



Letter to the Editor

Reproductive toxicology

Dear Sir,

The article published in *Reproductive Toxicology* entitled, *In vitro and in vivo reproduction toxicology of 12 monoaminergic reuptake inhibitors: Possible mechanisms of infrequent cardiovascular anomalies* by Sloot et al. [1], presents the authors' views in determining the teratogenicity of a group of drugs utilizing whole embryo culture (WEC). The authors used a well-described protocol for WEC utilizing five exposures ranging from 0–9 µg/mL to 0–100 µg/mL. For example, the five exposures for fluoxetine and paroxetine were 0 µg/mL, 0.3 µg/mL, 1 µg/mL, 3 µg/mL and 9 µg/mL and for mazindol and venlafaxine were 0 µg/mL, 10 µg/mL, 30 µg/mL, 60 µg/mL, and 100 µg/mL. The rat embryos were harvested at 9.5 days and grown for 48 h at which time they were evaluated. The effects of the drugs were labeled as embryotoxic, potentially teratogenic or teratogenic. It is appropriate to label pathologic findings at 48 h as an embryotoxic effect. However you cannot label WEC pathologic findings as potentially teratogenic unless you have data indicating that the embryo will survive to a viable stage. Even more important you cannot label a chemical or drug as a teratogen unless you have evidence that the embryo will survive the exposure during organogenesis as a malformed fetus. Of course, some embryotoxins can cause the embryo to die with malformations before viability is reached.

Sloot et al. all believe that they can demonstrate a teratogenic effect using WEC since the authors labeled toxicological findings in the cultured embryos as evidence of a teratogenic effect. While it is true that the authors of this paper used the term embryotoxicity and potential teratogenicity in many places in the paper, they also describe paroxetine as a teratogen. Let me quote from the paper the following two sentences. "In vitro, paroxetine and the positive control retinol were the only compounds identified as a clear teratogen" (Abstract). In paragraph 3.1.2 under the Results section the following statement is also repeated, "Retinol and paroxetine were the only two compounds identified as clear teratogens." This conclusion is inappropriate because the whole-animal teratology studies cited by authors did not result in teratogenesis [2,3] and the exposures of paroxetine and fluoxetine in the WEC were greater than the exposures that occur in the human with these drugs. Furthermore, these authors were unable to determine whether the embryotoxic effects observed in WEC would result in lethality in vivo at these exposures. You cannot utilize only the results of WEC to determine human teratogenic risks, unless the investigator is certain that the exposure used in the WEC will not be lethal to the embryo in vivo.

The use of WEC can have the following positive goals or purposes.

- (1) Investigators who have produced congenital malformation in pregnant animals such as the rat after administering a drug or chemical can utilize whole embryo culture to determine the mechanism of action of the teratogenic effect. The first publication using the roller tube WEC technique was published in 1972 using teratogenic kidney antibodies that were potent teratogens when injected into pregnant rats early in gestation [4–9]. The article describing these experiments was published by New and Brent and demonstrated that the mechanism of embryotoxicity was interference with the yolk sac function and not a direct effect on the embryo. Once teratogenicity was demonstrated in a whole-animal model, exposures of yolk sac antibodies in WEC at the levels that occur in the circulation of the whole-animal model permitted the investigators to determine the mechanism of action (MOA). If WEC had been performed first, before there was evidence that teratogenic antibody had an effect in a whole-animal study, the results would only indicate that the antiserum was embryotoxic. We could not determine that it was teratogenic, because we would not know the level of teratogenic antibody that occurs in the whole animal in order to select the proper exposure in the embryo culture – and we would not know whether the embryos would survive to term at these exposures.
- (2) The second purpose of embryo culture is for screening. When a pharmaceutical company prepares a new drug, there frequently are not large quantities of the drug available and, therefore, the investigators cannot afford to utilize their restricted supply for whole-animal teratology studies. Utilizing WEC to screen for the embryotoxicity of the compound is perfectly appropriate; because it gives the investigators an idea at what serum levels they can observe or not observe embryotoxic effects.
- (3) WEC can be used to study many aspects of pharmacokinetics and determining the putative teratogen. For example, cyclophosphamide has been demonstrated to be teratogenic in humans and rodents. However, the putative teratogen of cyclophosphamide is its metabolic product phosphoramidate mustard, which is teratogenic in vivo and in WEC while cyclophosphamide is teratogenic in vivo but not in embryo culture [10,11].
- (4) WEC can be used to study many aspects of normal embryonic development. You cannot use embryo culture to determine whether an agent is going to be teratogenic in a whole-animal teratology study or in the human when you have no exposure data available in either the human or the animal model at the time that you initiate the WEC. The reason why utilizing WEC as the first project for determining teratogenicity and labeling an agent as a teratogen is inappropriate (when you do not know whether it is teratogenic in a whole-animal model) is because you have no idea whether the levels that produce an embryotoxic effect in the WEC would be lethal to the embryo in the whole-animal model. That is why the positive results

