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Current Issues

Cell sensitivity to transplacental carcinogenesis by *N*-ethyl-*N*-nitrosourea is greatest in early post-implantation development

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Abstract

In a clear demonstration of the changing sensitivity of the developing mammal to transplacental carcinogenesis, Ivankovic and Druckrey [S. Ivankovic, H. Druckrey, Transplacentare Erzeugung maligner Tumoren des Nervensystem: I. Äthyl-nitroso-harnstoff (ÄNH) an BD IX-Ratten, Z. Krebsforsch. 71 (1968) 320–360] exposed pregnant BD IX rats to a pulse of *N*-ethyl-*N*-nitrosourea (ENU), a reactive carcinogen with a half-life of 20 min. No tumors were seen with ENU exposure before gestation day 12, but the multiplicity of neurogenic tumors increased steadily thereafter and was greatest with treatment on day 20, followed by a decline in sensitivity for the last three days of gestation. Similarly, a transplacental study of ENU in the Syrian hamster [B.A. Diwan, S. Rehm, J.M. Rice, Age- and dose-dependent transplacental carcinogenesis by *N*-nitrosoethylurea in Syrian golden hamsters, J. Cancer Res. Clin. Oncol. 122 (1996) 643–652] found that the numbers of tumors induced were greatest after exposure of late fetal stages. While these observations suggested that embryonic cells are refractory to carcinogenesis, an alternative explanation could be that a significant tumor yield was not observed because too few target cells were present in the embryo. I have resolved this issue by combining these published data with others on the numbers of neuroectodermal cells in the developing BD IX rat brain [R. Müller, M.F. Rajewsky, Elimination of O^6-ethylguanine from the DNA of brain, liver, and other rat tissues exposed to ethylnitrosourea at different stages of prenatal development, Cancer Res. 43 (1983) 2897–2904] and total cell counts of successive developmental stages of the Syrian hamster fetus [P.J. Donovan, G.T. Smith, Cell sensitivity to transplacental mutagenesis by *N*-ethyl-*N*-nitrosourea is greatest during early gestation in the Syrian hamster, Mutation Res., 1999, this issue], allowing the risk per cell at different stages of gestation to be calculated. Sensitivity to carcinogenesis was found to be greatest early in gestation and to decrease as gestation proceeds. For the rat model, tumor frequency per cell changed from 1.3×10^{-6} at day 12 exposure to 2.6×10^{-6} at day 23 exposure, a 50-fold decrease. For the hamster model, the tumor-initiation rate decreased 1250-fold from 1.2×10^{-5} at day 7 exposure to 9.6×10^{-9} at day 13 exposure. Thus, two independent experiments with different rodent species demonstrate that sensitivity of individual cells to damage leading to transplacental carcinogenesis is greatest

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in the early fetus and lessens markedly as gestation proceeds, in parallel with changing sensitivity to mutation (Donovan et al., Mutat. Res., this issue).

Keywords: *N*-Ethyl-*N*-nitrosourea; Tumor; Embryo; Fetus

1. Introduction

One important effect of exposure of the developing fetus to certain chemicals is tumors after birth. *N*-Ethyl-*N*-nitrosourea (ENU), first used for transplacental carcinogenesis studies by Ivankovic and Druckrey [1], has been a particularly useful model compound. ENU is a water-soluble, highly reactive alkylating agent that is both mutagenic and carcinogenic [2]. It has a half-life of 20 min in physiological media [3] and thus has a pulse effect. Ivankovic and Druckrey [1] demonstrated that rat fetuses were 50-fold more sensitive to induction of neurogenic tumors than adults, and that the sensitivity varied with stage of gestation. Multiplicity of neurogenic tumors was highest when exposure to ENU was at day 20 of gestation and decreased after this point. No tumors were seen with exposures before gestation day 12.

Other rodent species develop different tumor types after transplacental ENU, but again the greatest number of tumors per animal has been seen after exposures near the end of gestation. In the mouse, transplacental ENU causes lung tumors with highest sensitivity on gestation day 16 [4]. Diwan et al. [5] reported that exposure of the developing Syrian hamster fetus to ENU induced mostly tumors of the peripheral nerves, again with highest incidence after treatment late in gestation. These data might seem to imply that cells in the embryo are resistant to the carcinogenic effects of ENU. However, Kleihues and Rajewsky [6] have pointed out that target cell numbers may be limiting at earlier developmental stages.

