Acta Chirurgica Hungarica, 31 (2), pp. 169–174 (1990)

Effect of Bacterial Endotoxin on Placentation of Rats

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(Received: March 14, 1989)

The effect of bacterial endotoxin on placentation in rats was studied on 160 CFY pregnant rats. Based on this experiment, it was concluded that *(i)* the endotoxin (1 mg/animal i.p.) inhibited placentation (in 90% of animal). *(ii)* The endotoxin-induced fetopathy almost exclusively resulted in abortion. *(iii)* The fetuses reacted to endotoxin with relatively the same degrees of suspectibility. *(iv)* The growth of surviving fetuses seemed to be undisturbed. *(v)* Endotoxin-induced damages in mothers first of all depend on the individual susceptibility of these pregnant animals and *(vi)* the endotoxin tolerance induced by radio-detoxified endotoxin (TOLERIN) significantly protects both the mothers and the fetuses against endotoxin challenge.

It has long been known that bacterial endotoxins may induce fetal death, fetal absorption, abortion and malformations in experimental animals, particularly in endotoxin-sensitive species (golden hamster, swine), but also in human studies [9, 10, 11, 13, 14, 18, 19, 22, 24, 25, 26].

The majority of earlier investigations were focussed on placental changes in the third trimester of pregnancy [9, 11, 12]. Only a few reports have been concerned with the effect and consequences of bacterial endotoxaemia during placentation [2, 9]. It is also known that numerous effects of endotoxin cannot be prevented or warded off by small doses of endotoxin administered parenterally [3, 5, 6, 7, 9, 16, 17]. This phenomenon is called endotoxin tolerance. Beside its useful (endotoxin tolerance-inducing) effect, bacterial endotoxin has several unwanted and dangerous (toxic, fever-producing) side-effects. That is why endotoxin-detoxification has long been the primary aim of research workers by retaining its beneficial effects. For this purpose a bacterial endotoxin preparation, TOLERIN, detoxified by ^{60}Co-gamma by Bertók et al. 14, 5, 8] has been in use in our laboratory for almost 15 years. Its clinical tests are currently underway. This preparation has also been applied in our experiment.

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In these experiments an answer has been sought to the questions as follows:

1. What is the effect of endotoxaemia on placentation?
2. What forms of fetal damage are resulted?
3. How do surviving fetuses develop?
4. What kind of maternal impairment is induced in relation to, or simultaneously with, fetal damage?
5. How can all these harmful effects be prevented by the previous induction of endotoxin tolerance?

Material and Methods

In the experiment female CFY rats (LATI, Gödöllő) of an average weight of 210 g were used. Based on vaginal smear tests, the females in proestrus were kept together with males, then, after repeated vaginal smear tests the 'sperm-positive' females were included into the experiment, a total of 160 animals divided into 4 equal groups. The day of seminal examination was the first day of pregnancy. The animals were kept on granulated rat food and tap-water *ad lib.*

Treatment: Each animal of group I was administered a 1.0 ml physiological saline solution i.p. on the 12th day of her pregnancy. Group II received 0.2 mg TOLERIN/animal endotoxin i.p. on day 10, group III 0.5 mg/animal endotoxin i.p. on the 12th, while group IV equal amounts of TOLERIN as group II, on the 10th day, then toxic endotoxin on day 12, similar to group III.

Endotoxin: LPS (*E. coli* 089)P_2 87061601 (OSSKI). The endotoxin was prepared from a fermentor culture of an *E. coli* 089 strain by using the warm phenol-water method of Westphal et al. [28].

TOLERIN: RD-LPS (P_2: 150 KGy) 87061601 (OSSKI).

Time of observation: From the administration of TOLERIN (from the 10th day of pregnancy) up to the 15th day following parturition. The animals were kept under standard laboratory conditions during the experiment, then from the 19th day of their pregnancy they were separated from each other. After the inoculations, the animals were continuously monitored for 48 hours, and the dead were immediately dissected. The visible changes were recorded. The uteri containing the small embryos arranged like a string of beads were fixed in 4% neutral formalin for histological study. Following paraffin-embedding they were stained with heamatoxylin-eosin. The offspring was weighed on the 15th day after birth and their sex was determined.

Results

The experimental results were summarized in two tables. These reveal that in group I, including animals given a physiological saline solution, and in group II given only radiodetoxified endotoxin, no maternal deaths occurred. There was no change in the animals' behaviour and 38 and 37 animals, respectively, delivered the fetuses.

In group III treated only with endotoxin, within 48 hours of their endotoxin exposure, 21 animals died and four of the survived animals gave birth to fetuses. In group IV, pretreated with TOLERIN, 13 animals died following endotoxin administration and subsequently 13 animals produced offspring.

TABLE 1

Group	No. of animals (sperm-positive)	Treatment (of pregnancy)		Deaths* within 48 hours after endotoxin challenge	Parturition
		TOLERIN on 10th day	endotoxin on 12th day		
I	40	−	−	−	38
II	40	+	−	−	37
III	40	−	+	21	4
IV	40	+	+	13	13

* No other deaths occurred

Pathological examination of animals died as a result of endotoxin challenge, disclosed typical organic changes characteristic of endotoxin shock, i.e., pulmonary edema, pulmonary haemorrhage, thymus bleeding, congestive enlargement of the liver, intestinal edema, segmental intestinal bleeding, thin colonic contents, mesenterial lymph node swelling and haemorrhage and swelling of the Peyer's plaques. Histology disclosed the following changes in the developing placenta: extensive necrosis in between the cells of both the chorionic and the trophoblastic layers, similar to those in the decidua. The invasion of fetal capillaries was moderate, with sporadic fibrin thrombi in the sinuses, on the maternal side of the labyrinthine layer.

