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Research Article

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Antiretroviral therapy and cancer: a comparative effect of efavirenz on embryonic cam and tissue morphology

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Abstract

Introduction: *The development of angiostatic drugs is a useful treatment strategy for diseases with angiogenic tendencies. In our previous work the anti-angiogenic (thus anti-oncogenic) properties of Efavirenz was discussed. Its effect on embryonic vascular patterning and tissue morphology is hereby reported.*

Methods: *Fertile eggs of the domestic fowl (Gallus gallus variant domesticus) were treated on day 3 of incubation (Hamburger and Hamilton's stage 21) with Efavirenz through a single needle puncture into the air cell, and were assessed on day 15 for embryonic viability and gross tissue morphology, in comparison to thalidomide and an untreated control group (n=5) [1].*

Result: *Exogenous Efavirenz and Thalidomide both inhibited CAM angiogenesis (Thalidomide-80%, Efavirenz-100%). Embryonic viability was 20% for thalidomide, and 0% in the Efavirenz group. There was no sign of erythropoiesis in Efavirenz-treated CAMs.*

Conclusion: *The anti-angiogenic potency of Efavirenz in chick CAMs surpassed that of thalidomide. Efavirenz is a potential anti-oncogenic agent with additional clinical prospects.*

Keywords: Angiogenesis, Chorioallantoic membrane (CAM), Efavirenz, Thalidomide, Cancer

Introduction

HIV-Cancer

Ten percent of HIV-infected persons developed cancer. While the rates of aids-defining cancers have reduced with HAART, a 4-fold increase of non-aids-defining cancers has been reported in the HAART era [2]. A 10-year study in Jos, Nigeria showed late stage cancer presentation in 60%-92% of cases [3]. Cancer increase has been attributed to increasing age, racial factors particularly with regard to increased skin cancers in Caucasians, and unavailability of HAART [2], the latter being a common finding in poor societies.

Morphogenesis

Morphogenesis is the biological process for the formation of branched structures in the body. It is a key feature found in both animals and plants [4]. Branching morphogenesis is found in organs such as the pancreas [5], lung, kidney tubules and mammary gland [4], in which case a distal epithelial bud continues to grow and bifurcate thus forming a ductal network (Kouros-Mehr and Werb, 2006. In cardiac morphogenesis, embryonic precursor cells are patterned to form a cyclic contracting muscular tube that is connected to blood vessels thus leading to specification of the complex and highly efficient four-chambered heart [6]. Morphogenesis is regulated by both cellular and molecular factors, also referred to as morphogens or growth factors. Growth factors are characteristically soluble proteins that diffuse through tissues, carrying signals that regulate cell differentiation along a concentration gradient. They act by binding to specific protein receptors. These transcription factor proteins determine the fate of cells by interacting with their DNA, thereby controlling cellular behaviours such as cell migration, adhesion and contractility, as seen during gastrulation [7].

Angiogenesis and Cancer

Angiogenesis and vascular morphogenesis encompass complex mechanisms and processes that cause the formation of new blood and lymphatic vessels. Angiogenesis enables the supply of oxygen and nutrients and so, is a critical determinant for mammalian cell survival [6] and wound healing [8]. During embryonic development blood vessels form brand new from mesenchyme by vasculogenesis, but further growth occurs by angiogenesis;

sprouting and non-sprouting angiogenesis, the latter is also referred to as intussusception or intussusceptive angiogenesis [6,9]. Angiogenesis undergoes multiple specific steps with formation of vascular networks. It includes and culminate in vascular assembly, maturation, and remodeling Makanya, *et al*.[9]; Augustin and Reiss [10]. Many genes regulate endothelial cell behavior and thus regulate angiogenesis. For example, Fan *et al*.[11], characterized the role of Apold1 (Apolipoprotein L domain containing 1) in angiogenesis, both in vivo and in vitro, and found that while Apold1 is a key regulator of pathological angiogenesis, it has no significant role in developmental angiogenesis. They concluded that Apold1 research may hold a promising role in further clinical research [11].

It is known that vascular endothelial growth factor (VEGF) promotes endothelial cell proliferation, differentiation, and so, vascular sprouting. It has been implicated in both developmental and pathological angiogenesis [12]. Abnormal angiogenesis on the other hand, promotes major diseases such as cancer, rheumatoid arthritis and metabolic disorders such as diabetes mellitus [13], not to mention inflammatory diseases of the skin and eye [8].Hypoxia-induced angiogenesis is the common ploy of cancers [8]. Haspel *et al*. [12] suppressed VEGF expression with SU5416, thus blocking angiogenic sprouting in an endothelial model of angiogenesis. Anti-angiogenic drugs therefore reduce cancer-related mortality [2]. Because Pathologic angiogenesis is widespread and is associated with several diseases, the usefulness of developing angiostatic drugs as treatment strategies of these diseases cannot be over emphasized [14].

