

Effect Of Aminophylline On Serum Total Protein, Albumin, Iron And Transferrin In Albino Rats

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Abstract

This study was carried out to establish the effect of aminophylline (theophylline ethylenediamine) synthetic analogue of theophylline on serum total protein, albumin, iron, total iron binding capacity (TIBC) and transferrin in Wistar rats. The experimental animals were divided into 4 groups of 6 rats each. Group 1 received physiological saline as a placebo and served as a control. Groups II, III, and IV received 2.5 mg/kg, 5.0 mg/kg and 7.5 mg/kg body weight respectively of aminophylline daily for 5 days. Blood obtained from the sacrificed animals 24 hours after the last treatment was transferred into iron-free centrifuge tube, allowed to clot and serum obtained was used for analyzing. The results showed that there was a significant dose dependent increase ($P < 0.05$) in mean serum iron and significant dose dependent decrease ($P < 0.05$) in TIBC and serum transferrin in Wistar rats treated with different concentration of aminophylline when compared with control rats. However, mean values of total protein, and albumin in experimental animals showed no significant difference when compared with the control. The results of this study suggest that acute administration of aminophylline may affect serum iron, transferrin and TIBC levels and the consequences of this effect are discussed in relation to iron transport and erythropoiesis.

INTRODUCTION

Aminophylline also called theophylline ethylenediamine, a synthetic analogue of theophylline, is a pure alkaloid. Theophylline (1,3-diethylxanthine) is the xanthine usually employed in clinical medicine (Rang et al, 2000). Typical alkaloids are derived from plant sources e.g. tea leaves (*Thea sinensis*) and cocoa where it is in combination with caffeine and small amount of theobromine. They have marked physiological action on man and other animals (Elejo et al, 2004). Xanthine derivatives aminophylline and theophylline used primarily for their bronchodilatory effects often in patients with myocardial failure and/or pulmonary oedema (Bracket et al, 1990). They directly relax smooth muscles in the bronchi and pulmonary vasculature, increase gastric acid secretion and inhibit uterine contraction. They also have weak chronotropic and inotropic action, stimulate CNS and can cause respiratory stimulation (Brater et al, 1993).

There have been reports in the literature about the several effects aminophylline exerts on different organs and systems of both the human body and animals. These effects include its diuretic and natriuretic properties explained by its requirement of intact adenosine A_1 receptors (Rieg et al, 2004), bronchodilation in the bronchioles, increase in heart rate, central nervous system stimulation (Goel et al, 2004).

Its effect on serum electrolytes and glucose; skin, urinary tract (Brater et al, 1981), where hypokalaemia, hypophosphataemia, hypercalcaemia, hyperglycaemia and metabolic acidosis; cutaneous allergic reactions and urinary retention respectively are the results. Albumin binding and inhibition of iron absorption by administration of beverages containing theophylline is also documented (Goodman et al, 1996).

However, there has been no previous work relating to the effect of aminophylline (theophylline ethylenediamine) synthetic analogue of theophylline on iron transport and erythropoiesis. On this account it is therefore the aim of this work to relate the effect of this drug on the serum total protein albumin, iron and transferrin which could aid in discovering some of the adverse effect of aminophylline on iron transport and erythropoiesis.

MATERIALS AND METHODS

Twenty four white albino rats of Wistar strain were bought from Food Science and Technology Department of Federal University of Technology, Owerri, Nigeria and were held in the Animal House of College of Medicine and Health Sciences, Imo State University, Owerri, Nigeria. They had free access to water and commercial diet (Agro feed)

products of Guinea Farms Ltd, Ibadan, Nigeria. The animals were acclimatized with laboratory conditions for one week. The weight of the animals prior to the study ranged between 200 g – 300 g.

DRUGS

Aminophylline injections (Maxheal Pharmaceutical Company, India) were purchased from a standard pharmacy shop in Owerri, Nigeria. They were stored in the refrigerator until they were used for the experiment.

EXPERIMENTAL DESIGNS

The rats were randomly allocated to 4 experimental groups (n=6 in each group). Each group were numbered and housed individually in wired screen bottom cages made of stainless steel and equipped to separate urine and faeces of the rats.

Group I (control) animals received a placebo (physiologic saline)

Group II received aminophylline (2.5 mg/kg/day),

Group III received aminophylline (5.0 mg/kg/day)

Group IV received aminophylline (7.5 mg/kg/day).

The drug was administered to the animals by oral compulsion for 5 days. Food and water were provided ad libitum throughout the period.

