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Review

Transgenerational effects from exposure to environmental toxic substances

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ABSTRACT

Exposure of mouse germ cells to radiation and chemicals results in mutation, malformation, cancer and other adverse effects (*e.g.*, functional disorders) in the offspring, though these findings have not been proven in human studies. Environmental toxic substances such as urethane (ethyl carbamate) which had been injected subcutaneously to 50 million people as a co-solvent of analgesics and dioxin (an endocrine disruptor) have been found to be associated with adverse effects in the progeny of mice after parental exposures. There are some reports on congenital malformations in the progeny of fathers who had been exposed to dioxin. However, these substances have not shown mutagenicity in *in vitro* assay systems such as bacterial systems even with S9, cell transformation assays, *etc.*, in spite of their potent teratogenicity and carcinogenicity in *in vivo* systems. Urethane was negative in the mouse specific locus test for germ cell mutations, but elicited a significant response at the same loci in the offspring of mice treated during pregnancy. Further, urethane is a mutagen in *Drosophila* germ cell tests, specifically inducing point mutations. Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) does not induce *in vivo* somatic mutations in mice and rats. It does not induce chromosomal aberrations when the mouse and/or human sperm are treated, but induces mutations at ESTR (expanded simple tandem repeat) loci in mice at low frequencies and also congenital malformations.

In this paper, we first present an overview of the results of our studies on transgenerational effects of these toxic substances, compare the results with those obtained after radiation exposure, and then discuss our subsequent studies to reconcile the problems underlying their mutagenicity, teratogenicity and carcinogenicity.

Contents

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1. Introduction

Radiation and environmental toxic substances induce various types of adverse effects (*e.g.*, abortion, malformation, mutation, and cancer) in the progeny of mice and rats after transplacental (*in utero*) exposures or parental (germ cell) exposures. Extensive studies on *in utero* exposures were conducted from late 1960s to mid-1970s, and 41 chemicals were proven to induce cancers in the offspring [1–9]. These animal studies gained support from human studies which showed that exposure to diethylstilbestrol during pregnancy for threatened abortions was associated with a higher risk of cancer among children [10]. This was also true of *in utero* exposure of children to radiation from the atomic bombs [11]. Other adverse effects of *in utero* exposure include those on gonadal development and organ function [6,12] and hypersensitivity of the organ for the future development of tumors by postnatal environmental factors [13,14].

It is expected that exposure of germ cells to radiation or chemical mutagens may cause adverse effects such as cancer, malformation, abortion, *etc.* in the offspring derived from treated germ cells. However, only a very limited number of studies had been focused on this question and on the potential causal mechanisms. In order to fill this gap in knowledge, in 1967, we launched the first and largest series of mouse experiments with the ICR strain. We found that urethane (ethyl carbamate), 4-nitroquinoline-1-oxide (4NQO), and X-rays induced germ cell alterations causing tumors, malformations and embryonic deaths in the offspring [1,15–24]. These studies had been referred to under the headings of "Transgenerational Carcinogenesis and Teratogenesis", "Paternal Toxicology", or "Male-mediated Developmental Toxicology" [12,15–23,25,26]. Preconceptional exposure of females also induced such effects in the offspring [15–17,20].

Exposures of male mice and rats before mating, to chemical carcinogens (urethane, *N*-ethylnitrosourea, *N*-nitrosodiethylamine, 4-nitroquinoline-1-oxide, diethylstilbestrol, or cyclophosphamide), chromium or to X- or neutron irradiation can result in significant increase in the incidence of tumors in various organs (nervous system, lung, lymphoid tissue, ovary, uterus, liver, intestine, skin, forestomach, *etc.*) in the progeny, depending on the strains used, and sometimes in later generations (see Ref. [27–32]).

In humans, numerous epidemiological studies have suggested possible correlations between paternal exposure to chemical carcinogens or radiation and incidence of childhood cancers (see Ref. [29,33]). This association has been particularly frequent and strong for parental occupational exposures to metals [28,33]. A higher risk of leukemia and congenital malformations was reported in the children of fathers who had been exposed to radionuclides in the nuclear reprocessing plants and to diagnostic radiations [22,30,31,34–40]. However, no increases in adverse effects (mutations, malformation, cancer, *etc.*) have been demonstrated in the children of atomic bomb survivors in Hiroshima and Nagasaki, who had been exposed to higher doses of atomic radiations [41].

