td>5</td>
<td>67</td>
<td>46</td>
</tr>
<tr>
<td>CV-3988</td>
<td>4.0</td>
<td>5, 6</td>
<td>6</td>
<td>6</td>
<td>58</td>
<td>51</td>
</tr>
<tr>
<td>+ C-PAF</td>
<td>1.0 µg k g⁻¹</td>
<td>5, 6</td>
<td>6</td>
<td>5</td>
<td>59</td>
<td>45</td>
</tr>
<tr>
<td>BN52021</td>
<td>0.2</td>
<td>5, 6</td>
<td>6</td>
<td>6</td>
<td>56</td>
<td>48</td>
</tr>
<tr>
<td>BN52021</td>
<td>2.0</td>
<td>5, 6</td>
<td>6</td>
<td>5</td>
<td>67</td>
<td>46</td>
</tr>
<tr>
<td>SR63, 441</td>
<td>2.0</td>
<td>5, 6</td>
<td>6</td>
<td>5</td>
<td>52</td>
<td>37</td>
</tr>
<tr>
<td>WEB2086</td>
<td>0.2</td>
<td>5, 6</td>
<td>5</td>
<td>5</td>
<td>46</td>
<td>40</td>
</tr>
<tr>
<td>WEB2086</td>
<td>2.0</td>
<td>5, 6</td>
<td>6</td>
<td>5</td>
<td>66</td>
<td>55</td>
</tr>
<tr>
<td>WEB2086</td>
<td>4.0</td>
<td>5, 6</td>
<td>5</td>
<td>5</td>
<td>65</td>
<td>51</td>
</tr>
<tr>
<td>TCV-309</td>
<td>0.1</td>
<td>2, 3, 4</td>
<td>6</td>
<td>6</td>
<td>61</td>
<td>47</td>
</tr>
<tr>
<td>TCV-309</td>
<td>0.1</td>
<td>5, 6</td>
<td>6</td>
<td>5</td>
<td>62</td>
<td>58</td>
</tr>
</tbody>
</table>

aValues are means ± SEM.
bDose given twice a day i.v. on days stated.
cImplantation rate in parentheses calculated excluding nonpregnant animals.
dOn day 7.
Materials and Methods

Chemicals

The PAF antagonists studied were: CV-3988, (rac-3-(N-n-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazoloethy phosphate), some of which was a gift from M. Nishikawa, Takeda Chemical Industries, Ltd, Osaka and some purchased from Wako Chemicals, Bioproducts, Richmond, VA; BN52021, (3-t-butyl-3-hydroxy-4,7,11-trihydroxy-8-methyl-9H,1,7a-epoxyxymethano-1H,6a-H-cyclopenta[c]furol [2,3-b]furo[3′,2′: 3,4]cyclopenta[1,2-D]furan-5,9,12(4H)trione), which was a gift from P. Bruket, Institut Henri Beaufour, Le Plessis Robinson, France; SRL63441, (cis (±) - 1 - 2 - [hydroxy - [tetrahydro - 5 - (octadecylaminocarbonyloxy)methyl][methylene]furan - 2 - yl] - methoxy-phosphoryloxy ethyl]quinoline hydroxide), which was a gift from D. A. Handley, Sandoz Research Institute, East Hanover, NJ; WEB2086, (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f]1,2,4]triazolo-[4,3-q][1,4]diazepin-2-yl]-1-(4-morpholinyl)-1-propanone), which was a gift from H. Heuer, Boehringer Ingelheim KG, Germany; TCV-309, (3-bromo-5-[N-phenyl-N-2-[(2,3,4-tetrahydro-2-isquinolylcarbonyloxyethyl]-carbomoyl]ethyl]carbamoyl-1-propylpyridinium nitrate), which was a gift from C. Hatanaka, Takeda Chemical Industries Ltd, Osaka; and C-PAF, (1-O-alkyl-2-N-methylcarbamyl-sn-glycero-3-phosphorylcholine), which is a non-metabolizable analogue of PAF, was purchased from Calbiochem Corporation (La Jolla, CA).