BLOOD SAMPLE COLLECTION

Twenty four hours after the last doses were administered, the animals were anaesthetized with chloroform vapour, quickly brought out of jar and sacrificed. Whole blood was collected by cardiac puncture from each animal into clean, dry centrifuge tubes. The blood were allowed to stand for about 30 minutes to clot, and further centrifuges at 10,000 rpm for 5 minutes using Wisperfuge model 1384 centrifuge (Samson, Holland). Serum was separated from clot with Pasteur pipette into sterile serum sample tubes and used for biochemical assays. Collection of sample was done between 8-10 am since serum iron levels is affected by the time of the day among other parameters (Laurell et al,1949).

BIOCHEMICAL ASSAY

Serum total protein was measured using the Biuret method of Doumas (1975). Serum albumin was measured by the modification of Bromocresol Green method of Mcpherson and Everalld (1972). Serum iron was determined using the method of international committee for standardization in haematology, ICSH (1978). In this method, the serum sample is treated with a buffered reagent, which prevents precipitation of proteins and provides an acid medium for

the dissociation of the Fe^{3+} - transferrin complex and reduction of Fe^{3+} to Fe^{2+} . Addition of coloured reagent results in the formation of a deeply coloured ferrozine – Fe^{2+} complex with an absorbance maximum at 562nm. The presence of thiourea in the colour reagent binds copper and prevents formation of a ferrozine- CU complex for the determination of the Total iron binding capacity, TIBC using the method of ICSH (1978), the serum is treated with excess iron. Part of the iron binds with apotransferrin and the remainder is removed by passing the treated sample through a column prepared with MgCO_3 . The iron in the filtrate is determine in the same manner as the iron in serum and is a measure of the TIBC. Serum transferrin concentration was calculated from TIBC concentration using the formular $\text{TIBC}/1.45$.

Statistical Analysis: statistical evaluation of data was performed by using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Duncan, 1957).

RESULTS

The mean values of serum total protein, albumin and globulin in both normal and experimental animals are shown in table I. The mean values of total protein, albumin and globulin in serum showed no significant difference ($P>0.05$) in animals treated with different concentrations of aminophylline when compared with the values from control group. Hence the aminophylline has little or no effect on the levels of serum total protein, albumin and globulin.

Table II shows the mean values of serum iron, TIBC and transferrin concentrations in both the experimental and control groups. The results indicate that the mean values of serum iron for the test group II, III and IV were higher ($P<0.05$) when compared with the mean value of the control (group I). This shows that aminophylline induces a significant increase in serum levels of iron in rats and the effect was found to be dose dependent. However, there was a statistically significant decrease in both TIBC and serum transferrin levels in the test group ($P<0.05$) when compared with the value of the control.

DISCUSSION

The results obtained in this study showed that the mean values of total protein and albumin in the experimental group; group II treated with 2.5 mg/kg/day of aminophylline group III treated with 5.0 mg/kg/day and group IV treated with 7.5 mg/kg/day were not significantly different ($P>0.05$)

when compared with the control group treated with placebo (physiological saline). It could be inferred that aminophylline has no effect on the total protein and albumin levels in serum.

From the results also there was a significant increase in the serum iron in the experimental groups II, III, IV ($P<0.05$) when compared with the mean value of serum iron in the control group (I) and this was found to be dose dependent. Also there was a significant decrease in both serum total iron binding capacity (TIBC) and serum transferrin levels in the experimental groups (II, III IV) ($P<0.05$) when compared with control group.

Aminophylline has been found to have both diuretic and natriuretic properties (Rieg et al, 2004). Theobromine, another methyl xanthine has also been found to raise serum iron and lower serum TIBC and transferrin (Elego et al, 2004) conforming this study, but this is no literature on the effect of aminophylline on serum iron, TIBC, and transferrin in both humans and animals. The mechanism of action of this result is not clear. However, since almost all of the iron in serum is bound to transferrin in the form of Fe^{3+} -protein complex (Murray et al, 2000), it is expected that with raised serum iron, the level of transferrin should also be elevated but evidence obtained in this study is to the contrary hence elevation in serum iron and a fall in TIBC and serum transferrin. Joseph (1954) had reported that methyl xanthines are transported in blood bound to proteins. Aminophylline, a methyl xanthine is bound to plasma protein and since transferrin constitute part of the globulin fraction of plasma protein, such a binding of aminophylline unto transferrin could probably inhibit or block the receptor site for iron, thus iron is not bound and serum levels of iron are elevated (Eleogo et al, 2004). This may explain the observed rise in serum iron and the decrease in serum transferrin and TIBC in rats treated with different concentrations of aminophylline.