In group III one of the 21 died animals was proved at dissection to be non-pregnant.

Discussion

It can be made probable from the data of groups I and III and from the literature [1, 15, 20, 21, 27] that pregnancy occurs in at least 90% of sperm-positive female rats. Consequently, in our experiment pregnant rats were supposed to be found at least in this proportion in groups III and IV. (This

TABLE II

Group	No. of newborn	Mean litter no. (min. and max. litter nos)	Sex ratio Male : female	Mean weight, g at 15 days
I	278	7.3 (2–14)	1.1 : 1	37.1
II	265	7.2 (2–14)	1.1 : 1	36.1
III	27	6.75(3–10)	0.9 : 1	36.7
IV	92	7.1 (2–11)	1 : 1	36.6

empirical fact is important because a 10-day pregnancy in rat cannot be safely established by non-invasive methods.) At the same time, data of group II show that TOLERIN in the applied dose does not have a permanent toxic effect either on the mother or the fetus [9].

The endotoxin administered during placentation killed a considerable part of the mothers in group III (a total of 21 animals died, but on dissection one was found not to be pregnant). Four of the surviving animals littered, much less than they were supposed to do. So it can be concluded that endotoxin killed a considerable part of the fetuses in the period of placentation. In summary, half of the mothers died, the other half survived endotoxin exposure and about one-fifth of the survivors gave birth to healthy fetuses, while four-fifths aborted.

The data of group IV revealed that preliminary induction of endotoxin tolerance afforded the mothers some protection against endotoxaemia (only 13 died of 40). At the same time the extra protection for the mother implied also an extra protection for the fetus.

In summary, two-thirds of the mothers died, two-thirds survived endotoxaemia, and half of the survivors produced healthy fetuses, while the other half aborted.

Changes disclosed by the pathological and histological studies of the died animals corresponded to the changes induced by edotoxaemia already described by us in the literature [9, 10, 12, 13, 18, 19].

Comparing the four groups, there was no difference concerning litter number and the average weight of the fetuses measured at their age of two weeks at a 5% concordance level. Concerning the sex rate, there was only one thing to be noted, namely that it shifted towards females in group III. However, this information was obtained on the basis of 4 litters and so it cannot be considered specific for the group.

In conclusion, a certain amount of endotoxin exerts its effect during placentation in a way that, due to a single applied dose, a considerable part of the mothers (about half of them in our experiment) died, some survived but aborted, while some survived and produced healthy fetuses. Apparently, in this process, the mother's individual sensitivity is predominant, therefore

the 'all but none' law seems to be valid, i.e., if the mother and her fetuses survive endotoxin exposure in the period of placentation then practically all fetuses are born healthy. This means that the fetuses of a mother respond with the same degree of individual sensitivity to endotoxin. The development of the viable fetuses up to the 15th day after parturition shows that a single endotoxin exposure does not cause a notable difference in growth.

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Untersuchung der Wirkung in der der Periode der Plazentation ausgelösten bakteriellen Endotoxämie bei der Ratte

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Untersucht wurde die Wirkung des bakteriellen Endotoxins in der Periode der Plazentation bei der Ratte. Die Experimente führten zu folgenden Feststellungen: 1. Im Falle einer, mit der i.p. Gabe von 1 mg/Tier Dosis Endotoxin ausgelösten Endotoxämie ist die Plazentation in bedeutendem Maße (bis zu 90%) gehemmt. 2. Die sich entwickelnde Fruchtschädigung ist fast ausschließlich eine Fehlgeburt. 3. Die Früchte reagieren mit einer relativ identischen individuellen Empfindlichkeit auf das Endotoxin. 4. Die Entwicklung der überlebenden Früchte scheint ungestört zu sein. 5. Die Grundlage der mütterlichen Schädigung ist die individuelle Endotoxinempfindlichkeit des Muttertiers. Ein Teil der trächtigen Rattenweibchen geht ein, ein Teil bleibt am Leben, bei ihnen kommt es aber zu einer Fehlgeburt, während einige Tiere gesunde Früchte auf die Welt bringen. Die vorangehende Auslösung der Endotoxintoleranz mittels TOLERIN (strahlendetoxiziertes Endotoxin) bot einem bedeutenden Anteil der Mütter und auch der Früchte einen Schutz gegenüber den katastrophalen Folgen.

[Исследование в экспериментах на крысах эффекта бактериальной эндотоксемии, вызванной в период плацентации

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В экспериментах на крысах авторы изучали действие бактериального эндотоксина в период формирования плаценты. Результаты экспериментов показали, что: 1) эндотоксемия, вызванная интраперитонеальным введением 1 мг эндотоксина, значительно (примерно в 90%) тормозит развитие плаценты. 2) Во всех случаях без исключения повреждение плода приводит к выкидышу. 3) Плоды примерно с одинаковой чувствительностью реагируют на эндотоксин. 4) В основе повреждения материнского организма лежит индивидуальная чувствительность к эндотоксину. Часть матерей погибает, другая часть остается в живых, но у них происходит выкидыш, несколько животных донашивает и рождает здоровых детенышей. 6) Предварительное выявление толерантности к эндотоксину с помощью ТОЛЕРИНА (детоксицированный облучением эндотоксин) спасло от гибели значительную часть как матерей, так и плодов.