Effect of Thalidomide on Brain Oxygenation

A. V. BERAN^[40], J. STRAUSS, D. R. SPERLING, A. C. NORTON, V. P. GARWOOD, AND J. YAMAZAKI

Department of Pediatrics, University of California, Irvine, College of Medicine, Irvine, and Childrens Hospital of Los Angeles, University of Southern California School of Medicine, Los Angeles, California, USA

Extract

This study was undertaken to evaluate the effect of thalidomide on oxygenation of maternal and fetal brain tissue. Oxygen availability (O_2a) was determined polarographically using acutely and chronically implanted Pt and AgCl electrodes. Partial pressure of oxygen (pO_2) was measured polarographically *in vitro* from blood samples, and blood flow was determined *in vivo* by the hydrogen diffusion rate method. Thalidomide increased concentrations of O_2a in the brain of adult guinea pigs and fetuses by an average of 33 and 200%, respectively. Simultaneously, jugular venous blood pO_2 was increased, and arterial-venous (A-V) difference of O_2 content, blood flow, and O_2 consumption were decreased. Arterial blood pO_2 , pH and hemoglobin levels, body temperature, heart rate, and blood pressure were not affected by thalidomide. Niacinamide or hydrocortisone administered 30 to 60 min before the injection of thalidomide either decreased or prevented the thalidomide-induced response.

Speculation

It is postulated that thalidomide (or its metabolites) decreases cellular oxygen consumption by interfering with intermediary energy metabolism, and that this in turn could lead to developmental anomalies of the organism.

Introduction

The tragic story of thalidomide and its association with birth defects is now well known [14, 16, 18, 20, 27]. Although clinical use has been discontinued, thalidomide is a useful experimental tool in biological research for the study and modification of developmental [3, 6, 7, 17, 19] and basic metabolic processes [9]. In the present study, evaluation of oxygen availability (O_2a) in tissue, blood flow, and oxygen consumption have been used to determine the effect of thalidomide on such basic processes.

Methods and Materials

Recording Techniques and Calculations

Oxygen availability (O_2a) in tissues was measured polarographically using a siliclad-coated [31] platinum-

(Pt) cathode and an Ag-AgCl reference anode. This technique depends upon the fact that molecular O_2 is reduced at the Pt electrode by addition of two electrons (e^-), developing a current that is directly proportional to the amount of oxygen reduced [5, 21, 22]. In the present experiments the current was amplified [32] and recorded on a chart recorder [33].

Blood flow as measured by the hydrogen diffusion method, using platinum covered with platinum chloride as the active electrode and Ag-AgCl as the reference electrode. This electrode system generates a negative voltage that is directly proportional to the number of H_2 molecules present at the tip of the active electrode. When an animal breathes a gas mixture containing H_2 , the potential difference (ΔV) reaches a maximum when equilibrium is established between the H_2 concentration around the active electrode and that in arterial blood. The linear slope time (LS), which is inversely

proportional to the H_2 diffusion rate, was taken as the index of tissue blood flow. The H_2 was introduced in an inspired gas mixture composed of 5% H_2 , 20% O_2 , and 75% N_2 [2, 4, 8, 23, 25].

The content and consumption of O_2 were calculated from partial pressure of oxygen (pO_2) and hemoglobin data, using a rabbit blood O_2 dissociation curve [1, 11, 13], as follows:

- 1) The O_2 content is equal to the physically dissolved O_2 plus the O_2 chemically combined in oxy-hemoglobin.

$$C_{O_2T} = C_{O_2d} + C_{O_2Hb}$$

- a. Physically dissolved O_2 (C_{O_2d}) was calculated by the following formula: $C_{O_2d} = pO_2 (100 \cdot S/760)$ where S is the Bunsen solubility coefficient for O_2 in plasma at 37° with dimensions ml/ml/atm and a value of 0.02356.
- b. Chemically combined O_2 (C_{O_2Hb}) was expressed using the following formula: $C_{O_2Hb} = S_{O_2} \times O_2$ cap. where O_2 cap. is oxygen capacity ($gHb \times 1.34$ ml O_2 at 100% saturation), and S_{O_2} is oxygen saturation, derived from the rabbit blood O_2 dissociation curve using both pO_2 and pH values.
- 2) Coefficient of O_2 consumption (10) was calculated by the following formula:

$$C\dot{V}_{O_2} = \frac{C_{aO_2} - C_{jvO_2}}{C_{aO_2}} \times F$$

$C\dot{V}_{O_2}$: Coefficient of O_2 consumption

C_{aO_2} : Common carotid or femoral artery O_2 content (vol %)

C_{jvO_2} : Jugular vein O_2 content (vol %)

F : Blood flow

Arterial blood pO_2 and pH, rectal temperature, arterial blood pressure, heart rate, and blood hemoglobin levels were measured by conventional methods.

