Studies of Thalidomide's Effects on Rodent Embryos from 1962-2008

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Thalidomide is a sedative drug introduced to European markets on 1 October 1957 after extensive testing on rodent embryos to ensure its safety. Early laboratory tests in rodent populations showed that pregnant rodents could safely use it, so doctors prescribed Thalidomide to treat morning sickness in pregnant women. However, in humans Thalidomide interfered with embryonic and fetal development in ways not observed in rodent tests. Pregnant women who take Thalidomide are at greater than normal risk for spontaneous abortion and for giving birth to children with developmental anomalies such as shortened, absent, or extra limbs, as well as a variety of heart, ear, and internal organ defects. The failure of rodent models to inform scientists of Thalidomide's teratogenicity in humans ignited debate about the proper use of cross-species testing during drug development.

Thalidomide was first marketed on 1 October 1957 by the West German pharmaceutical company Chemie Grünenthal, headquartered in Achen, Germany. Chemie Grünenthal hailed Thalidomide as multipurpose drug capable of treating morning sickness, restlessness in children, loss of vision, and some forms of cancer. By the late 1950s, the company was marketing Thalidomide in forty-six countries. In early 1961 doctors noticed an extraordinary increase in documented cases of children with a variety of birth defects, and they soon hypothesized that maternal exposure to Thalidomide during pregnancy caused these often-severe congenital abnormalities. By March 1962 many countries had banned Thalidomide, however by then greater than 10,000 babies worldwide were born with birth defects attributed to Thalidomide.

Those birth defects mystified researchers and government entities alike, because pre-marketing tests in lab mice and rats had showed no signs of teratogenic risk. Public outrage ensued over the lack of strict regulations in regard to drug testing. That outrage motivated a scientific debate over the efficacy of using model organisms, like mice and rats, in drug development. By 1962, researchers across scientific disciplines began studies on Thalidomide's teratogenic effects in various organisms. The most commonly used embryological model organisms during this time included rabbits, rats, and mice.

A 1962 study titled "Thalidomide and Congenital Abnormalities," by Victor Knapp, George Christie, and Mary Seller, all working in the UK, looked at the teratogenic effects of Thalidomide on rats, mice, and rabbits, and the study reported no abnormalities in the offspring of these animals after researchers had exposed the pregnant females to the drug. The authors noted that the study provided no grounds to think that drugs containing Thalidomide were safe for human use, and they argued that the only method guaranteed to safely deal with drugs of unknown teratogenicity would be to completely refrain from using them unless absolutely necessary. In 1963, Joseph A. DiPaolo, working in the US, discussed various birth defects found in mice fetuses whose mothers were fed Thalidomide daily, but
he found only one kind of anomaly called fetal resorption, or the partial or complete dissolution of fetal tissues after some embryos had died *in utero*.

In 1965, J.D. McColl and colleagues in Canada further illustrated the limitations of animal studies for the use of predicting human responses to potential teratogens. In their study titled "Effect of Some Therapeutic Agents on the Developing Rat Fetus," McColl and colleagues observed in rats an increase in resorption rate but no incidences of *phocomelia* [7], the absence or abnormal development of the limbs. In *humans* [3] limb truncation in offspring is a common congenital abnormality arising from Thalidomide use by pregnant women. This abnormality is caused by exposure to the drug in a short time period in early human embryonic development. Similar to Knapp, Christie, and Seller's statement from three years prior, McColl and colleagues stated that the factors involved in *embryogenesis* [8] and drug interaction are so complex that straightforward predictions from animal models to *humans* [3] were not possible.

From these studies, and many others, the scientists formed a consensus about the key feature of rodent embryos that grants them the resistance to Thalidomide's teratogenic effects. Mouse and *rat* [9] embryos both possess superior antioxidants than those in *humans* [3]. These *rat* [9] and *mouse* [10] antioxidants protected their embryos from the damaging free radicals that Thalidomide introduces into developing embryos.

Studies performed by William McBride [11] in New Zealand, and by Raymond Cahen in France, in 1961 and 1966 respectively, demonstrated that mice given megadoses of Thalidomide didn't exhibit classic toxicity. A 2004 study by Jun Lu, Lai-Ming Ching, and colleagues in New Zealand found that organisms from different species metabolize Thalidomide in different ways. The half-life for Thalidomide in blood plasma is considerably shorter in mice than in *humans* [3], and other model organisms, such as rabbits, display half-lives on a continuum between human and *mouse* [10]. The greater the half-life, the longer Thalidomide remains active, thereby causing more damage over a longer period of time. A 2004 study by Francisco Chung, Lai-Ming Ching, and colleagues in New Zealand argued that the half-life of Thalidomide in *mouse* [10] embryos was approximately half an hour while that of human embryos was 7.3 hours.