HAART

Efavirenz, a non-nucleoside reverse transcriptase inhibitor (NNRTI) and first-line drug in HIV1 treatment, is a stable component of Highly active antiretroviral therapy (HAART) [15]. HAART is a combination treatment consisting of two nucleoside (NRTI) backbone given together with either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a ritonavir-enhanced protease inhibitor (PI/r). The introduction of HAART in HIV management in 1996 has not only decreased AIDS mortality but has also changed the clinical profile of HIV infection from a sub-acute and lethal, to a chronic but ambulatory disease. In most patients HAART reduces plasma viral load to an undetectable level [16]. Furthermore, it has been reported that some antiretroviral drugs (ARDS) possess antiangiogenic properties, [17-19] that have led to spontaneous resolutions of HIV-lung cancers. The protease inhibitor (PI), indinavir (also known as saquinavir) blocked Kaposi Sarcoma-like lesions in mice and VEGF-induced angiogenesis in chorioallantoic membrane assay at a potency level equated with the antitumour agent paclitaxel (marketed as taxol). Paclitaxel is commonly used to treat human malignancies [18,19].

Animals

Embryonic CAMs of the domestic fowl (*Gallus gallus* variant *Domesticus*) were used as a vascular test environment as follows: 15 fertile eggs were randomly allocated (n=5) to three groups; Thalidomide, untreated Control group and Efavirenz and placed in a thermostatically regulated incubator with humidity maintained by sponges soaked in water baths. They were turned twice each day.

Drug Treatment

The embryos were grouped and treated by a single dose injected into the air cells as follows: Efavirenz was obtained from Sequoia Research Products (Pangbourne, UK, 200 µg [17]. Thalidomide Negative control group were treated with thalidomide, 20 mg/ embryo [17,20].

The eggs were wiped down with 70% ethanol in cotton wool and treated with the respective drugs on day 3 of incubation (estimated Hamburger and Hamilton's stage 21), by a single injection given into the air cells. Control embryos received equal volumes of physiological saline. On day 15 of incubation (the eggs had observatory windows of 1.5.x 1.5 cm dimensions cut in their egg shells. Antibiotics (Penstrept (50 µl/50ml chick ringer) drops were instilled into the windows for sterility and to help the CAM to sink below the egg shell. CAMs images were captured using a Nikon microscope camera. CAM blood vessels were compared to that of control embryos (Figure 1).

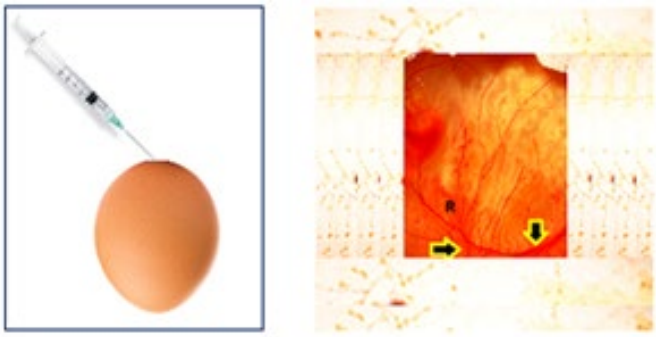

Figure 1: Illustrates both the treatment and observation techniques.

The embryos were then harvested, grossly inspected for physical anomalies. They were then dissected and internal organs were examined and photographed. Results were analyzed using one-way ANOVA and Fisher exact test (Figure 2-6).

