Rat alpha-fetoprotein in experimental utero-placental ischaemia*

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Summary. Retardation of growth and death of fetal rats were produced after utero-placental ischaemia was induced by surgical ligation of the uterine arteries. Changes in maternal plasma levels of alpha-fetoprotein (AFP) were measured by radioimmunoassay. In rats in which the uterine blood supply was totally occluded, the resultant increase in maternal plasma AFP was due to resorption of fetal elements, because AFP levels in maternal rat plasma did not increase following hysterectomy in a control group. Maternal plasma AFP levels in rats with a partly occluded blood supply (and therefore some dead and some live fetuses) paralleled those of sham-operated rats, suggesting that increased placental transfer of AFP to maternal plasma may have offset the anticipated decline of AFP due to a decreased number of live fetuses.

Introduction

The presence of alpha-fetoprotein (AFP) in human fetal serum was first reported by Bergstrand & Czar (1956). Subsequently, the same protein fraction was identified in a number of mammalian species (Gitlin & Boesman, 1967a). Human AFP was thought to be a fetus-specific serum protein (Bergstrand & Czar, 1957), but high levels of this protein in adult human serum are also associated with primary liver cancer and other hepatic diseases (Kew, 1974). AFP is synthesized in the fetal liver and yolk sac of the fetal rat and is secreted into the fetal circulation (Gitlin & Boesman, 1967b), ultimately appearing in the maternal circulation. Feto-maternal transfer of radiolabelled rat AFP occurs via amniotic fluid and the placenta (Sell & Alexander, 1974). We have recently established the normal gestational pattern for rat AFP in fetal liver, fetal plasma, amniotic fluid and maternal serum using a radioimmunoassay (Lai, Forrester, Hancock, Hay & Lorscheider, 1976).

Several authors have reported that abnormal levels of AFP in human maternal serum are associated with fetal death or fetal distress (Seppala & Ruoslahti, 1973; Garoff & Seppala, 1973; Cohen, Graham & Lau, 1973) but the mechanisms responsible for altering maternal serum AFP levels are poorly understood. The purpose of the present study was to investigate the relationship between changes in maternal rat plasma AFP levels and a specific fetal stress, surgically induced utero-placental ischaemia.

Materials and Methods

Nulliparous female Sprague–Dawley rats (Canadian Breeding Laboratories, Constant, Quebec), weighing approximately 260 g, were maintained at 22 ± 1 °C with 12 hr light daily. After a controlled breeding schedule, surgery was performed on the 15th day of gestation, the rats being randomly

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allocated to four groups of 6 each. Animals were anaesthetized by an i.p. injection of sodium pentobarbital (30 mg/kg) and a mid-line abdominal incision was made. The arterial vessels supplying the uterus were ligated with silk suture thread at the ovarian and cervical ends of both uterine horns of rats in Group 1 (total occlusion) and at the cervical ends only for those in Group 2 (partial occlusion). In Group 3, all fetuses were removed by hysterectomy and Group-4 rats were sham-operated and handled in the same way as were the experimental animals. The number of live fetuses present in each animal was noted during surgery and the rats were then caged singly. Blood was withdrawn from the tail vein into a heparinized microhaematocrit tube immediately before and at specified times following surgery. Plasma (10–20 μl) was stored at −20°C in 1 ml 0·05 M-phosphate buffer (pH 7·5) containing 0·5% bovine serum albumin. Radioimmunoassay of plasma AFP was as previously described (Lai et al., 1976). The rats were asphyxiated with carbon dioxide on Day 20 of gestation and each live fetus and placenta was removed and weighed.

Results

The post-operative recovery of rats in Groups 2, 3 and 4 was uneventful. One rat in Group 1 died at 96 hr after surgery and data from this animal were excluded.

At autopsy on Day 20, all the fetuses in Group 1 were dead, macerated and partly resorbed; the uteri were necrotic with adhesions to the gut and mesenteries. In Group 2, the few fetuses proximal to the sites of ligation (36%) were dead and partly resorbed and the placentae were dark and necrotic. Fetal death had probably occurred shortly after ligation. The live fetuses distal to the sites of ligature appeared to be small for their age and the conceptus tended to be larger towards the ovarian end of the uterine horns. In the sham-operated animals (Group 4), 98·3% of the fetuses were alive. Comparison of the mean ± S.E.M. weights of the live fetuses and their placentae in rats in Groups 2 and 4 showed that those of Group 2 (fetal wt, 2·89 ± 0·15 g; placental wt, 0·61 ± 0·02 g) were significantly ($P < 0·001$) lower than those of Group 4 (fetal wt, 3·92 ± 0·19 g; placental wt, 0·78 ± 0·02 g).