By 2008, scientists had shown that Thalidomide interfered with the Wnt/beta-catenin pathway between cells, a pathway that helps regulate the development of limbs and eyes in embryos. For the Wnt/ beta-catenin pathway, a Wnt ligand outside of a cell interacts with molecules in a cell membrane to free within that cell a protein called beta-catenin from a cluster of molecules. The beta-catenin protein then travels into the cell's *nucleus* [12] and interacts with *genes* [13] to produce new proteins. Those proteins disperse through the cell to help regulate the development of new cells, or to help cause cells to die in the developing eyes and limbs of embryos, a process called *apoptosis* [14]. When Thalidomide interferes with the Wnt/beta-catenin pathway in developing embryos, those embryos develop abnormally small eyes, or microphthalmia, which often leads to blindness. Thalidomide also increases the communication between cells that use bone morphogenetic proteins (Bmps), thereby increasing the activity of the gene in cells regulated by Bmp. This process simultaneously increases the amount of the protein Dickkopf1 (Dkk1) produced in the cell. The increase in the amount of Dkk1, which inhibits the normal functioning of Wnt receptors, in turn causes the increased cell death during eye and limb development. This cell death ultimately leads to Thalidomide's developmental irregularities.
In rodent embryos, however, scientists have shown that Thalidomide doesn't increase the amount of Bmp or Dkk1 proteins in cells, thereby preventing the induction of cell death, or apoptosis. In a 2008 Jürgen Knoblach and colleagues in Germany compared the effects of Thalidomide exposure in chick, embryonic fibroblasts, human embryonic fibroblasts, and mouse embryonic fibroblasts. They argued that these comparisons showed that Thalidomide does not induce apoptosis in developing mice, but that it does in chicks and humans. Further studies suggested that rabbits, humans, and chicks are Thalidomide-sensitive, while mice are Thalidomide-resistant.

Like many chemical compounds, Thalidomide appears in two different conformations, or distinctive arrangements of the elements composing a molecule. In the case of Thalidomide, the two conformations are mirror images of one another called enantiomers. These enantiomers scientists call right- or left-handed, an instance of chemical chirality. By convention, the two conformations are named R+ and S- based on the direction that they rotate polarized light, with "+" rotating light clockwise and "-" rotating light counterclockwise. The R+ conformation of Thalidomide is safe for humans and aids in the anti-inflammatory response easing morning sickness; the S- conformation contributes to Thalidomide’s toxicity. Specifically, the S- conformation inhibits release of the cell signaling protein tumor necrosis factor alpha (TNF-?), which helps regulate programmed cell death. Malfunction of the tumor necrosis factor’s release causes adverse developmental effects.

One proposed solution to Thalidomide’s extreme side effects is to use only R+ conformation drugs. Research has shown, however, that Thalidomide is unstable in solutions over a pH of 6.0, at which point it spontaneously shifts between its two conformations. This transformation often occurs in the liver. The S- conformation of Thalidomide also reversibly inserts itself into DNA through a mechanism called intercalation. Because the drug is similar in structure to adenine and guanine, the purine bases of DNA, it binds to purine-rich regions of DNA as a substitute for these bases, affecting in particular the gene FGF-2. This gene helps form new blood vessels in a process called angiogenesis. Because angiogenesis is part of limb development, disruption of its proper functioning results in abnormal limbs.

A 2008 study by Knoblach and colleagues demonstrated how superoxides can prevent Thalidomide from causing apoptosis. When scientists blocked the antioxidant glutathione from binding to its receptors in laboratory mice, they counteracted the mice’s resistances to Thalidomide, a result that indicates that glutathione helps to repress Thalidomide's effects. Studies artificially inducing the expression of glutathione in humans corroborate this hypothesis through a reduction in Thalidomide’s adverse effects. These results further substantiate glutathione's and other antioxidants' protective effects. Due to these effects, Thalidomide does not cause limbs to shorten in all species. In mice, limbs and blood vessels don't develop abnormally.

Thalidomide spurred researchers to examine the methods and reasons by which they select animal models for tests, and it showed that drug interactions could differ drastically from species to species. Thalidomide problematized the claim that mice and rats are suitable analogues to humans in all cases.
Sources

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