Results

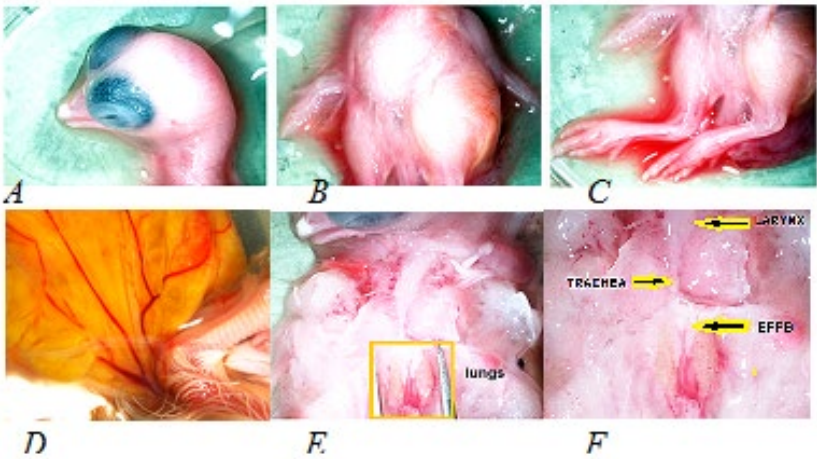

Figure 2: Shows well-formed blood vessels in untreated control CAM. The embryonic tissues showed good tissue perfusion. Internal organs all appeared grossly normal. Note the lungs as highlighted and the airway (EPPB- extra pulmonary primary bronchi).

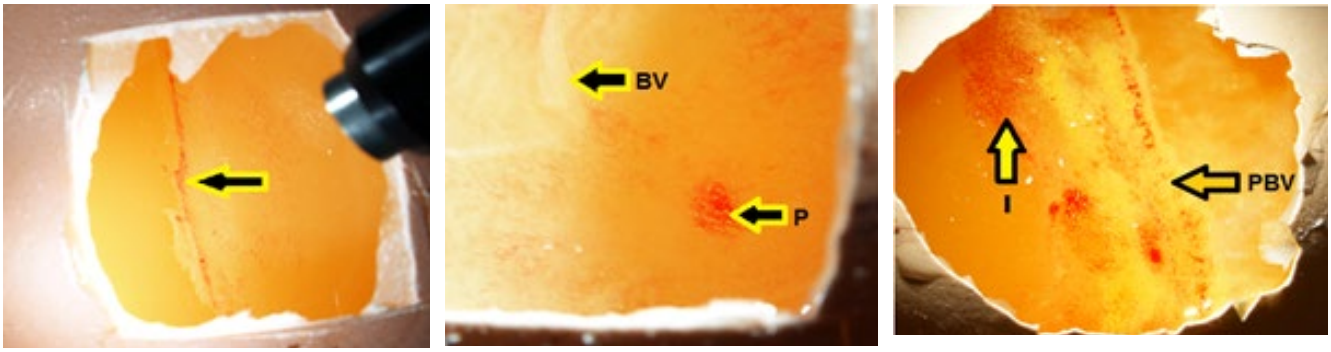

Figure 3: Shows Thalidomide-treated CAMs had poorly patterned and (scanty CAM blood vessels (PBV). Erythropoietic activity is present in patches (P) on CAM surfaces.

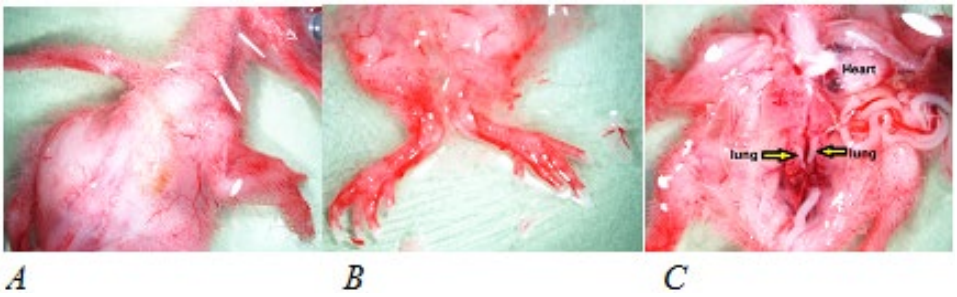

Figure 4: Shows the gross appearance of thalidomide-treated embryos, A) The limbs were abnormally positioned, B) The embryos were stunted with an abnormal reddish coloration that maybe from tissue attempt at increased angiogenesis and erythropoiesis; to overcome tissue hypoxia from angiogenic suppression. C) embryonic tissues were also frail and immature. Note the heart and lungs were rather rudimentary in comparison to that in Control embryos. On dissection of the abdomen the organs in some subjects were not distinguishable.