Results of transplacental tumorigenesis assays have customarily been expressed on a tumors-per-animal basis, because of the usefulness of these values for risk studies. However, evaluation of the sensitivity of individual cells is of critical importance for understanding the underlying mechanism. Since the total number of cells at various stages of gestation has been determined for the fetal rat brain [7]

Table 1
Neuroectodermal tumors per target cell induced in BD IX rat offspring on transplacental exposure to ENU

Day of exposure	Number of target cells per embryo/fetus[a]	Neuroectodermal tumors per offspring[b]	Tumors per target cell
7	–	0	0
8	–	0	0
9	–	0	0
10	3.2×10^3	0	0
11	3.7×10^4	0	0
12	2.3×10^5	0.3	1.3×10^{-6}
13	2.3×10^6	1.0	4.4×10^{-7}
14	7.8×10^6	1.5	1.9×10^{-7}
15	1.8×10^7	1.8	1.0×10^{-7}
16	2.7×10^7	2.2	8.1×10^{-8}
17	3.7×10^7	2.4	6.5×10^{-8}
18	5.2×10^7	2.5	4.8×10^{-8}
19	6.2×10^7	2.6	4.2×10^{-8}
20	7.8×10^7	2.7	3.5×10^{-8}
21	7.8×10^7	2.6	3.3×10^{-8}
22	8.5×10^7	2.6	3.1×10^{-8}
23	8.5×10^7	2.2	2.6×10^{-8}

[a] Data from Ref. [7].
[b] Data from Ref. [1].

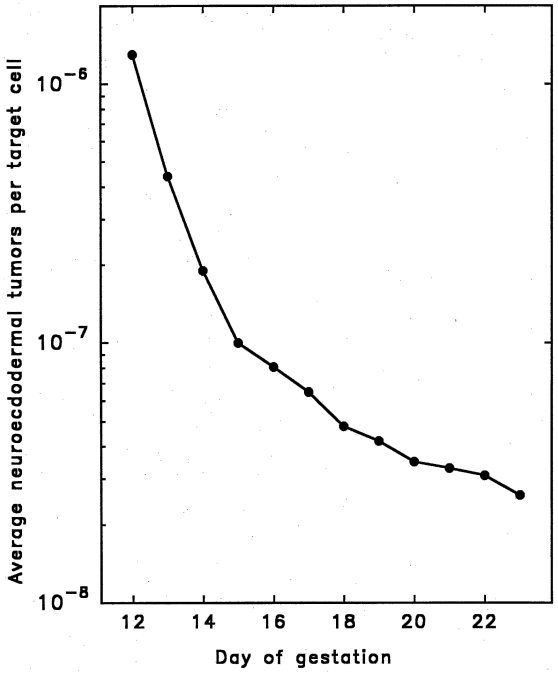

Fig. 1. Neuroectodermal tumors per target cell induced in BD IX rat offspring exposed transplacentally to ENU.

and now for the Syrian hamster fetus [8], these numbers can now be combined with the published tumor results for the two species [1,5] to estimate a tumor risk per cell.

2. Discussion

The number of neuroectodermal tumors per BD IX rat after transplacental exposure to ENU (60 mg/kg) was estimated from the data of Ivankovic and Druckrey [1] (Table 1). The maximum number of tumors per animal was 2.7, after exposure on gestation day 20. The numbers of neuroectodermal cells of the developing BD IX rat as determined by Müller and Rajewsky [7] are also listed in Table 1, and the values are combined in the plot of tumor frequency in Fig. 1. Risk of tumor initiation per neuroectodermal cell was greatest on gestation day 12, 1.3×10^{-6}, and declined thereafter to 2.6×10^{-8} at day 23, a 50-fold decrease. The curve appeared to be biphasic, with a reduction in line slope on gestation day 15, reflecting the slower growth rate of the brain after that time.

For hamsters exposed transplacentally to ENU (23 mg/kg), the average number of tumors per animal has been calculated from the data of Diwan et al. [5]. These were mainly neurogenic tumors, plus occasional neoplasms of thyroid, pancreas, skin, kidney, and other sites. The total tumors per animal varied from 0.05 when exposure was at day 7 to 1.8 when exposure was at day 14. For this model, the total number of cells in the fetuses as determined earlier [8] is listed in Table 2. As for the rat, the tumor frequency per cell was greatest at the earliest stage of gestation tested, day 7, at 1.3×10^{-5}, and declined exponentially to day 11, with a further decrease at day 13 to 9.3×10^{-9} (Fig. 2). The decline in sensitivity per cell between day 7 and day 13 is 1400-fold in the hamster.