of WEC results are described as an embryotoxic effect. Teratogenicity infers that the abnormalities will be present at term in the liveborn fetus or as a dead fetus later in pregnancy.

- (5) WEC can be used to describe the actual developmental changes in the embryo that occur during organogenesis when exposed to an embryotoxic agent.

If the authors had examined the package insert or the information available in the Physician's Desk Reference [12] they would have found the following information under the section of Pharmacokinetics. When a human being is administered 30 mg of paroxetine that reaches the steady state, the C_{max} level for paroxetine is 61.7 ng/mL. Following a single dose of fluoxetine of 40 mg, the peak plasma concentration ranges between 15 and 55 ng/mL. In the WEC experiments with paroxetine and fluoxetine the investigators used the following concentrations: for paroxetine and fluoxetine; 0 μ g/mL, 0.3 μ g/mL, 1 μ g/mL, 3 μ g/mL and 9 μ g/mL (Table 1 in the Sloot paper). The results of these embryo culture experiments with paroxetine and fluoxetine are stated as follows. "Paroxetine at 3 μ g/mL induced specific malformations (fused brachial bar, swollen posterior neuropore) without signs of embryotoxicity, demonstrating a teratogenic potential. At higher concentration of 9 μ g/mL, specific malformations such as displaced/additional otic system and again brachial bar defects (fused or swollen) were apparent. In the case of fluoxetine, one embryo showed irregular formed branchial bars at 1 μ g/mL without signs of embryotoxicity."

In the embryo culture experiments using paroxetine the investigators had concentrations of paroxetine of 0.3 μ g/mL, 1 μ g/mL, 3 μ g/mL and 9 μ g/mL. This is respectively 5 times, 16 times, 48 times and 145 times the concentration that would be present in the human. Similarly for fluoxetine, if the same concentrations in the embryo culture of 0.3 μ g/mL, 1 μ g/mL, 3 μ g/mL and 9 μ g/mL were used these concentrations are equal to 6 times, 20 times, 60 times and 160 times the clinical serum levels of fluoxetine.

It is interesting that the authors did not use the WEC that contained serum concentrations that occur in humans who are treated with these medications. Furthermore, they demonstrated quite clearly that they had to have very high levels of paroxetine and fluoxetine to produce any effects in embryo culture. There is no drug or chemical that would not produce an embryotoxic effect if the concentration were raised to very high levels. But teratogens have threshold exposures below, which no deleterious effects are produced [13] and the authors demonstrated that the NOAEL (no adverse effect level) for embryotoxicity was far above the usual human exposures.

The most that we can conclude from WEC experiments if we have no evidence of teratogenicity from animal studies [2,3] or inconsistent evidence from human epidemiology studies is to indicate that a particular concentration of a drug or chemical is or is not embryotoxic, since we do not know if the embryo will survive [14,15]. The most important aspect of WEC is to utilize a serum concentration in the WEC that is similar to the serum level in the exposed human or exposed animal model that has demonstrated teratogenesis.

Although the animal teratology studies utilizing paroxetine and fluoxetine were negative [2,3], the authors still attempted to produce a "teratogenic effect" in a WEC model.