PAF antagonists were prepared as recommended by the suppliers. CV-3988 and TCV-309 were dissolved in 0.9% (w/v) saline at the appropriate concentration. CV-3988 is very insoluble; the suspension therefore required heating to 50°C for 5 min. The solution was then allowed to cool and used immediately. SRL63441 was dissolved in chloroform/methanol at a concentration of 10 mg ml⁻¹, and then diluted with saline to the appropriate concentration. BN52021 was dissolved in dimethyl sulfoxide at a concentration of 10 mg ml⁻¹, and then diluted with saline. WEB2086 was dissolved in 1 mol HCl l⁻¹ at a concentration of 300 mg ml⁻¹, rapidly neutralized with 1 mol NaOH l⁻¹, and then diluted with saline. C-PAF was dissolved in ethanol at a concentration of 10 mg ml⁻¹, and then diluted with saline. The final concentrations were adjusted so that the volume injected at any one time ranged from 0.1 to 0.5 ml (depending on the weight of the animal and the dose administered). As CV-3988 can be haemolytic, the solution was injected slowly. Doses chosen were in the ranges that were reported to be active in vivo following i.v. administration (Terashita et al., 1983; Handley et al., 1986; Casals-Stenzel et al., 1987; Takatani et al., 1990).

Animals and treatments

Mature New Zealand White–Cambridge crossbred female rabbits (body weight > 3.0 kg; Penn Acres, Wimberley, TX) were caged individually in a controlled environment with a photoperiod of 14 h light:10 h dark. They were fed 170 g of rabbit pellets day⁻¹, and provided with water ad libitum. Animals at oestrus were selected after examination for vaginal engorgement and inseminated with 0.5 ml of a mixed sperm suspension collected, via an artificial vagina, from fertile bucks immediately before use and diluted 1:1 with Krebs–Ringer bicarbonate buffer, pH 7.4. After insemination, the females were injected i.v. with 50 i.u. hCG (Sigma Chemical Co., St Louis, MO). In initial experiments with CV-3988, several groups of animals (three to eight animals per group for control and four to six control animals) were treated. Control animals received vehicle and treated animals CV-3988 (2 mg kg⁻¹ i.v. twice per day at 11:00 and 16:00 h) on days 5 and 6 of pregnancy. Over 15 months (March 1990–July 1991), a total of 27 treated and 22 control animals were studied contemporaneously. In this initial series of experiments, the animals were killed by an overdose of pentobarbitone sodium, given i.v., on day 7 of pregnancy. In the second series of experiments, groups of eight animals were inseminated at any one time, two of which acted as controls and received only the same vehicle used to dissolve the test compound i.v. at the same times as the six experimental animals received one dose of one test compound i.v. C-PAF alone or in combination with the PAF antagonists was injected twice per day on different days of pregnancy. In this second series of experiments, all animals were killed on day 8, except for one group given CV-3988 which was killed on day 7 in an attempt to replicate exactly the initial experiments. At autopsy, the number of implantation sites in each uterine horn and the number of corpora lutea on each ovary were recorded. In animals in the most advanced stages of pregnancy in the first series of experiments, the diameters of the implantation swellings were measured with vernier calipers. Where implantation did not occur, the uteri were flushed with 0.9% saline to detect the presence of unimplanted blastocysts. In the second series of experiments, results from control animals for each group were pooled, as there were no differences between implantation sites in control animals. χ² analysis was used to test for differences between percentages of oocytes ovulated and blastocysts implanting, and Student’s t test was used for differences between mean numbers of corpora lutea, implantation sites and their diameters.

Results

Effect of CV-3988

In the first series of experiments, implantation was partially inhibited after treatment with CV-3988 (Table 1). The CV-3988-treated animals that remained pregnant had normal numbers of implantation sites in the uterus and this suggested an all-or-none effect. Furthermore, the mean diameter (±SEM) of the implantation sites for 16 pregnant control animals of 7.70 ± 0.23 mm per animal (or 7.68 ± 0.20 mm per pregnant horn; n = 30) was not different from that for eight treated animals (7.48 ± 0.40 mm per animal, or 7.50 ± 0.30 mm per horn; n = 15). However, it was noted that when the uteri of the CV-3988-treated animals without obvious implantation sites were flushed on day 7, blastocysts were usually easily dislodged, but because of the flushing procedure was collapsed. Even in the treated animals with implantation swellings, dislodged blastocysts could often be recovered by flushing. In pregnant control animals, blastocysts were rarely dislodged when flushing was done at an equivalent time.
Table 2. Effects of C-PAF, a platelet-activating factor (PAF) analogue, on implantation in rabbits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose&lt;sup&gt;b&lt;/sup&gt; (μg kg&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Days</th>
<th>Number of animals</th>
<th>Total number</th>
<th>Number per pregnant animal&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated</td>
<td>Pregnant</td>
<td>Corpora lutea</td>
</tr>
<tr>
<td>None</td>
<td>Vehicle</td>
<td></td>
<td>12</td>
<td>10</td>
<td>105</td>
</tr>
<tr>
<td>C-PAF</td>
<td>1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5, 6</td>
<td>5</td>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td>C-PAF</td>
<td>1.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5, 6</td>
<td>5</td>
<td>4</td>
<td>55</td>
</tr>
<tr>
<td>C-PAF</td>
<td>0.5</td>
<td>5, 6</td>
<td>6</td>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td>C-PAF</td>
<td>1.0</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>63</td>
</tr>
<tr>
<td>C-PAF</td>
<td>1.0</td>
<td>5, 6</td>
<td>6</td>
<td>6</td>
<td>65</td>
</tr>
<tr>
<td>C-PAF</td>
<td>1.0</td>
<td>5, 6, 7</td>
<td>6</td>
<td>6</td>
<td>53</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± SEM.

