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ORIGINAL ARTICLE

In vitro adaptability of *Plasmodium falciparum* to different fresh serum alternatives

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Abstract To reduce the dependency on fresh AB^+ serum in continuous culture of *Plasmodium falciparum*, a comparative study was undertaken to assess the in vitro adaptability of *P. falciparum* to media supplemented with fresh AB^+ serum from whole blood, AB^+ plasma, serum derived from AB^+ plasma, AB^+ human serum from Sigma, Albumax II, fetal bovine serum and new born calf serum, independently and in different combinations. Combinations were used to analyze whether two different substitutes demonstrate any synergistic effect on the growth of the parasites. Our findings exhibited that the combination of fresh human serum and Albumax II showed good growth pattern in comparison to that of fresh serum and can thereby be instrumental in reducing the role of fresh human serum in continuous parasite maintenance. Culture maintained with Albumax II with or without hypoxanthine showed average growth.

Keywords Continuous parasite culture · *Plasmodium falciparum* · Human serum · Plasma · Albumax II

Introduction

The continuous in vitro culture of malaria parasites is an important tool in malaria research. However, proper maintenance of the parasite cultures requires essential nutrients for their growth which are mainly obtained from fresh human serum which is difficult to procure. Earlier attempts to replace human serum requirement have met with limited success (Geiman et al. 1966; Ifediba and Vandenberg 1980). Studies using different animal sera as an alternative to fresh human serum have resulted in mixed results (Jensen 1979; Sax and Rieckmann 1980; Divo and Jensen 1982; Grellier et al. 1991). Horse serum was found to show better results than other animal sera (Butcher 1979; Srivastava et al. 2007). The most valuable application of parasite cultivation is the in vitro drug sensitivity assays to screen alternative anti malarial drug candidates, monitoring the sensitivity of a drug and identification of cross-resistance patterns against *P. falciparum* (Ringwald et al. 1999; Singh et al. 2007). Even though the continuous in vitro cultivation of *P. falciparum* has provided a huge contribution in malaria research capabilities (Trager and Jensen 1977) the limited availability of non-immune human serum has become a major hurdle in this area (Asahi and Kanazawa 1994). Studies were undertaken to grow *P. falciparum* in a plasma free medium in presence of adenosine and supplemented with unsaturated C-18 fatty acids and bovine serum albumin which resulted in slow growth of the parasites and lasted for a month (Willet and Canfield 1984). Externally supplied lipoproteins obtained from human serum by ultracentrifugation indicated their need in the development of the parasite (Grellier et al. 1990; Schrevel et al. 1992). Commercial preparations like the lipid-enriched bovine albumin called Albumax, have shown average to good results as compared to human serum and fetal calf serum in several isolates (Cranmer et al. 1997) whereas serum supplements like AmnioMax and Ultroser are reported to have supported parasite growth relatively well (Basco 2003).

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Materials and methods

RPMI-1640, HEPES, gentamycin, amphotericin, D-glucose, hypoxanthine and AB^+ human serum were obtained from Sigma-Aldrich. Albumax II was obtained from Life Technologies while NBCS and FBS were obtained from Gibco life tech. Human AB^+ plasma was obtained from blood bank while fresh serum and O^+ red blood cells were obtained from healthy voluntary donor. The AB^+ plasma was kept at a temperature of −20 °C overnight and thawed at room temperature the next day. Serum was separated from plasma using freshly prepared and syringe filtered (0.22 µm) 10 % calcium chloride, centrifuged and made into aliquots of 50 ml and heat inactivated. Incomplete media (IRPMI) was prepared as 400 ml stock using externally supplied HEPES, D-Glucose, amphotericin and gentamycin. Ten millilitre aliquots of the prepared media were made and completed (CRPMI) using freshly prepared 5 % sodium bicarbonate and 15 % serum (Jensen and Trager 1977; Trager and Jensen 1980). FBS and NBCS media were prepared in the same concentration as that of fresh serum. The culture was maintained in triplicates at 37 °C and 5 % CO_2 concentration following the in vitro culture techniques of Trager and Jensen with slight alterations.

Percentage parasitaemia was calculated by the formula: No. of parasitized RBC's/Total No. of RBC's counted × 100. (www.cdc.gov/dpdx/resources/pdf/benchAids/malaria/Parasitemia_and_Lifecycle.pdf).

Results

The culture maintained with fresh AB^+ plasma showed healthy growth (3–5 % parasitemia) as compared to that of fresh serum (4–6 % parasitemia). Culture maintained with fresh serum and Albumax II in a combination of 15, 10, 5 and 0.5 % respectively showed almost the same growth rate as the control while plates maintained only in 0.5 % Albumax II with hypoxanthine showed moderate growth (1–2 % parasitemia) for a week. Addition of AB^+ serum in different concentrations to the media with 0.5 % Albumax II and hypoxanthine enhanced the growth rate of the parasites; plates with 15 % serum showed the best growth. The serum supplied by Sigma did not contribute to parasite growth. The serum obtained by the addition of 10 % $CaCl_2$ to the plasma was found to show moderate growth of parasites; the only concern being it interfering with parasite growth. The combination of AB^+ plasma and Sigma serum showed insignificant (0–0.1 % parasitemia) results. Both NBCS and FBS did not support any growth of the parasites individually or in combination (1:1). A combination of the Sigma serum and NBCS (1:1) showed 0–0.1 % growth. Plates with combination of FBS and Sigma Serum (1:1) and combination of NBCS and FBS (1:1) also yielded insignificant results. Parasites showed varied growth depending on the different media used. It was seen that the fresh plasma offers better growth nutrients to the parasites than the $CaCl_2$ separated serum. Differences in growth rates of the parasites using the different nutritional sup-