Electrodes

For recording O_{2a} of brain and bone marrow tissue in the adult, the active electrode (75- μ Pt siliclad-coated wire) was 7 mm long and insulated with epoxy resin except for 1 mm at the tip; the reference electrode (500- μ Ag-AgCl wire) was 2 mm long and uninsulated. Electrodes used in the fetus were thin, flexible wires coated with epoxy resin; a ball of resin was formed at the end of each electrode, and the insulation was scraped off a 1-mm section 4 mm from the end of the wire. The active cathode was 75- μ siliclad-coated Pt wire; the reference anode was 175- μ Ag wire with a bare

section 2 mm long, chlorided electrolytically in 0.1 N HCl. For recording blood flow in brain tissue, the electrodes were similarly constructed, except the active Pt electrode was coated electrolytically with platinum black in a 3% solution of platinum chloride.

Surgical Techniques

A total of 106 guinea pigs (66 nonpregnant adults, 14 pregnant females, and 26 fetuses) and 12 adult rabbits were used in these studies.

Holes were drilled in the skulls of adult animals, and electrodes were inserted into the parietal brain cortex and the parietal bone marrow. The electrodes were soldered to external connectors which then were fixed to the skull with dental cement.

In the fetus, the abdomen of the mother was opened, the uterus was exposed, and the head of the fetus was located by palpation. A no. 25 syringe needle was passed through the parietal region of the fetal head. Electrodes were threaded through the needle until the end with the ball of resin rested snugly against the fetal skull. The needle was removed and the electrode was left with its bare portion inside the brain. After the abdomen was closed, up to 6 hr was allowed for recovery from anesthesia and surgical trauma; fetal records were obtained for 1–2 days thereafter.

During the experiments, cannulae were inserted to draw blood samples from the femoral or ear lobe arteries and from the jugular vein. Three blood samples were obtained in the control period; thalidomide [35] was then injected; samples were repeatedly drawn as soon as the increase in brain O_{2a} was recorded, when brain O_{2a} neared a maximum, and at 1- to 2-hr intervals during recovery.

Drugs

A suspension of 100 mg thalidomide and 100 mg methylcellulose [36] in 2 ml saline was used in these experiments. Niacinamide [37] (150–200 mg/kg) or hydrocortisone [38] (200 mg/kg) was injected 30–60 min before administration of a standard 100 mg/kg dose of thalidomide. All injections were given intraperitoneally.

Results

Studies *in vitro* indicated that O_2 - and H_2 -sensitive electrodes were unaltered by thalidomide.

Effects of Thalidomide on O_{2a} Levels in Brain and Bone Marrow

The typical response of brain tissue was a gradual increase in O_{2a} beginning 20 min after injection, reach-

Fig. 1. Effect of thalidomide on oxygen availability in brain of a maternal and a fetal guinea pig.

ing a maximum 2 hr after injection, and returning to normal values within 24 hr (Fig. 1). Though there was no difference between the response of pregnant and nonpregnant animals, the response in fetuses was greater in amplitude and duration than in adult animals (Table I).

Thalidomide administration also induced the appearance of large amplitude fluctuations of the O_{2a} base line at the rate of 2–3/min. Smaller waves or “slow waves” [5, 12, 21, 29] at the rate of 14/min were superimposed on the base-line fluctuations. The increase in large base-line fluctuations appeared about 2 hr after injection of thalidomide with the increase in brain O_{2a} (Fig. 2, C through E); these fluctuations were similar to those observed in fetuses during uterine contractions [21, 22], and in animals exposed to O_2 pressures of 45 pounds per square inch gauge (psig).

The effect of thalidomide on O_{2a} in bone marrow (used as an indication of changes in tissues other than brain) of eight adult rabbits was greater than that observed in brain tissue; the increase averaged 50% ($\pm 30\%$). The O_{2a} in bone marrow remained elevated when daily injections of thalidomide were administered and returned to normal within 24 hr after the last injection.