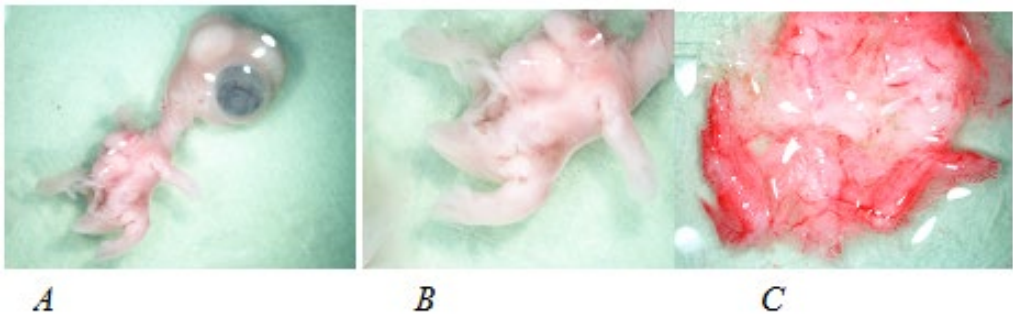

Figure 5: Shows A) Thalidomide-induced maceration. B) is a higher magnification of C) Dissection showed early embryonic death with tissue maceration.

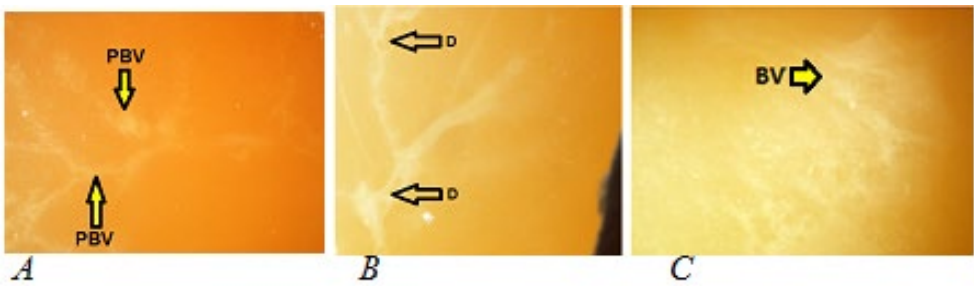

Figure 6: A)Shows Efavirenz-treated CAMs to be rather "'quiet" with neither angiogenic nor erythropoietic activities. Note thin thread-like cords with no sign of erythropoiesis. B) probably arrested blood vessels seen as fibrotic structures on the CAM surface, that is better seen in image. C) There is increased fibrotic tissue reaction in the CAM in image C.

Considering angiogenesis alone, the results showed that there was no significant difference between the anti-angiogenic effect of Efavirenz and Thalidomide. However, Efavirenz suppressed both angiogenesis and erythropoiesis in chick CAM.

	N	Mean	Standard deviation	F	p-value
Thalidomide	5	0.17	0.41	1	0.341
Efavirenz	5	0	0	-	-
Total	10	0.08	0.29	-	-

Table 1: Statistical Analysis.

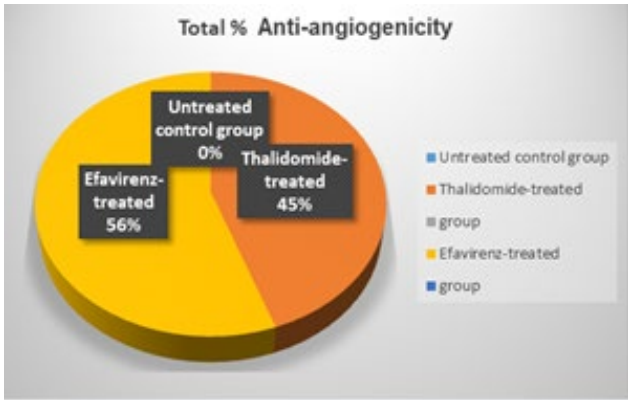

Figure 7: Shows Efavirenz had absolute antiangiogenic property in chick CAM, which surpassed this property of Thalidomide.

