THE ACTION OF FURAZOLIDONE ON PREGNANCY

BY D. JACKSON AND J. M. ROBSON

From the Department of Pharmacology, Guy’s Hospital
Medical School, London, S.E. 1

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SUMMARY

1. Furazolidone (0.75 g/kg) will interrupt pregnancy in mice.
2. Foetal tissue is particularly sensitive to the drug at the time of or before implantation of the ovum.
3. Intra-amniotic injection of furazolidone in rabbits causes foetal death and resorption.
4. Furazolidone applied locally does not antagonize the proliferative effect of progesterone on rabbit endometrium.
5. The mode of action of furazolidone on pregnancy is discussed. It is suggested that it acts directly on the foetus.

Furazolidone (\(N\)-5(nitro-2-furfurylidene)-3-amino-2-oxazolidone) is one of a series of nitrated furan derivatives which are being used against a variety of microorganisms in both animals and man.

Nitrofurazone (5-nitro-2-furaldehyde semicarbazone) has been used in the treatment of testicular tumours [Wildermuth, 1955]. The same nitrofuran has been shown by Green & Friedgood [1948] and Friedgood & Green [1950] to depress the rate of growth of fibrosarcomata in mice.

Nissim [personal communication] has shown that the administration of furazolidone reduces the rate of growth of mice, and Rogers, Belloff, Paul, Yurchencho & Gever [1956] have demonstrated a similar effect in young rats. Didcock, Jackson & Robson [1956] have shown that a number of cytotoxic drugs can affect pregnancy, and it was thought of interest to investigate whether furazolidone has an effect on gestation, since at this time there is rapid new growth in the body.

METHODS

The experiments were performed on mice (albino C strain) and rabbits, using the methods described by Didcock et al. [1956]. In mice the duration of pregnancy was dated from the finding of the vaginal plug and in rabbits from the observed mating.

Furazolidone was administered to mice either in the diet or by gastric instillation of a suspension in olive oil. The drug was well mixed with M.R.C. diet 41 [Parkes, 1946] and each animal was given 5 g of the mixture each day throughout the experiment, the treatment being started at various stages of pregnancy. Furazolidone is only slightly soluble in olive oil and so was given partly in solution and partly in suspension, the mixture containing 100 \(\mu\)g/ml. The total amount was given in a single dose by stomach tube.

Furazolidone was administered to rabbits by intra-amniotic injection as a suspension in normal saline, the volume of every injection being 0.1 ml.
The drug was also applied directly to the rabbit's endometrium according to the method described by Höhn & Robson [1950]. For 7 days before insertion of the pellet the animal was given a daily subcutaneous injection of 5 μg oestradiol in 0.1 ml olive oil. On the day of insertion of the pellet and for the 3 succeeding days the animal received a daily subcutaneous injection of 0.25 mg progesterone in olive oil. Compressed implants of 50% furazolidone in a base of 75% lactose and 25% magnesium stearate were used. The animal was killed 4 days after insertion of the pellet and both the treated segments and pieces of the opposite uterine horn were taken for histological examination.

RESULTS

Effect of furazolidone on pregnancy in mice

The results are shown in Table 1. It will be seen that the foetus is more sensitive to the toxic action of furazolidone when the drug is administered before the 8th day of pregnancy. A dose of 1·0 g/kg interrupted pregnancy in all animals treated on the 7th day and when the drug was administered on the 1st day, pregnancy occurred in only one of ten animals treated as compared with fifteen of the twenty untreated animals. However, when 1·0 or 1·25 g/kg was given on the 10th day, interruption of pregnancy occurred in only two out of nine animals. The toxic effect on pregnancy developed quickly with doses of 2·0 g/kg, vaginal bleeding and abortion occurring within 24 hr of the administration of furazolidone. With smaller doses the effect was more gradual, there being a progressive loss in weight with resorption of the foetuses. There was no difference in the results obtained with the two methods of administration.

The litters produced by the mothers receiving furazolidone were all well below normal weight, but no congenital abnormalities were detected. Post-mortem examination of the mothers revealed no abnormalities. Some of these animals, subsequently interbred, produced normal litters.

Table 1. Effect of furazolidone on pregnancy in mice

<table>
<thead>
<tr>
<th>Mode of administration of furazolidone</th>
<th>Day of pregnancy on which treatment started</th>
<th>No. of abortions or foetal deaths/total studied</th>
<th>Maternal deaths/total studied</th>
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<tbody>
<tr>
<td>Nil</td>
<td>-</td>
<td>5/20</td>
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</tr>
<tr>
<td>0·2</td>
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<td>0/3</td>
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<td>0/10</td>
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<tr>
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<td>7</td>
<td>4/4</td>
<td>1/4</td>
</tr>
<tr>
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<tr>
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<tr>
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<tr>
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</tr>
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</tr>
<tr>
<td>-</td>
<td>1·0</td>
<td>2/6</td>
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</tr>
</tbody>
</table>
Effect of intra-amniotic furazolidone in rabbits

It seemed possible that the effect of furazolidone on pregnancy was due to a direct toxic action on the foetus, the foetal tissue being more sensitive to the drug than the maternal organism. The drug was therefore applied directly to the intrauterine tissues by intra-amniotic injection.