Blockage of Thalidomide Effect by Niacinamide and Hydrocortisone

Niacinamide (150–200 mg/kg) was injected into seven adult, seven fetal, and five pregnant guinea pigs 30–60 min before administration of a standard 100 mg/kg dose of thalidomide. The effect of niacinamide on O_{2a} levels in brain and in cerebral blood flow was varied and insignificant. In contrast to the marked increase of O_{2a} normally following administration of thalidomide (Fig. 1), there was no change in the O_{2a} level in five adult, five fetal, and four pregnant female guinea pigs receiving pretreatment (Fig. 3). In the

Table I. Effect of thalidomide on oxygen availability in brain of adult, pregnant, and fetal guinea pigs

	Adult average	Maternal average	Fetal average
No. of exp.	28	9	14
Onset of response, min	25 $\pm$ 10 ¹	30 $\pm$ 15 ¹	50 $\pm$ 30 ¹
Maximum change, min	145 $\pm$ 70	185 $\pm$ 80	210 $\pm$ 120
O_{2a} increase, %	33 $\pm$ 13	32 $\pm$ 15	200 $\pm$ 130
Duration, hr	13 $\pm$ 5	13 $\pm$ 6	18 $\pm$ 8

¹ $\pm$ SD.

Fig. 2. Effect of thalidomide on oxygen availability in brain of an adult rabbit. Note that as O_{2a} increases, base-line fluctuations appear. Amplitude of the fluctuation increases to reach a maximum at the height of the O_{2a} level and disappears as the O_{2a} level returns to normal. A, B: control. C, D, E: during effect of thalidomide. F: O_{2a} back to normal.

remaining five animals, the thalidomide-induced O_{2a} increase occurred, but was 50% less than the increase observed in animals without pretreatment. No differences were observed among the adult, fetal, or pregnant female animals. Pretreatment of six adult nonpregnant guinea pigs with hydrocortisone (200 mg/kg) produced a blockage of the thalidomide effect similar to that produced by pretreatment with nicotinamide; hydrocortisone alone decreased O_{2a} in brain tissue by 8% ($\pm 3\%$). The large base-line fluctuations, observed after administration of thalidomide, were not blocked by pretreatment with either nicotinamide or hydrocortisone.

Effect of Thalidomide on Cerebral Blood Flow

Thalidomide (100 mg/kg) was injected into five pregnant and five adult nonpregnant guinea pigs. In all animals, LS increased by 40% ($\pm 8\%$) and ΔV decreased by 25% ($\pm 8\%$). Both of these indices sug-

gest a decrease in cerebral blood flow following administration of thalidomide (Fig. 4).

Effect of Thalidomide on Blood pO_2 , O_2 Content, and O_2 Consumption

Arterial (A) and venous (V) blood samples were analyzed for pO_2 , and their differences were calculated. Changes in A-V pO_2 differences in all six rabbits were similar to changes in brain O_{2a} (Fig. 5). Differences in A-V pO_2 decreased from an average of 44 ± 10 mm Hg during the control period to an average of 35 ± 10 mm Hg after administration of thalidomide. No change was observed in arterial blood pO_2 , but venous blood pO_2 increased. The maximum decrease in A-V pO_2 difference was $20 \pm 10\%$.

The average A-V difference of O_2 content was 4.98 ± 0.35 ml/100 ml. After thalidomide administration, the A-V difference of O_2 content decreased to an average of 2.89 ± 0.77 ml/100 ml, or by $42 \pm 12\%$.

The average coefficient of O_2 consumption was

Fig. 3. Effect of thalidomide, alone and after administration of 150 mg/kg nicotinamide, on oxygen availability in brain of an adult guinea pig.

Fig. 4. Effect of thalidomide on cerebral blood flow of an adult guinea pig. LS: linear slope. ΔV : potential difference.

23.2%. After administration of thalidomide, it decreased by 62% to an average coefficient of 8.8%.

Effect of Thalidomide on Body Temperature, Blood Pressure, and Heart Rate

No changes in body temperature, blood pressure, or heart rate were observed during or after the increase of mean brain O_{2a} produced by thalidomide.

Discussion

The changes in O_{2a} reported in the present study were reproduced under a variety of conditions. The

effects observed *in vivo* cannot be attributed to electrochemical artifacts, since the *in vitro* experiments demonstrated that neither thalidomide nor the other drugs used had any direct effect on O₂ current. Justification of the use of polarographic methods for measurement of tissue O₂ [5, 21, 22, 24, 29] and the potentiometric H₂ diffusion method for measurement of tissue blood flow [2, 4, 8, 23, 25] has been previously established.

