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## Exposure to excess phenobarbital negatively influences the osteogenesis of chick embryos

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Phenobarbital is an antiepileptic drug that is widely used to treat epilepsy in a clinical setting. However, a long term of phenobarbital administration in pregnant women may produce side effects on embryonic skeletogenesis. In this study, we aim to investigate the mechanism by which phenobarbital treatment induces developmental defects in long bones. We first determined that phenobarbital treatment decreased chondrogenesis and inhibited the proliferation of chondrocytes in chick embryos. Phenobarbital treatment also suppressed mineralization in both in vivo and in vitro long bone models. Next, we established that phenobarbital treatment delayed blood vessel invasion in a cartilage template, and this finding was supported by the down-regulation of vascular endothelial growth factor in the hypertrophic zone following phenobarbital treatment. Phenobarbital treatment inhibited tube formation and the migration of human umbilical vein endothelial cells. In addition, it impaired angiogenesis in chick yolk sac membrane model and chorioallantoic membrane model. In summary, phenobarbital exposure led to shortened lengths of long bones during embryogenesis, which might result from inhibiting mesenchyme differentiation, chondrocyte proliferation, and delaying mineralization by impairing vascular invasion.

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