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Determination of the therapeutic potential of human umbilical cord blood

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Abstract

This research was conducted to evaluate the therapeutic potential of human umbilical cord blood, by determining their effect on bacterial pathogens which included: *Streptobacillus* sp, *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Escherichia coli*. Cord blood samples were obtained from hospitals following consent of the women. The antimicrobial activity was determined by the agar-well diffusion method. There was significant difference ($p < 0.05$) in the activity observed between the different concentrations of the samples against the test bacteria, while there was no significant difference ($p > 0.05$) observed in the activity of the different samples against the test isolates. Gram-positive and Gram-negative bacteria were sensitive to the samples at varying concentrations. Sample A did not yield any significant activity against most of the test organisms, possibly due to denaturation during preservation as a result of power failure. At 100 % concentration, *S. aureus* was most susceptible to sample B (21.7 ± 0.3 mm) and sample C (22.0 ± 0.6 mm) while *E. coli* was the least susceptible to sample C (11.7 ± 0.5 mm). *E. coli* and *S. typhimurium* with no zone of inhibition were observed to be the least susceptible to sample B. The MIC ranged from 12.5 % to 100 % while the MBC ranged from 25 % to 100 %. Results revealed that human cord blood could complement synthetic drugs in the fight against bacterial diseases.

Keywords: Antibacterial; Umbilical cord blood; Hematopoietic stem cells

INTRODUCTION

Diseases which are of genetic origin have been studied to be cured by stem cell technology (Rocha *et al*., 2001), as an alternative to bone marrow transplant. However, infectious diseases such as pneumonia, typhoid and otitis media have not been tested with this technology. Scientists have provided evidence that the human umbilical cord contains blood that is enriched with stem cells, known as hematopoietic (blood-forming) stem cells. Originally thrown away after the delivery of the baby, umbilical cord blood (UCB) was first considered as a source of stem cells in the 1970s (Mayani *et al*, 2003). The most recent significant constituents of UCB are erythrocytes, endothelial cells, mesenchyma stem cells (MSCs), hematopoietic stem cells (HSCs) (Eapen *et al*., 2007). Recent advances in the potential of UCB have revealed therapeutic uses of hematopoietic stem cells (HSCs) (Eapen *et al*., 2007).

Stem cells are immature tissue precursor cells which are able to self-renew and differentiate into multiple cell lineages (Graf, 2002). They serve as a repair system for other body cells and the immune system.

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They are able to multiply and transform into blood, bones, tissues and organs of the body (Watt and Hogan, 2000). They have the ability to find and replace damaged cells of the body. There are basically three sources of stem cells, which are: bone marrow, human embryo and cord blood (Graf, 2002).

Cord blood (sometimes called umbilical cord blood) is the blood that remains in the placenta and umbilical cord after a baby is born. It is the richest source of stem cells. According to Weiss *et al*. (2005), umbilical cord blood remains an inexhaustible, non – controversial source of stem cells for therapy. Most cord blood transplants have been performed in patients with blood and immune system diseases. Cord blood transplants have also been performed for patients with genetic or metabolic diseases. The two types of stem cells are the embryonic stem cell (which are the earliest type of stem cells, and they reach their embryonic stem cell phase four to five days after fertilization. Collection of these stem cells results in the destruction of the embryo); and the adult stem cells (which are found in the body after development and they replenish dying cells and regenerate dying tissues. They are found in the bone marrow, placenta and cord blood) (Ballen *et al*., 2001).

Cord blood can be collected *In-utero* or *Ex-utero*. *In-utero* cord blood collection, is the collection of cord blood from the umbilical vein after delivery while the placenta remains in the uterus. *Ex-utero* cord blood collection is the collection of cord blood after the placenta has been delivered (Ballen *et al*., 2001; Fraser *et al*., 1998). Cord blood offers a number of advantages, it is readily available; it is a richer source of hematopoietic stem cells than bone marrow; there are no risks involved during cord blood collection after birth; there is a lower risk of infectious disease transmission; there is tolerance of partial human leucocyte antigen (HLA) matching between donor and recipient and there is a lower incidence of acute graft versus host disease despite HLA disparity (Eapen, 2007).

Preservation of stem cells is critical for both research and clinical application of stem cell-based therapies. Possible sources of contamination of stem cells include asymptomatic bacteremia of the patient or donor at the time of collection, improper aseptic technique during collection or processing (Schwella *et al.,* 1994).