Table 1 Observation of parasite growth in different nutritional supplements

Category	No. of replicates	No. of * pRBCs per 1000 RBCs			
		Median (0 h)	Median (24 h) (range)	Median (48 h) (range)	Median (72 h) (range)
AB + ve fresh serum	3	3	5 (4–6)	16 (15–18)	46 (45–52)
Sigma serum	3	3	3 (2–3)	2 (1–2)	1 (0–2)
Plasma	3	3	6 (4–7)	14 (12–17)	41 (34–43)
$CaCl_2$ sep. serum	3	3	4 (4–5)	9 (4–11)	2 (1–11)
NBCS	3	3	2 (1–2)	1 (0–1)	0
FBS	3	3	1 (0–2)	0 (0–1)	0 (0–1)
Albumax II	3	3	3 (2–4)	7 (5–10)	12 (9–16)
Albumax II + 15 % serum	3	3	4 (3–6)	13 (11–18)	29 (25–33)
Albumax II + 10 % serum	3	3	4 (4–6)	12 (11–13)	28 (23–29)
Albumax II + 5 % serum	3	3	4 (3–4)	9 (6–14)	16 (10–27)
NBCS + FBS	3	3	1 (0–1)	1 (0–1)	0 (0–1)
NBCS + sigma serum	3	3	1 (0–1)	1 (0–1)	0
FBS + sigma serum	3	3	1 (0–2)	1 (0–1)	0
Plasma + sigma serum	3	3	3 (2–4)	3 (3–4)	1 (0–2)

* *pRBC's* parasitized RBC's

Table 2 Summary of study

Sl. No.	Serum supplements	Observation up to first 72 h	Inference
1.	Control serum (from whole blood)	Excellent growth of parasites*	Best medium for proper growth of parasites
2.	AB^+ plasma	Healthy growth of parasites observed	Can be used as a serum substitute
3.	Serum obtained from plasma	Moderate growth#	Can be an acceptable candidate
4.	NBCS	No parasites seen in culture	NBCS does not promote parasite growth
5.	FBS	No parasites observed	FBS does not promote parasite growth
6.	Albumax II	Moderate parasite growth observed	Acceptable as a serum substitute for short duration
7.	Sigma AB^+ serum	Absence of parasite growth	Not a favourable candidate for parasite culture
8.	FBS + NBCS	Parasite growth absent	Combination not suitable for culture purpose
9.	Plasma + sigma serum	Insignificant number of parasites observed^	The combination was not favourable for culture growth
10.	NBCS + sigma serum	Insignificant parasite growth	Not supportive for parasite growth
11.	FBS + sigma serum	Insignificant growth of parasites	Not suitable for parasite culture
12.	Albumax II + serum	Healthy parasite growth observed+	Acceptable combination for parasite culture

* Excellent growth: 4–6 % parasitemia

Moderate growth: 1–2 % parasitemia

^ Insignificant growth: 0–0.1 % parasitemia

+ Healthy growth: 3–5 % parasitemia

plements were duly observed and noted for a period of 72 h (Table 1). Albumax II worked better on continuous cultures than those obtained from cryopreservation. It was noticed that parasites initially cultivated in fresh human serum develop a better adaptability to Albumax II if initially used in combinations with fresh serum and gradually decreasing the serum concentrations instead of directly using Albumax II on the culture plates. A summary of the observations has been duly made and reported. (Table 2).

Discussion

The search for the perfect serum substitute to facilitate healthy growth of *P. falciparum* in vitro is still in process. Experiments on the replacement of human serum with various animal sera (Grellier et al. 1990) have not been fruitful so far. Our study using Albumax II in combination with human sera has shown results similar to that obtained with human serum and can thereby be instrumental in reducing complete dependency on fresh human sera. Successive generations cultivated in combination with fresh human sera showed better adaptability of the parasites to grow alone in Albumax II (0.5 %) later on. Combinations were used in the hope of fulfilling the nutritional requirements of the parasite and thereby increase its longevity. Different ratios of serum and Albumax II were used in order to gradually increase the adaptability of the parasites to Albumax II since prior to this study the cultures were entirely dependent on the supply of fresh human serum. The AB^+ serum obtained from Sigma did not show any marked parasite growth while fresh human plasma showed satisfactory growth (3–5 % parasitemia). $CaCl_2$ separated serum demonstrated 1–2 % parasite growth. FBS and NBCS were found to be not supportive for parasite growth. The commercially available Albumax II can be one of the acceptable alternatives for short term cultures. The study would be helpful to researchers in establishing in vitro malaria parasite cultures for experimentation purpose.

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Compliance with ethical standards

Conflict of interest The authors report no conflict of interest.

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