The increased O_{2a} observed in brain and bone marrow following administration of thalidomide could be caused by either an increase in the supply of O₂ or a decrease in the consumption of O₂. The former has

Fig. 5. Effect of thalidomide on oxygen availability in brain and on A-V blood pO_2 of an adult rabbit.

been rejected, since no increase in arterial pO_2 was noted and, furthermore, a decreased blood flow through the brain was recorded. The latter mechanism, decreased cerebral consumption of O_2 , is supported by the results of measurement and calculation (62% decrease in O_2 consumption). Venous blood returning from the brain showed an increase in C_{O_2} that paralleled an increase in O_{2a} .

The finding that nicotinamide blocks the thalidomide-induced increase of O_{2a} adds further support to the above interpretation and also suggests a possible mechanism for the decreased O_2 consumption. The role of nicotinic acid as a coenzyme in cellular respiration and intermediary energy metabolism is well established. Interference with these systems could be expected to decrease O_2 consumption, producing an accumulation of O_2 at the tissue level. It has been reported that thalidomide interferes with metabolism in protozoa and that this interference is related to nicotinic acid [9].

Thalidomide induced two types of changes in O_{2a} : increase in the O_{2a} level and increase in base-line fluctuations. The overall increase of O_{2a} was completely or partially blocked by niacinamide and hydrocortisone, but the fluctuations were essentially unaffected.

The increase in base-line fluctuations could be attributed to the alternate opening and closing of the precapillary sphincter as a response of autoregulatory mechanisms to the increase in O_{2a} [12]. The decrease in tissue blood flow could be explained as a response

of autoregulatory mechanisms to the increased O_{2a} , as postulated for brain and muscle [12, 15, 26, 30].

In conclusion, the interpretation most consistent with the present findings is that thalidomide interferes with intermediary metabolism; the resulting decrease in O_2 consumption results in an increase in tissue O_2 levels, which in turn triggers autoregulatory mechanisms by decreasing the local tissue blood flow. This effect was not limited to brain tissue and was greater in the fetus than in the adult. It seems reasonable that the suggested interference with intermediary metabolism could lead to developmental anomalies.

Summary

The effect of thalidomide on brain oxygen availability (O_{2a}), blood flow, and oxygen consumption was studied in a series of normal adult, pregnant, and fetal guinea pigs. The O_{2a} was significantly increased in all experiments following administration of thalidomide. This effect was decreased or prevented by the prior administration of niacinamide or hydrocortisone. The O_2 consumption in brain and A-V O_2 content difference were decreased significantly, suggesting that the drug interfered with oxygen consumption in both fetal and adult brains.

References and Notes

1. ALTMAN, P. L., GIBSON, J. F., JR., AND WONG, C. C.: *Handbook of Respiration*, p. 55 (Saunders, Philadelphia, 1958).
2. AUKLAND, K. B., BOWER, B. F., AND BERLINER, R. W.: *Meas-*