The maternal plasma AFP levels in the various groups of animals are illustrated in Text-fig. 1. The plasma level of maternal AFP dropped precipitously for animals in Group 1 in the initial 6 hr after total occlusion of the uterine arteries but increased steadily after 12 hr and reached a peak at 36 hr. The AFP level in maternal plasma of animals in Group 2 after partial occlusion of the uterine arteries remained only slightly elevated until 48 hr after surgery when it more closely paralleled the level observed in sham-operated control rats. Plasma AFP in hysterectomized rats (Group 3) decreased steadily with a half-life of approximately 27 hr between 24 and 72 hr after surgery, and the pattern of maternal plasma AFP levels during pregnancy in the sham-operated controls (Group 4) was similar to that previously reported for normal pregnant rats (Lai et al., 1976).

Discussion

Fetal growth is dependent on the genetic potential of the fetus, the fetal environment and, in particular, the maternal blood supply to the placenta. The fetal growth rate is obviously affected by the rate of exchange of nutrients and metabolites across the placenta. Experimental reduction of maternal blood to the placenta has been shown to cause retardation of fetal growth in rats (Wigglesworth, 1964) and in sheep (Creasy, Barrett, de Swiet, Kahanpaa & Rudolph, 1972). In the present study, significant intrauterine growth retardation of fetal rats was experimentally induced by the partial occlusion of the uterine arteries and was similar to that previously reported (Wigglesworth, 1964).

It was necessary to totally ligate the uterine arteries of both uterine horns to produce death of all fetuses. In these animals (Group 1), the initial decrease in maternal plasma AFP immediately after ligation was probably due to the physical occlusion of the uterine circulation and to the continued maternal catabolism of AFP. The subsequent increase in maternal plasma AFP concentration in these rats can be explained by the resorption of fetal elements into the maternal circulation. Theoretically, this phase of increase in maternal blood concentration of AFP could be explained by an
increase in maternal production of this protein because of changes in maternal hormonal or metabolic status associated with cessation of the pregnancy. This interpretation can be excluded, however, since plasma AFP levels continued to decrease in hysterectomized rats. The significance of these results is that, firstly, maternal catabolism of AFP and resorption of fetal elements play a role in altering the maternal plasma levels of AFP after fetal death, and, secondly, serial sampling of maternal blood is essential in attempts to utilize AFP measurement in the antenatal diagnosis of possible fetal compromise. Previous investigators (Garoff & Seppala, 1973) have speculated that the variability in human maternal serum AFP concentrations, which they measured in association with intrauterine fetal death, might be due to the degree of resorption of fetal elements as a function of the estimated time of fetal death. The findings in the present study indicate that maternal resorption of AFP from the rat fetus is a consequence of fetal death and that the variable levels of AFP manifest in maternal plasma are a function of the time of fetal death.

In pregnant rats with partial uterine arterial occlusion (Group 2), the levels of maternal plasma AFP are slightly higher than those of the sham-operated control rats during the first two postoperative days. This probably reflects the additive effect of resorption of some fetuses with the continued development of the majority; the anticipated decline in maternal serum AFP levels due to a decrease in the number of live fetuses is offset by the resorption of AFP from dead fetuses. Levels of AFP in maternal blood have been shown to increase with the number of normal fetuses in rats (Masseyeff, Gilli, Krebs, Calluad & Bonet, 1975). The subsequent period of decreasing maternal AFP in this experimental group between 48 and 72 hr after surgery is perhaps due to the declining contribution of AFP from resorption of dead fetuses. The increase in maternal AFP between 72 and 120 hr after surgery may reflect an increased transfer of AFP across the feto-maternal barrier which could change as a function of gestational age.

Several mechanisms have been postulated for increased AFP levels in abnormal human pregnancies during the third trimester (Adinolfi, Adinolfi & Lessof, 1975; Lau & Linkins, 1976). The
results of the present study show that the resorption of fetal elements into the maternal circulation can explain the alteration in maternal blood AFP levels in some circumstances.

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