The incidence of antibiotic resistance among bacterial isolates has been increasing at a rapid rate, thereby greatly limiting the armamentarium of medications available to treat patients with their various diseases. Drug resistance is an alarming issue worldwide and is spreading rapidly due to overuse, self-medication or the non-therapeutic use of antimicrobials (Slama *et al*., 2005). Usually, the emergence of single or multiple antibiotic resistances are closely associated with misuse or abuse of the various antimicrobials used (Manjusha and Sarita, 2012). Therefore, given the large public health impact of infectious diseases, novel therapies are needed. This research therefore seeks to evaluate the therapeutic potential of human umbilical cord blood. The objectives were to study the effect of umbilical cord blood on some clinical isolates and to determine the minimum inhibitory and bactericidal concentrations of the cord blood against the different test microbial isolates used in this study.

EXPERIMENTAL

Collection of samples. Cord blood was collected for the research from three consenting donors before delivery of the placenta by medical doctors from tertiary hospitals in Benin City, Edo State. The umbilical vein was cleaned with alcohol, after which the cord blood was collected into an anticoagulant solution. The cord blood samples were stored at 4–6^{0}C before laboratory analysis.

Collection of test bacteria. The test bacteria were clinical bacterial pathogens collected from the Department of Medical Microbiology, University of Benin Teaching Hospital, (UBTH), Benin City, Nigeria. They included: *Streptobacillus* sp, *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*. Their identity was confirmed using standard biochemical tests as prescribed by Jolt *et al*., 1994 and Cheesbrough, 2006. The test bacteria were maintained on nutrient agar slants at 4 ^{o}C.

Description of test bacteria. The test organisms: *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis* and *Shigella dysenteriae*, have been previously described (Prescott *et al*., 2005; Akinnibosun *et al*., 2008a, 2008b).

Antimicrobial sensitivity bioassay. The antimicrobial activity of the cord blood was determined by the agar-well diffusion method. The bioassay was conducted to determine the tolerance of the test organisms to the cord blood samples. Sterile agar plates were aseptically inoculated with a loopful of the test pathogens. Each inoculum was spread evenly over the surface of the agar plate with sterile swab. With a flamed cork borer, the antimicrobial sensitivity wells prepared were made in the agar. Undiluted cord blood (100%) samples were diluted in two-fold to obtain 50 %, 25 %, 12.5 %, and 6.25 % concentrations. Into the agar wells of the inoculated plates, 0.2 ml of the different concentrations of the cord blood was carefully placed, at a distance of 20.0 mm apart from each well to prevent over lapping, and allowed to stand for 5 mins (to enable the cord blood permeate into the medium) before being incubated at 37 ºC for 24 h. The plates were observed for the presence of inhibition zones around the cord blood diffusion wells. The extent of inhibition was determined by measuring the diameter of the inhibition zone using a transparent meter rule. The mean zone of inhibition of the three replicates was expressed in millimeters. Standard antibiotic was used as positive control.

Determination of minimum inhibitory concentration (MIC). The MICs of the cord blood were determined by diluting the cord blood samples double-fold with Mueller-Hinton broth in a series of test-tubes and each of the tubes were inoculated with 1 ml of the test suspension. The tubes were incubated at 37 ºC for 24 h. Controls were prepared by inoculating tubes without the cord blood but with the cell suspensions. The tubes were then examined for the presence of turbidity after the incubation. The least concentration with no observable growth when compared with the control was considered as the Minimum Inhibitory Concentration.

Determination of minimum bactericidal concentration (MBC). Double dilution containing different concentrations as used in the MIC determination was carried. To the stock solution (100 %), 0.5 ml of the test organism was added. To the other tubes containing different concentrations of the cord blood, 0.5 ml of each test organism was added. The tubes were incubated then. After 24 h, incubated samples were streaked from the tubes into nutrient agar plates to determine the minimum concentration of the cord blood required to kill the organisms. These concentrations were indicated by failure of the cord blood to kill the organisms. The lowest concentration that prevented bacterial growth after two days of incubation was recorded as minimum bactericidal concentration.

Statistical analysis. The Statistical Package for Social Scientists (SPSS, version 16.0) was used for the analyses of the data obtained (Ogbeibu, 2005). The means were compared with Duncan multiple range test at probability level five percent ($P < 0.05$).