- urement of local blood flow with hydrogen gas. *Circ. Res.*, 14: 164 (1964).
3. BOYLEN, J. B., HORNE, H. H., AND JOHNSON, W. J.: Teratogenic effects of thalidomide and related substances. *Lancet*, i: 552 (1963).
 4. CLARK, L. C., JR., AND MISRAHY, G. A.: An electrochemical method to measure hydrogen gas in biological tissue (Abstract). *Int. Physiol. Congr.*, 20th, Brussels, No. 177 (1957).
 5. CLARK, L. C., JR., MISRAHY, G., AND FOX, R. P.: Chronically implanted polarographic electrodes. *J. Appl. Physiol.*, 13: 85 (1958).
 6. COOK, M. J., AND MOORE, D. F.: The effect of thalidomide on the development of rat fetus. *Brit. J. Exp. Pathol.*, 48: 150 (1967).
 7. DEBOCK, C. A., AND PETERS, A.: Effect of thalidomide on the development of the chick embryo. *Nature*, 199: 1204 (1963).
 8. FIESCHI, C., BOZZAO, L., AGNOLI, A., NARDINI, M., AND BARTOLINI, A.: The hydrogen method of measuring local blood flow in subcortical structures of the brain: Including a comparative study with the ¹⁴C antipyrine method. *Exp. Brain Res.*, 7: 111 (1969).
 9. FRANK, O., BAKER, H., ZIFFER, H., AARONSON, S., HUTNER, S. H., AND CEEVY, C. M.: Metabolic deficiencies in protozoa induced by Thalidomide. *Science*, 139: 110 (1963).
 10. FULTON, J. F.: A Textbook of Physiology, p. 800 (Saunders, Philadelphia, 1949).
 11. GEORGE, W. C.: Comparative hematology and the function of leucocytes. *Quart. Rev. Biol.*, 16: 426 (1941).
 12. GUYTON, A. C., ROSS, J. M., CARRIER, O., JR., AND WALER, J. R.: Evidence for tissue oxygen demand as the major factor causing autoregulation. *Circ. Res.*, 15 (suppl. 1): 60 (1964).
 13. JORDON, H. E.: Comparative hematology. In: *Handbook of Hematology*, Vol 2, p. 703 (Paul B. Hoeber, New York, 1938).
 14. JUNG, H.: Clinical experience with a new sedative, *Arzneimittel-forschung*, 6: 430 (1956).
 15. LASSEN, A. N.: Autoregulation of cerebral blood flow. *Circ. Res.*, 15 (suppl. 1): 201 (1964).
 16. LENZ, W.: Thalidomide and congenital abnormalities. *Lancet*, i: 45 (1962).
 17. LUCYS, J. F.: Thalidomide: Effect upon pregnancy in the Rhesus monkey. *Science*, 139: 1295 (1963).
 18. LUNZ, W., KELLER, H., AND MUCKTER, H.: N-Phthalylglutamic acidimide. *Arzneimittel-forschung*, 6: 426 (1956).
 19. LUTWAK-MANN, AND HAY, M. F.: Effect on the early embryo of the agents administered to the mother. *Brit. Med. J.*, 1: 944 (1962).
 20. MELLIN, G., AND KATZENSTEIN, M.: The saga of thalidomide. *New Engl. J. Med.*, 267: 1184, 1238 (1962).
 21. MISRAHY, G. A., BERAN, A. V., AND HARDWICK, D. F.: Fetal and neonatal brain oxygen. *Amer. J. Physiol.*, 203: 160 (1962).
 22. MISRAHY, G. A., BERAN, A. V., SPRADLEY, J. F., AND GARWOOD, V. P.: Fetal brain oxygen. *Amer. J. Physiol.*, 199: 959 (1960).
 23. MISRAHY, G. A., BERAN, A. V., STRAUSS, J., AND GARWOOD, V. P.: Diffusion of hydrogen to the fetal brain and neonatal brain and kidney. *Exp. Neurol.*, 8: 361 (1963).
 24. MISRAHY, G. A., HARDWICK, D. F., BROOKS, C. J., GARWOOD, V. P., AND HALL, W. P.: Bone, bone marrow and brain oxygen. *Amer. J. Physiol.*, 202: 226 (1962).
 25. NEELY, A. W., TURNER, M. D., HARDY, D. J., AND GODFREY, W. D.: The use of hydrogen electrode to measure tissue blood flow. *J. Surg. Res.*, 5: 363 (1965).
 26. RAPELA, E. C., AND GREEN, D. H.: Autoregulation of canine cerebral blood flow. *Circ. Res.*, 15 (suppl. 1): 205 (1964).
 27. SPEIRS, A. L.: Thalidomide and congenital abnormalities. *Lancet*, i: 303 (1962).
 28. SPERLING, D. R., NATHANIEL, D., STRAUSS, J., BERAN, A. V., AND MISRAHY, G.: Oxygen toxicity in newborn and adult mice. (Manuscript in preparation.)
 29. STRAUSS, J., BERAN, A. V., BROWN, C. T., AND KATURICH, N.: Renal oxygenation under "normal" conditions. *Amer. J. Physiol.*, 215: 1482 (1968).
 30. STRAUSS, J., GARWOOD, V. P., BERAN, A. V., AND MISRAHY, G. A.: Variations in brain and blood oxygen during prolonged hypoxia. *Clin. Res.*, 13: 117 (1965).
 31. Clay-Adams, Inc., New York, N. Y.
 32. HP8875A differential DC amplifier, Hewlett-Packard Company, Palo Alto, Calif.
 33. Esterline-Angus AW, Esterline-Angus, Indianapolis, Ind.
 34. Epoxylite Corporation, El Monte, Calif.
 35. Kevadon, The Wm. S. Merrell Company, Cincinnati, Ohio.
 36. Methocel, Dow Chemical Company, Midland, Mich.
 37. El, Lilly & Company, Indianapolis, Ind.
 38. Solu-Cortef, Upjohn Company, Kalamazoo, Mich.
 39. Supported by National Institutes of Health Grants nos. HE 09351 and NB 03453.
 40. Request for reprints should be addressed to: A. V. Beran, Department of Pediatrics, University of California, Irvine, College of Medicine, Irvine, Calif. (92664) USA.
 41. Accepted for publication July 15, 1970.