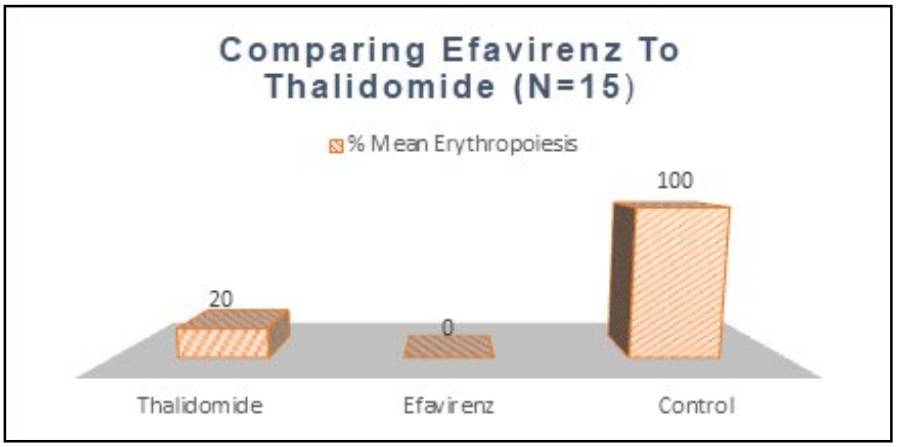

Figure 8: Shows that unlike Thalidomide, Efavirenz blocked erythropoiesis in chick CAM.

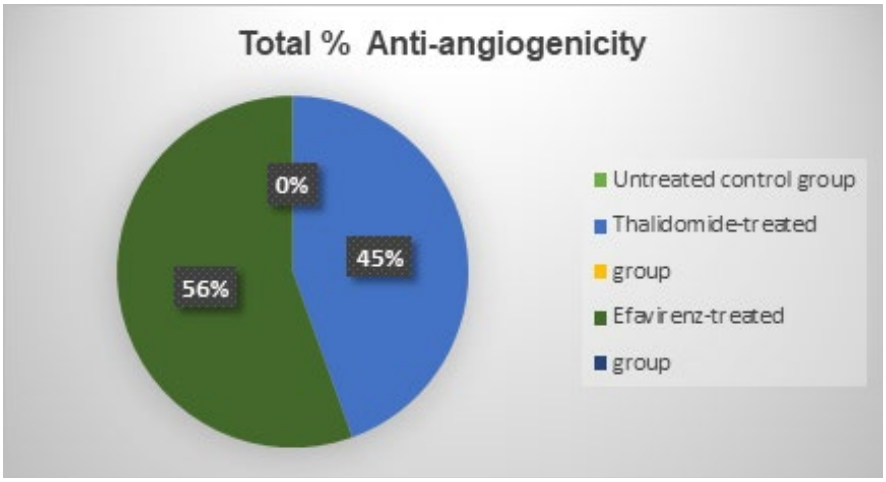

Figure 9: Shows that, while Efavirenz had absolute anti-angiogenic effect on chick CAM, Thalidomide failed to suppress CAM angiogenic activities in 20% subjects.

Discussion

During early embryogenesis, blood islands form from angioblasts. Angioblasts and progenitor blood cells, haematoblasts both differentiate from the mesenchyme. Angiogenesis is regulated by both activator and inhibitor molecules [21]. isolated angiogenic factors from both human and animal tumors which has led to the discovery of genes for VEGF, tumor necrotic factor, epidermal growth factor, transforming growth factor and angiogenin [22]. Angiogenic factors directly promote endothelial cell proliferation or through inflammatory cells cause the release of these endothelial mitogens [23]. It has been demonstrated that deletion of a single allele of VEGF is not compatible with life [24]. In mice, inactivation of VEGF or its receptor led to death in utero [25,26] as a result of the non-development of yolk sac blood islands and lack of organized blood vessels at all developmental stages [27]. Inhibitors of the VEGF signaling pathway (angiogenesis inhibitors) are useful in cancers management [22].

As expected, control embryos had well-formed blood vessels. Thalidomide- Treated embryos presented with results similar to that found with Efavirenz in that in some subjects, the CAM blood vessels did not form. There was no well-established blood vessel network seen in the thalidomide group. Blood vessels when seen were scanty. Efavirenz suppressed CAM blood vessels in all embryos and also prevented CAM erythropoiesis. This caused early embryonic death and resolution of the 3-day embryos. No remnant of the embryonic tissue found in any of the group. Thalidomide failed as an anti-angiogenic agent in 20%, and did not suppress erythropoiesis in 60% of subjects. Efavirenz showed more potency as an anti-oncogenic agent than thalidomide.

Conclusion

The incidence of non-AIDS-defining cancers such as Hodgkin's disease, basal cell carcinoma of the skin, squamous cell carcinoma of the tongue and anus pediatric leiomyosarcoma and seminoma are increasing in HIV-infected patients [28,29]. And several anti-angiogenic agents have been evaluated for the treatment of cancers but not much is known about the anti-oncogenic properties of ARDs. This work helps confirm that Efavirenz is a potential anti-cancer agent. However, additional studies will be necessary to ascertain the effects of Efavirenz on specific HIV-cancers and non-AIDS-defining cancers.

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