RESULTS AND DISCUSSION

In this present study, we investigated the effects of human umbilical cord blood on some clinical bacterial isolates. Mesenchyma stem cells have been demonstrated to provide protection against acute inflammatory tissue and organ injuries; however, their potential role in the setting of bacterial infections have not been well studied, this study therefore focuses on the therapeutic use of the human umbilical cord blood against clinical bacterial pathogens. Our results showed that human umbilical cord blood samples were effective in inhibiting the growth of the test organisms as seen in tables 1, 2, 3, 4 and 5. This is in agreement with the findings of Gupta *et al*. (2012), who observed clearance of bacterial cells upon exposure to mesenchyma stem cells. Antibacterial activity was demonstrated by the zones of inhibition exhibited by the umbilical cord blood samples against the test organisms. HSCs are multipotent and have the capability to self-renew and differentiate into all mature blood cells including myeloids such as granulocytes, monocytes, leukocytes, erythrocytes, and megakaryocytes, and lymphoids such as T-lymphocytes and B-lymphocytes (Higuchi *et al*., 2010). These constituents have been considered to be responsible for the inhibitory properties of the human umbilical cord blood. HSCs in UCB are similar to those in bone marrow and peripheral blood in their capacity for differentiation. They can self-renew and they contain erythroid and granulocyte-macrophage progenitor cells when examined in an *in vitro* colony assay (Broxmeyer *et al*., 1989). Tables 1 -5 shows the sensitivity of the bacterial isolates to the cord blood samples. There was however, a significant difference ($p < 0.05$) in the activity observed between the different concentrations of the cord blood samples against the test bacteria, while there was no significant difference ($p > 0.05$) observed in the activity of the different cord blood samples against the test isolates. Recent studies have demonstrated that mesenchymal stem cells (MSCs) not only attenuate the inflammatory responses but also enhance bacterial clearance in bacterial pneumonia and/or sepsis (Gluckman *et al.*, 1989; Gupta *et al*., 2012). These findings suggest that MSCs could be a promising novel therapeutic modality for bacterial pneumonia. Nonetheless, Gram-positive and Gram-negative bacteria were both sensitive to the samples at varying concentrations. This is in consonance with the findings of Krasnodembskaya *et al*. (2010), who observed the broad spectrum activity of mesenchyma stem cells against Gram-positive and Gram-negative bacteria. They demonstrated that human mesenchyma stem cells from the bone marrow possessed intrinsic antimicrobial properties which showed marked inhibition of bacterial growth. The activity of cord blood samples against the test bacteria was dose-dependent. This is similar to the observations of Krasnodembskaya *et al*. (2010), who observed a dose-dependent antimicrobial activity of mesenchyma stem cells against both Gram-positive and Gram-negative bacteria.

The zone of inhibition (mm) of the standard antibiotic (Ciprofloxacin) against the test bacteria is shown in Table 6. This was used as positive control in this study. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the cord blood samples used in this study are shown in Tables 7 – 9.

Cord blood sample A did not yield any significant activity against most of the microorganisms used. This could be due to many factors, such as denaturation during preservation due to power failure, stem cell source and/ or collection technique or during transportation to the laboratory. *Staphylococcus aureus* was most susceptible to sample B (21.7 ± 0.3 mm) and sample C (22.0 ± 0.6 mm) at 100 % concentration while

Escherichia coli was the least susceptible to sample C at 100 % concentration. *Escherichia coli* and *Salmonella typhimurium* with no zone of inhibition were observed to be the least susceptible to sample B at 100 % concentration.

The MIC ranged from 12.5 % to 100 % while the MBC ranged from 25 % to 100 % for all the test isolates. Bacterial infections are the most common cause of death worldwide and treatment is increasingly hampered by antibiotic resistance. Findings from this research, revealed the promising nature of human umbilical cord blood as an excellent source of broad spectrum protection against Gram-positive and Gram-negative clinical bacterial pathogens.

Table 1: Zone of inhibition (mm) of cord blood against *Streptobacillus* sp.

Concentration (%)	Zone of inhibition (mm)			*P value*
	Sample A	Sample B	Sample C	
100	-	19.0 ±0.6	20.0 ± 0.6	0.30
50	-	17.7 ± 0.3	18.7 ± 0.9	0.25
25	-	16.3 ± 0.7	15.0 ± 1.2	0.23
12.5	-	11.0 ± 0.6	11.0 ± 1.2	0.90
6.25	-	-	-	
		P < 0.00001	P < 0.00001	

Key: - = no zone of inhibition**;** *Values are mean ± standard error (SE.)