Increased erythropoiesis is associated with an increase in iron supply from the plasma (Katzung et al, 2001) and to meet this increase demand for iron, transferrin normally one-third saturated with iron will have to bind and transport more iron to the bone marrow, however, with aminophylline affecting the binding of iron to transferrin, this essential metal cannot be transported to the erythroid marrow, thus adversely affecting erythropoiesis and may induce iron deficiency anaemia. It is however, suggested that further research be done on humans and proper advice given to health workers since aminophylline is a common drug given

to ashmatic patients especially in developing countries like Nigeria.

Figure 1

Table 1: Mean values of total protein and albumin in both aminophylline treated and control animals

	Control (I)	Group II	Group III	Group IV
Serum total Protein (g/L)	79±036	73.9±6.0	73.9±075	69.3±8.5
Albumin (g/L)	45.4±2.0	41.6±1.9	43.8±44.9	41.6±4.6

* Significant different from control ($P<0.05$)

Figure 2

Table 2: Mean values of serum iron, TIBC transferrin in both aminophylline treated and control Animals

	Control (I)	Group II	Group III	Group IV
Iron (µmol/L)	6.8±1.68	8.16±2.01*	10.21±2.66**	13.65±3.20***
TIBC (µmol/L)	47.2±2.11	40.44±1.86*	34.3±1.56**	28.25±0.98***
Transferrin (g/L)	11.3±0.89	10.56±1.20	8.42±1.32**	6.08±0.14***

* Significantly different from control ($P<0.05$)

** Significantly different from control and Group II ($P<0.05$)

*** Significantly different from control, Group II and III ($P<0.05$)

References

- r-0. Brackett, L.E., Shamim, M.T. and Daly, J.W. (1990). Activities of Caffeine, theophylline and enprofylline analogs as tracheal relaxants. *Biochemical pharmacology*. 39: 1897-1904.
- r-1. Brater, D.C., Kaojaren, S. and Chennavasin, P. (1983). Pharmacodynamics of the diuretic effects of aminophylline and acetazolamide alone and combined with furosemide in normal subjects. *Journal of pharmacology and experimental therapeutics*. 227: 92-97.
- r-2. Doumas, B.T., Peters, T.J., and Bio-monle, G.T. (1975). Protein (Total protein) in serum, urine, and cerebrospinal fluid. In: *Selected methods of clinical chemistry Vol 9*, W.R. Faulkner, S. Meites, Eds. Washington D.C. pp. 822-828.
- r-3. Elejo B.M., Eteng, M.U., Omale, J. and Olajide, J.E. (2004). Effect of theobromine on serum total protein, albumin, iron and transferrin in albino rats. *Nig. J. Biochem. and Mol. Biol.* 16(2): 102-105.
- r-4. Goel, V., Chang, C., Slama, J., Barton, R., Gahler, R., and Basu, T. (2004). Respiratory Drugs, Bronchodilators, xanthine derivatives. *Annals of pharmacotherapy*. 33: 502-510.
- r-5. Goodman, G.A., Ruddon, R.W., Molinoff, B.P., Hardman, G.J. and Limbird, L.E. (1996). Methylxanthines in the pharmacological basis of therapeutics. Ninth Edition. Mc Graw-Hill Companies, London. Pp 671-679.
- r-6. ICSH (1978). The measurement of total and unsaturated iron binding capacity in serum *Journal of Haematology*: 35(38): 281-290.
- r-7. Joseph, R.D. (1954). Xanthine, Drill pharmacology in Medicine. Fourth Edition. Varro tyler Co. London. Pp 533-549.
- r-8. Katzung, B.G. (2001). Aminophylline in basis and clinical pharmacology. Eight edition McGraw-Hill Companies Inc. USA pp 337-340.
- r-9. Laurell, C.B. (1947). Diurnal variation of serum iron concentration. *Journal of clinical laboratory investigation*. 5:115-121.
- r-10. McPherson, I.G. and Everal, D.W. (1972). Serum albumin estimation: modification of the bromocresol green methods. *Clin. Chem. Acta*. 37. 117-121.

r-11. Murray, R.K. Granner, D.K., Mayes, D.A. and Rodwell, V.W. (2000). Harpers Biochemistry 25 th edition. McGraw Hill Company, U.S.A. pp 740-742.
r-12. Rang, H.P., Dale, M.M. and Rilter, J.M. (2000). Xanthine drugs in pharmacology. 4 th Edition. Harcourt

publishers. ChurchHill Livingstone, Edinburgh pp. 334-350.
r-13. Rieg, T., Steigele, H., Schnermann, J., Richter, K. Oswald, H. and Vallon, V. (2004). Requirement of intact Adenosine A₁ -receptors for diuretic and natriuretic action of the methylxanthines theophylline and caffeine. Journal of pharmacology and Experimental therapeutics 313. 403-404.

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