Could the authors explain how a drug can be teratogenic in WEC but not in vivo in an animal model? One of the reviewers of this Letter commented on Sloot et al.'s definition of teratogenicity. "A basic principle of toxicological hazard identification is to identify lesions in tissues following high doses of xenobiotic. A no adverse effect level is then defined in the species concerned. Regulatory in vivo developmental toxicity studies are expected to show a degree of toxicity at the highest dose levels [5]. Sloot et al. were justified in taking a similar approach in their in vitro experiments. The restric-

tive definition of teratogenicity used in this paper," i.e. "A specific malformation in the absence of effects on growth or development" is valid within the context of the stated objectives of the experiment." The problem with this restrictive definition of teratogenicity is that it is not a scientifically valid definition and does not apply to all teratogens.

You can have malformations caused by environmental agents that are not associated with growth retardation and there may not even be an increase in fetal loss during development. You can have exposures that produce growth retardation and death but do not result in live fetuses with congenital malformations. Why did these investigators not expose the WEC to levels that occur in the human when being treated with antidepressants. It is obvious that if Sloot et al. would have found no effect at those levels the topic of teratogenic potential and teratogenicity would not be relevant.

Another area of concern is the fact that the authors have described malformations of the branchial arches as indications that paroxetine can produce congenital heart disease. They provide no data to support this concept. While neural crest migration problems and other hypothetical causes of congenital heart disease are plausible mechanisms, they provide no evidence to support their hypothesis. Furthermore, they did not observe abnormalities of cardiac development in the WEC, even with very high concentrations of paroxetine.

The final paragraph in the author's abstract states the following: "It is suggested that observed specific malformations in vitro (e.g. branchial bars deformed, displaced or additional otic system), not noted in any (historical) controls, may be early ontogenetic indicators for infrequent CV-anomalies observed in vivo. Despite the low incidence of anomalies in vitro or in vivo, they may yet be clinically relevant as in the case of paroxetine."

Scientists should not draw conclusions from hypotheses. They must have objective evidence in order to support their conclusions. These authors have no evidence that in their WEC model that the cardiovascular malformations were produced at even highly toxic exposures. Furthermore, they have no evidence that at lower exposures that are still much higher than would be experienced by humans, that cardiovascular abnormalities or any malformations would be observed in viable fetuses in later stage pregnancies.

1. Summary

1. WEC has many useful scientific purposes.
2. You cannot utilize WEC to label a drug or chemical as a teratogen if the exposures utilized will result in embryonic or fetal death in vivo.
3. WEC can indicate that a drug or chemical is embryotoxic.
4. If the exposure level for clinical use of a drug or environmental exposure of a chemical is known, it should be used in WEC studies along with higher exposures as well.
5. You cannot predict that a drug is a cardiac teratogen from WEC when no cardiac malformations are observed, as in this study. You can generate many hypotheses, but unfortunately the hypotheses generated by Sloot et al. will not make cardiac malformations appear in their WEC experiments.
6. If whole-animal teratology studies are negative with clinically appropriate exposures, WEC cannot provide information that will label the drug as a teratogen.

Conflict of interest statement

I was contacted by Lilly Pharmaceuticals 5 years ago to comment on the results of an ATSDR (Agency for Toxic Substance and Disease Registry) committee report that reviewed the developmental effects of fluoxetine. The committee was convened by ATSDR

to examine whether the water supply content of fluoxetine represented a reproductive risk. I was paid a consultation fee for my review.

I was contacted by Glaxo-Smith Kline 2 years ago to review the literature dealing with the developmental effects of paroxetine. I have not received any payment for services for this consultation except for travel expenses to one meeting.

Sincerely,

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Robert L. Brent^{a,b,*}

^a Distinguished Professor of Pediatrics, Radiology, Pathology, Anatomy and Cell Biology, Louis and Bess Stein Professor of Pediatrics, Emeritus Chairman of the Department of Pediatrics, Jefferson Medical College, United States

^b Head of the Laboratory of Clinical and Environmental Teratology, Research Department, Alfred I. duPont Hospital for Children, United States

*Correspondence address: Room 308. R/A, Box 269, Wilmington, DE 19899, United States. Tel.: +1 302 651 6880; fax: +1 302 651 6888. E-mail address: rbrent@nemours.org

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