Table 2: Zone of inhibition (mm) of cord blood against *Corynebacterium diphtheriae*

Concentration (%)	Zone of inhibition (mm)			*P value*
	Sample A	Sample B	Sample C	
100	-	20.3 ±0.9	19.3 ± 0.7	0.43
50	-	19.3 ± 0.03	18.3 ± 0.9	0.35
25	-	16.0 ± 0.6	17.3 ± 1.2	0.60
12.5	-	12.0 ± 0.6	12.0 ± 1.2	0.99
6.25	-	-	-	
		P= 0.03	P=0.003	

Key: - = no zone of inhibition**;** *Values are mean ± standard error (SE.)

Table 3: Zone of inhibition (mm) of cord blood against *Staphylococcus aureus*

Concentration (%)	Zone of inhibition (mm)			*P value*
	Sample A	Sample B	Sample C	
100	-	21.7 ± 0.3	22.0 ± 0.6	0.67
50	-	19.3 ± 0.3	20.3 ± 1.2	0.46
25	-	15.0 ± 1.0	16.3 ± 1.5	0.51
12.5	-	-	11.3 ± 0.3	0.00
6.25	-	-	-	

Key: - = no zone of inhibition**;** *Values are mean ± standard error (SE.)

Table 4: Zone of inhibition (mm) of cord blood against *Salmonella typhimurium*

Concentration (%)	Zone of inhibition (mm)			*P value*
	Sample A	Sample B	Sample C	
100	12.7 ± 0.9	-	21.3 ± 0.9	0.80
50	-	-	18.7 ± 1.2	0.00
25	-	-	15.0 ± 0.6	0.00
12.5	-	-	11.3 ± 0.3	0.00
6.25	-	-	-	-
	-	-	P = 0.008	

Key: - = no zone of inhibition *Values are mean ± standard error (SE.)

Table 5: Zone of inhibition (mm) of cord blood against *Escherichia coli*

Concentration (%)	Zone of inhibition (mm)			*P value*
	Sample A	Sample B	Sample C	
100	17.7 ± 0.10	-	11.7 ± 0.5	0.0
50	-	-	-	-
25	-	-	-	-
12.5	-	-	-	-
6.25	-	-	-	-

Key: - = no zone of inhibition *Values are mean ± standard error (S.E.)

Table 6: Zone of inhibition (mm) of standard antibiotic against the test organisms (positive control)

Test organisms	Ciprofloxacin
Streptobacillus sp	20.0
Corynebacterium diphtheria	17.0
Staphylococcus aureus	19.0
Salmonella typhimurium	21.0
Escherichia coli	18.0

Table 7: Minimum inhibitory and bactericidal concentrations of sample A

Test organisms	MIC (%)	MBC (%)
Streptococcus sp.	-	-
Corynebacterium diphtheriae	-	-
Staphylococcus aureus	-	-
Salmonella typhimurium	100	100
Escherichia coli	100	100

- = no zone of inhibition MIC = Minimum Inhibitory concentration MBC = Minimum bactericidal concentration

Table 8: Minimum inhibitory and bactericidal concentrations of sample B.

Test organisms	MIC (%)	MBC (%)
Streptococcus sp.	12.5	25
Corynebacterium diphtheriae	12.5	25
Staphylococcus aureus	25	50
Salmonella typhimurium	-	-
Escherichia coli	100	100

- = no zone of inhibition MIC = Minimum Inhibitory concentration MBC = Minimum bactericidal concentration

Table 9: Minimum inhibitory and bactericidal concentrations of sample C.

Test organisms	MIC (%)	MBC (%)
Streptococcus sp.	12.5	25
Corynebacterium diphtheriae	12.5	25
Staphylococcus aureus	12.5	25
Salmonella typhimurium	12.5	25
Escherichia coli	100	100

- = no zone of inhibition MIC = Minimum Inhibitory concentration MBC = Minimum bactericidal concentration

Conclusion. This research emphasizes the importance of human umbilical cord blood in the treatment of diseases, a measure that is easy to attain, more available, and requires less compatible with the patient, compared to other methods of treatment such as the bone marrow transplant. Findings from this research will encourage the country as a whole to have a cord blood bank for quick, efficient and cost-effective treatment of infectious diseases. It has also been observed that human umbilical cord blood could complement synthetic drugs in the fight against infectious bacterial diseases since cord blood samples had therapeutic effect on the bacterial isolates. This will help in curbing the

problem of antibiotic resistance among microorganisms.