These clear and striking correlations demonstrate that the sensitivity of the individual cells of the developing embryo/fetus to tumor initiation by a genotoxic agent is greatest early in gestation, as

Table 2
Embryonic cell sensitivity to carcinogenesis in Syrian hamster offspring exposed transplacentally to ENU

Day of exposure	Total number of cells per embryo/fetus[a]	Average tumors per offspring[b]	Tumors per cell
7	4.0×10^{3}	0.05	1.3×10^{-5}
8	5.3×10^{4}	0.23	4.3×10^{-6}
9	8.9×10^{5}	0.15	1.7×10^{-7}
10	7.3×10^{6}	0.39	5.3×10^{-8}
11	3.2×10^{7}	0.62	1.9×10^{-8}
12	4.6×10^{7}	1.2	2.6×10^{-8}
13	1.4×10^{8}	1.3	9.3×10^{-9}

[a] Data from Ref. [8].
[b] Data from Ref. [5].

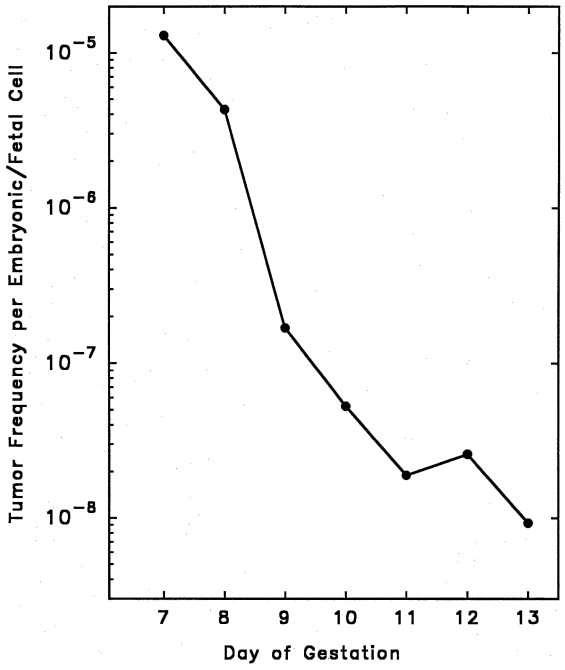

Fig. 2. Average tumors per embryo/fetal cell induced in Syrian hamster offspring exposed transplacentally to ENU.

might be predicted from the high rates of cell division and the undifferentiated character of the cells. In adult animals, dividing cells are known to be more susceptible to chemical carcinogenesis [9,10]. The results parallel the changing sensitivity of the embryonic/fetal cells to the mutagenic effect of ENU [8]. In the rat study, the lack of tumors resulting from exposure on gestation day 11 or earlier may be ascribed to the small number of tumor target cells and possibly to cell lethality due to the high dose of ENU used [6].

In several studies, the higher sensitivities of embryonic and earlier fetal stages has been apparent even from tumors-per-animal data. In the only strain of mouse that is susceptible to transplacental causation of neurogenic tumors by ENU, C3Hf/HeN, Wechsler et al. [11] reported 0.35, 0.36, 0.35, and 0.02 tumors per offspring after exposure on gestation days 13, 14, 15 and 18, respectively. In rabbits, ENU caused more neurogenic tumors when given on gestation days 8–10 than on days 15–25 [12]. Of particular relevance to humans, the fetal Patas monkey was susceptible to causation of brain tumors by ENU only if treatment started in the first trimester of gestation, before day 60 [13].

It is of interest in this context that qualitatively unique sensitivities have also been noted for early-gestation exposure. Goerttler et al. [14] found that leukemias were induced in mice by 7,12-dimethylbenz[a]anthracene only during the embryonic period.

3. Summary

The cells of the mammalian embryo and early fetus are not refractory to carcinogenesis, as is sometimes concluded from the absence or low incidence of tumors in offspring after exposure at these times. Their sensitivity to a direct-acting carcinogen is in fact high, as predicted from mutagenicity results [8].

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