<sup>b</sup>Dose given twice a day i.v. on days stated.

<sup>c</sup>Implantation rate in parentheses calculated excluding nonpregnant animals.

<sup>d</sup>First injection of 2 μg kg<sup>-1</sup> i.v. caused adverse reactions, and subsequent three doses were reduced to 1 μg kg<sup>-1</sup>.

<sup>e</sup>First injection was supposed to be 1 μg kg<sup>-1</sup> i.v., but one animal died rapidly owing to adverse reactions, and the subsequent three doses of 1 μg kg<sup>-1</sup> were from a freshly prepared solution.
Effect of CV-3988 and other PAF antagonists

Further experiments with CV-3988 at 0.2, 2.0 and 4.0 mg kg\(^{-1}\) on days 5 and 6 with autopsy on day 8 did not show an effect of CV-3988 on implantation, unlike the results achieved in the first series of experiments with 2 mg kg\(^{-1}\) with autopsy on day 7 (Table 1). Exact repetition of the first protocol with autopsy on day 7 was therefore performed to study the possibility that the apparent success in the first protocol was due to an induced delay of implantation of less than 24 h (rather than to inhibition) (Table 1). As in the second series of experiments, CV-3988 at a dose of 2 mg kg\(^{-1}\) i.v. given on days 5 and 6 of pregnancy did not inhibit implantation.

Experiments were conducted to study the action of 2 mg CV-3988 kg\(^{-1}\) and of 1 µg C-PAF kg\(^{-1}\) administered together. There was an indication of an inhibition (only four out of six animals became pregnant, and 56% of blastocysts implanted) with this combination. With the combination of 4 mg CV-3988 kg\(^{-1}\) and 1 µg C-PAF kg\(^{-1}\), instead of the expected greater inhibition, less inhibition was observed.

The actions of other PAF antagonists were also examined. None of these compounds inhibited implantation at any of the doses tested, although in the groups where an animal was not pregnant, the implantation rate was apparently reduced. However, if the implantation rate is calculated solely for the pregnant animals, on the assumption that pregnancy failed through chance and not treatment, it is clear that no reduction occurred. A few control animals also failed to become pregnant.

A final experiment was done with a low dose of TCV-309 given twice a day on days 3–4 of pregnancy to explore the possibility that starting treatment on day 5 of pregnancy was too late to inhibit some critical event. This treatment was also without effect.

Effect of C-PAF

The effect of a non-metabolizable PAF analogue, C-PAF, on the implantation process was studied (Table 2). The first group was scheduled to receive a dose of 2 µg C-PAF kg\(^{-1}\) i.v. twice a day, but adverse reactions were noted immediately after the first dose, and the subsequent three doses were reduced to 1 µg kg\(^{-1}\). Only one out of six animals remained pregnant. Neither 0.5 µg C-PAF kg\(^{-1}\) on days 5 and 6 nor 1 µg C-PAF kg\(^{-1}\) on day 6 only had any effect on implantation. These results then raised the question of the validity of the first experiment, in which toxicity had occurred, and the experiment was repeated. Again when toxicity was seen (one animal died after the first injection) implantation was inhibited. This result, combined with the previous result, strongly suggested that the inhibition seen was due to general toxicity and not to a specific effect of C-PAF on implantation. This contention was confirmed by two final experiments, in which 1 µg C-PAF kg\(^{-1}\) (freshly made solutions) was given twice a day on days 5 and 6, or 5, 6 and 7. In neither of these experiments was any toxicity or effect on implantation seen.