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REFERENCES

Akinnibosun, F. I., Akinnibosun, H. A., Ibeh, I. N. and Osaghae, F. (2008a). Antibacterial activity of *Phyllanthus amarus* Schum and Thonn. on five vegetative organisms. *Plant Archives* **8(2):** 563-568

Akinnibosun, H. A., Akinnibosun, F. I. and German, B. E. (2008b). antibacterial activity of aqueous and ethanolic leaf extracts of *Peperomia pellucida* (L.) H, B and K (Piperaceae) on three Gram-negative bacterial isolates. *Science World Journal* **3(4):** 33-36.

Ballen, K. K., Wilson, M. and Wuu, J. (2001). Bigger is better: maternal and neonatal predictors of hematopoietic potential of umbilical cord blood units. *Bone Marrow Transplant* **27:** 7-14.

Broxmeyer, H. E., Douglas, G. W., Hangoc, G., Cooper, S., Bard, J., English, D. (1989). Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proceedings of the National Academy of Sciences of the United States of America*, **86(10):** 3828-3832.

Cheesbrough, M. (2006). District laboratory practice in tropical countries. Cambridge University Press. 434 pp.

Eapen, M., Rubinstein, P. and Zhang, M. J. (2007). Outcome of transplantation of unrelated donor umbilical cord blood and bone marrow of children with acute leukemia: a comparison study. *Lancet* **369**: 1947-1954.

Fraser, J. K., Cairo, M. S. and Wagner, E. L. (1998). Cord blood transplantation study (COBLT): cord blood bank study operating procedures. *Journal of Hematotherapy* **7**: 521-561.

Gluckman, E., Broxmeyer, H. E., Auerbach, A. D., Friedman, H. S., Douglas, G. W., Devergie, A. (1989). Hematopoietic reconstitution in a patient with fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *New England Journal of Medicine*, **321(17):** 1174-1178.

Graf, T. (2002). Differentiation plasticity of hematopoietic cells. *Blood* **99 (9):** 3089 – 3101.

Gupta, N., Krasnodembskaya, A., Kapetanaki, M., Mouded, M., Tan, X., Serikov, V., and Matthay, M.A. (2012). Mesenchymal stem cells enhance survival and bacterial clearance in murine Escherichia coli pneumonia. Thorax **67:** 533–539.

Higuchi, A., Yang, S., Li, P., Tamai, M., Tagawa, Y., Chang, Y. (2010). Direct ex vivo expansion of hematopoietic stem cells from umbilical cord blood on membranes. *Journal of Membrane Science* **351(1-2):**104-111.

Jolt, J. G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T. and Williams, S. T. (1994). Bergey's manual of systematic bacteriology. Williams & Wilkins Co. Baltimore, Maryland 9th edition 786 pp.

Krasnodembskaya A., Song Y., Fang X., Gupta N., Serikov V., Lee J.W., Matthay M.A. (2010). Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. Stem Cells **28:** 2229–2238.

Manjusha, S. and Sarita, G. B. (2012). Characteristics of plasmids from multiple antibiotic resistant *Vibrio* sp. isolated from mulluscs and crustaceans. *Korean Journal of Microbiology and Biotechnology* **37:** 157-169.

Ogbeibu, A. E. (2005). *Biostatistics: A practical approach to research and data handling.* Mindex press, Benin City, Nigeria, 264pp.

Prescott, M. L., Harley, J. P. and Klen, D. A. (2008). General Microbiology. McGraw Hill Publishing Company New Delhi 7th edition pp 163-850.

Rocha, V., Cornish, J., Sievers, E. L., Filipovich, A., Locatelli, F., Peters, C. (2001). Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood* **97(10):** 2962-2971.

Schwella, N., Zimmermann, R., Heuft, H. G., Blasczyk, R., Beyer, J., Rick, O. (1994). Microbiologic contamination of peripheral blood stem cell autografts. *Vox sanguinis* **67(1):** 32-35.

Slama, T. G., Amin, A. and Brunton, S. A. (2005). A clinician's guide to the appropriate and accurate use of antibiotics: The Council for Appropriate and Rational Antibiotic Therapy (CARAT) criteria. *American Journal of Medicine* **118:** 1-6.

Watt, F. M. and Hogan, B. I. M. (2000). Out of Eden: stem cell and their niches. *Science* **287:** 1427 – 1430.

Weiss, P. H., Zilles, K. and Fink, G. R. (2005). When visual perception causes feeling: Enhanced cross-modal processing in grapheme-colour synesthesia. *Journal of Neurological Image* **28(4):** 859-868.