Four rabbits were given intra-amniotic injections of furazolidone on the 14th or 15th day of pregnancy. A laparotomy was performed 5 days after injection to determine the effect on pregnancy.

The injection of 1·0 mg furazolidone produced no effect on pregnancy (two sites in one animal), whereas a dose of 2·0 mg (four sites in two animals) and 2·5 mg (two sites in one animal) caused interruption of pregnancy at all the injected sites. When pregnancy was interrupted, abortion or absorption of the foetus occurred. The following example illustrates these effects. The uterus of a rabbit 14 days pregnant contained five foetuses. In two of these 2 mg furazolidone was injected into the amniotic fluid; the remainder were injected with 0·1 ml. saline. The mother was killed 6 days after injection and the uterine contents removed and examined. At the three sites where saline was injected the foetuses were alive and appeared normal. The weights of these foetuses were 2·87, 2·54 and 2·79 g, and the corresponding placental weights were 2·50, 2·47 and 2·66 g. Where furazolidone had been injected into the intra-amniotic fluid the foetuses had been absorbed, but the placentae remained and appeared normal; their weights were 1·53 and 2·24 g.

Effect of furazolidone on the progestational proliferation of the rabbit endometrium

It seemed possible that furazolidone produces its effect on pregnancy by direct hormone antagonism, and therefore the effect of the drug on the progestational proliferation of the rabbit endometrium was investigated.

One animal was used in this experiment. Two implants were placed in the left uterine horn, the right horn serving as control. Two separate segments of the untreated horn were examined and each showed a proliferation of grade 5 on the McPhail scale. Both implanted segments showed the same degree of proliferation as the control. Thus the local application of furazolidone does not antagonize the proliferative effects of progesterone on the rabbit endometrium.

DISCUSSION

Our experiments demonstrate that furazolidone will interrupt pregnancy in mice and rabbits. In the mouse the conceptus is much more sensitive to the action of furazolidone when the drug is administered before the 8th day of pregnancy, i.e. at or before the time of implantation of the fertilized ovum which occurs on the 6th day of pregnancy.

These results bear some similarity to those obtained with various anti-metabolic cytotoxic agents. Thus Thiersch [1954] found that when 6-mercaptopurine was administered to rats on the 12th or 13th day of gestation there was little effect on pregnancy, although stunted foetuses were produced. However, the foetus was much more sensitive at the time of implantation (7th–8th day) when 90 % foetal deaths were produced. A similar effect in mice was found by Didcock et al. [1956]. These workers
also found that the anti-metabolites D-glucosamine and 4-amino-\textsuperscript{N}^{10}-methyl pteroyl glutamic acid were almost completely inactive when given to mice between the 11th and 13th days of pregnancy. Thierson & Philips [1950] have shown that the folic acid antagonist aminopterin will interrupt pregnancy in mice and rats. As with 6-mercaptopurine the embryos were much more sensitive to the drug before the 10th day of pregnancy.

No abnormality was found at post mortem in the mothers which died following furazolidone administration, although death was usually preceded by convulsions. This is in agreement with the findings of Rogers et al. [1956] who, after a detailed investigation of the toxicity of furazolidone in rodents and dogs, concluded that the toxicity is manifested entirely as central nervous system symptoms and as effects on the spermatogenetic elements in the testis.

It is of interest that a reversible disintegration of the seminiferous tubules has been observed in man following the administration of nitrofurazone in doses of 5–7 mg/kg [Friedgood & Ripstein, quoted by Wildermuth, 1955]. Doses of 1·5–2·0 g/day (approximately 20–25 mg/kg) have been found to be effective against testicular neoplasms [Wildermuth, 1955]. This worker found vomiting and peripheral neuritis to be the most common toxic manifestations. Similar, though less pronounced, effects are seen with doses of 5–7 mg/kg in the treatment of urinary infections [Hasen & Moore, 1954].

The mode of action of furazolidone on pregnancy is uncertain. Paul, Paul & Kopko [1952] and Paul, Paul, Kopko, Bryson & Harrington [1954] showed that nitrofurazone interfered with the pyruvate-to-citrate stage of the Krebs cycle. This action could account for the similarity between the sensitivity of the foetus to furazolidone and anti-metabolic drugs.

It appeared possible that furazolidone might produce its toxic effect on pregnancy by direct hormone antagonism. According to Nissim [personal communication] furazolidone given in the diet antagonizes the vaginal effect of oestradiol given systemically. Since furazolidone is itself oestrogenic when applied locally to the vagina, we have been unable to investigate its relationship to oestrogen at the level of the target organ. It does not, however, antagonize the effect of progesterone on the rabbit endometrium. In their studies on the toxicity of furazolidone, Rogers et al. [1956] found no changes in the female reproductive organs with lethal doses of the drug. However, Friedgood & Green [1960] found atrophy of the ovaries following nitrofurazone administration.

The experiments with intra-amniotic furazolidone, in view of the comparatively small doses required, indicate that the drug produces its effect by a direct action on the uterine contents. When pregnancy is interrupted following intra-amniotic injection of furazolidone, the foetus is absorbed but the placenta remains intact. Furthermore, the placental weights are not significantly different from the controls. This would suggest that the effect of furazolidone on pregnancy is produced by a direct action on the foetus, which is more sensitive to the drug than the maternal organism.

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REFERENCES