In this paper, we focus two specific environmental toxic substances, namely, (1) urethane (ethyl carbamate) which had been injected subcutaneously to 50 million people in Japan and some other countries as a co-solvent of analgesics [42], *etc.*, and (2) dioxin, a widespread endocrine disruptor. The puzzling feature of these substances is that while their teratogenic and carcinogenic properties have been well-established, there is, as yet, no evidence regarding their mutagenic potentials: in bacterial mutagenicity tests, these compounds have been found to be negative (even with S9). Cell transformation assays were, likewise, negative [43–45]. Urethane presents a paradox in the sense that it is a germ-cell mutagen in *Drosophila* [46,47], but does not induce specific locus mutations in mouse germ cells [48], although transplacental treatments induce *in vivo* somatic mutations at the same gene loci used in specific locus tests (the spot test) [49]. In this article, we first present an overview of the results of our studies on transgenerational effects of these toxic substances and comparisons with such effects after radiation exposures, and then discuss our subsequent studies aimed at reconciling the findings on their mutagenicity, teratogenicity and carcinogenicity in mice and humans.

2. Urethane as a mutagen, teratogen and carcinogen

It is well-known that urethane induces a high frequency of tumors, malformations, and chromosome aberrations in experimental animals. Carcinogenic effects of urethane via placenta [1–6] and via mother's milk [3] have also been reported. Furthermore, tumors and malformations were transmitted to the next generation of mice subsequent to urethane treatment [1,15–17,20].

2.1. Transgenerational effects of urethane

There is a significant increase in congenital malformations in the progeny of parental mice treated with urethane [15–17]. Generally, a higher rate of anomalies is detected prenatally than after birth, because many of the anomalies (for example, cleft palate, exencephalus, gastroschisis and buphthalmus) are lethal shortly after birth (Table 1). Open eyelid and tail anomalies, the predominant types among non-lethal anomalies, were shown to be transmissible to further generations [17,19,31].

Both radiation exposure and urethane treatment cause a significant increase of the number of tumors in the progeny, regardless of which sex is treated. The majority of the induced

Table 1
Incidence of tumors and malformations in the offspring of mice exposed to X-rays or urethane

Treatment to parent			Incidence (%)	
Agent	Sex	Dose (Gy or mg/g)	Anomalies detected in 19-day-old fetuses	Tumors in offspring 8 months after birth
X-rays	M	0.36–5.04	48/2,201 (2.2)[a]	153/1,529 (10.0)[b]
X-rays	F	0.36–5.04	25/942 (2.7)[a]	101/1,155 (8.7)[c]
Urethane	M	1.5	65/2,923 (2.2)[a]	136/1,254 (10.9)[b]
Urethane	F	1.5	52/1,262 (4.1)[a]	139/963 (14.4)[a]
Untreated		0.0	4/1,026 (0.4)	29/548 (5.3)

X-irradiation was by Toshiba KC-18-2A, 180KVp at a rate of 0.72 Gy min^{-1}. Data are from Ref. [15–17] and totals for various dosages, fractionations and age of exposure. Urethane was given as a single subcutaneous dose [15–17]. Pathology was detected both by gross examination and by histological methods. The induced anomalies were: cleft palate, kinky and/or short tail, dwarf, open eyelid, exencephalus, hydrocephalus, gastroschisis, polydactyly, syndactyly, gigantic toe, buphthalmus, hydatidiform mole, atresia hymenalis, mislobulation of lung or liver, and hemiplegia. Among non-lethal anomalies, open eyelid, the predominant type (41%), was transmissible to F_2, but transmission of tail anomaly was inconsistent. Other non-lethal anomalies are sterile or too rare to determine exact heritability. See details in Ref. [18]. Types of tumors are given in the text.
Results were significantly different from the control value at [a]$p < 0.001$, [b]$p < 0.01$ and [c]$p < 0.05$ by χ^2-test.