Discussion

In the experiments reported by O’Neill et al. (1990), antifertility effects were produced in mice by daily i.p. administration on days 1–4 of pregnancy of the PAF antagonists, WEB2086, BN52021 and SRI63,441, in descending order of potency. However, these authors note that not all compounds are active at all doses, and that there is a very narrow window of efficacy. For example, WEB2086 was about 50% effective at a dose of 22 nmol day\(^{-1}\) (conversion to 726 nmol kg\(^{-1}\) on the basis of 30 g body weight), marginally effective at 1.82 µmol kg\(^{-1}\), and not effective at 3.63 µmol day\(^{-1}\). Similarly, BN52021 was effective at 779 nmol kg\(^{-1}\), but not at 1.94 or 3.89 µmol kg\(^{-1}\). In contrast, SRI63,441 was effective at doses of 0.33–1.32 µmol kg\(^{-1}\). Consideration of these results led the authors to conclude that the antifertility actions of these compounds were restricted to a narrow range, perhaps because at higher doses the partial agonism exhibited by such antagonists predominated. They also observed that none of these compounds produced complete inhibition of implantation, that there was little correlation between their potency for anti-platelet compared to antifertility activity, and that lloprost (a stable analogue of prostacyclin) exhibited greater anti-platelet and antifertility activity than did any of the PAF antagonists.

In contrast, in experiments in rats 10 nmol (conversion to 52.6 nmol kg\(^{-1}\) on the basis of 190 g body weight) administered into the uterine lumen in 100 µl vehicle on day 4 of pregnancy reduced the percentage of rats pregnant from 100 to 18 (Acke et al., 1988). Earlier or later treatment was much less effective, strongly suggesting a specific action on the implantation process. With this protocol, local administration of doses of 10 fmol–100 nmol (52.6 fmol kg\(^{-1}\) to 526 nmol kg\(^{-1}\)) all showed some effect, with the effectiveness increasing with the dose. However, when BN52021 was given orally on days 2–5 of pregnancy, only a 20% inhibition of implantation was seen with a daily dose of 4 mg kg\(^{-1}\) (9.4 µmol kg\(^{-1}\)) and no effect was seen with doses of 8 mg kg\(^{-1}\) or greater (Acke et al., 1988). Thus, systemic administration was much less effective than intrauterine administration, and there was evidence for a window of efficacy, as in mice.

The experiments reported here differ in several ways from those described above, i.e. species, days and route of administration and compounds. The mouse is not a good model for antifertility tests. The rat has been widely used, but is very susceptible to the presence or absence of oestrogens, and so a positive result in rats should be checked in another species, usually the hamster – a species that does not require oestrogen for induction of implantation and is resistant to excess oestrogenic stimulation (Harper, 1972). As rabbits, like hamsters, do not require oestrogen to induce implantation (Wu and Allen, 1959), this species was used for the antifertility tests reported here. We had already shown that within 2 min of an i.v. injection of [\(^{3}H\)] C-PAF, radioactivity was localized at the implantation sites in day 6 and 7 pregnant rabbits (Kudolo et al., 1991), and as i.p. administration of drugs to rabbits is difficult and oral administration of PAF antagonists to rats less effective than intrauterine administration (Acke et al., 1988), i.v. administration was chosen for the present experiments. The experiments of Acke et al. (1988) in rats appeared to indicate a specific timing for the anti-implantation action of BN52021 when given locally; the antagonists were therefore given during the 48 h before implantation, when changes in uterine vascular permeability would normally be occurring, i.e. especially on day 6 of pregnancy. Doses shown by others to have biological actions when given i.v. were chosen. In
consideration of O’Neill’s theory about a U-shaped curve for efficacy of such compounds, doses similar in concentration to those that he found effective, were also tried. The compounds selected included CV-3988 which competes effectively for endometrial PAF receptors, and BN52021 and SRI63,441 which do not (Kudolo and Harper, 1989). The activity of WEB2086 and TCV-309 in this regard is not known, but both have been reported to be much more active in other systems than have any of the other antagonists studied here (Casals-Stenzel et al., 1987; Takatani et al., 1990).

The results of the first series of experiments with CV-3988 seemed to provide the expected result. Indeed, morphological changes of the endometrium that correlated with the anti-implantation effect were observed (GB Kudolo, CJ Noris, and MJ Harper, unpublished). Our inability to repeat these results in further experiments is unexpected and difficult to explain. One possibility is differences in batches of CV-3988 used. Clearly, if the anti-implantation efficacy was related to batch, then in the first series where efficacy was seen in 16 out of 27 animals, those treated last with material from the second batch should have remained pregnant. However, this was not the case, as pregnancies occurred apparently randomly throughout the period of the experiment. Consequently, use of a different batch does not appear to be an explanation for the failure to repeat the initial experiments.

A second, and more possible, is that because the animals in the first series were killed shortly after blastocyst attachment, any delay in this process would have resulted in delayed attachment of blastocysts or implantation swellings not or minimally detectable on day 7, but detectable on day 8. As treatment, at least with CV-3988, ceased 22 h before autopsy, blastocyst implantation or uterine vascular permeability changes might have been delayed, but then occurred during the drug-free period. (Purchase of adequate CV-3988 to continue treatment through day 7 was prohibitively expensive, so this could not be tested directly.) Treatment with TCV-309, a related and more potent compound, on days 5, 6 and 7 was, however, tested and found not to be effective. In addition, repetition of the experiment with CV-3988 at 2 mg kg⁻¹ on days 5 and 6 with autopsy on day 7 also failed to replicate exactly the results obtained in the first series, except that delayed attachment of blastocysts owing to treatment was confirmed. Rabbits do not normally experience delayed implantation similar to that that can be induced in mice and rats, but following treatment with indomethacin a reduced degree of vascular permeability at the implantation sites on day 7 and a reduction in blastocyst size, such that day 7 blastocysts were similar in appearance to day 6 blastocysts, have been reported (Hoffman et al., 1978). This evidence implies that a temporary delay to the implantation process can occur in rabbits, but with indomethacin treatment subsequent fetal growth was also compromised (Hoffman, 1978).

PAF antagonists may be effective if a different protocol is used, e.g. a longer period of treatment or a different route of administration. In the successful experiments of O’Neill et al. (1990), the mice were treated for 4 days, while in the unsuccessful ones of Milligan and Finn (1990) one experiment involved treatment every hour for the 24 h before implantation, but another protocol also used treatment for 4 days with SRI63,441 at a daily i.p. dose of 1.3 mg kg⁻¹, which was similar to the highest dose used by Spinks and O’Neill (1988). The failure of Milligan and Finn (1990) to obtain the same results as Spinks and O’Neill (1988) using the same compound and protocol might be explained by the results of Scodras et al. (1991), who showed that not all strains of mice experience thrombocytopeia in response to challenge with PAF. The explanation for the different results in mice might therefore lie in strain differences.

There is also the issue of the narrow window of effective doses for implantation inhibition, and thus whether, at least in rabbits, doses within this range were used in this study is uncertain, although some were similar on a per kg body weight basis to those that were effective in the experiments of Spinks and O’Neill (1988) in mice. There is little information on the use of PAF antagonists in rabbits in vivo, except that BN52021 inhibits the early inflammatory response to thermal injury in rabbits when it is perfused at 5–20 mg kg⁻¹ i.v. (Braquet, 1986). Nevertheless, from experiments performed in vivo in rats, mice, and guinea-pigs with PAF antagonists given i.v. over a range of doses, it is clear that the doses used in the study reported here were within the range of those biologically active for inhibition of PAF action in a variety of pathological states (Braquet et al., 1987). Furthermore, the U-shaped response curve was seen only for inhibition of implantation in mice. The reason for this narrow window of effectiveness in mice is unclear, and it is not known whether the same phenomenon occurs in rabbits.

It therefore appears that if PAF antagonists do exert antifertility effects it is in only very narrowly defined circumstances, and perhaps only in a permissive role in the implantation process. However, there is ample evidence that PAF is secreted by, and exerts marked stimulatory actions on, early embryos, is concentrated at the implantation site, and interacts with high affinity receptors on endometrial epithelial cells and thus may still play an important and physiological role during the preimplantation period.

The authors thank the companies concerned for the generous gifts of PAF antagonists. These studies were supported by NIH grants HD 14048 and 25224.

References

Platelet-activating factor antagonists and implantation in rabbits


Handley DA, Tonesch JC and Saunders RN (1986) Inhibition of PAF-induced responses in the rat. guinea pig, dog and primate by the receptor antagonist STI 64-411. *Thrombosis and Haemostasis* 56 40–44


Downloaded from Bioscientifica.com at 12/18/2018 05:29:00AM via free access