BIPHASIC CULTURE STRATEGY BASED ON HYPEROSMOTIC PRESSURE FOR IMPROVED HUMANIZED ANTIBODY PRODUCTION IN CHINESE HAMSTER OVARY CELL CULTURE

김민수, 김노수*, 성윤희, 이균민
Animal Cell Engineering Lab., Dept. of Biological Science, KAIST, 한스바이오메드(주)*
Tel: (042) 869-2658, Fax: (042) 869-2610

SUMMARY

Hyperosmotic pressure increased specific antibody productivity (q_{Ab}) of recombinant CHO cells (SH2-0.32) while it depressed cell growth. Thus, the use of hyperosmolar medium did not increase the maximum antibody concentration substantially. To overcome this drawback, the feasibility of biphasic culture strategy was investigated. In the biphasic culture, cells were first cultivated in the standard medium with physiological osmolality(294 mOsm/kg) for cell growth. When cells reached the late exponential phase of growth, the spent standard medium was replaced with the fresh hyperosmolar medium (522 mOsm/kg) for antibody production. The q_{Ab} in growth phase with the standard medium was 2.1μg/ 10^6cells/day while the q_{Ab} in antibody production phase with the hyperosmolar medium (522 mOsm/kg) was 11.1μg/ 10^6cells/day. Northern blot analysis showed a positive relationship between the relative contenet of Ig mRNA and q_{Ab}, indicating that transcriptional regulation was involved in the response of rCHO cells to hyperosmotic pressure. Due to the enhanced q_{Ab} and increased cell concentration in biphasic culture, the maximum antibody concentration obtained in biphasic culture with 522 mOsm/kg medium exchange was 161% higher than that obtained in batch culture with the standard medium. Taken together, simple biphasic culture strategy based on hyperosmotic culture for improved foreign protein production from rCHO cells is effective in improving antibody production of rCHO cells.

INTRODUCTION

Hyperosmotic pressure has been recognized as being an economical solution to increase q in hybridoma (1,4), transfectoma (2), and rCHO cell cultures (5). Despite the potential of commercial strategies based on hyperosmotic pressure, the use of hyperosmolar medium has not been popular because cell growth is depressed at elevated osmolality. (1,4). Among various strategies to overcome depressed cell growth at elevated osmolality, the use of osmoprotective compounds such as glycine betaine is probably most feasible for improved foreign protein production (3,5). However, the efficacy of the simultaneous use of hyperosmotic pressure and glycine betaine was found to be variable

among rCHO cell lines (5).

In this study, we investigated the feasibility of simple biphasic culture strategy based on hyperosmotic culture for improved foreign protein production from rCHO cells. In the biphasic culture, the rCHO cells producing humanized antibody are first cultivated in the standard medium for cell growth and then in the hyperosmolar medium for antibody production.

MATERIALS AND METHODS

The rCHO cell line producing a humanized antibody against the S surface antigen of hepatitis B virus (SH2-0.32) was established in our laboratory. The medium for culture maintenance was α-minimal essential medium (α-MEM, Gibco, Grand Island, NY) supplemented with 10% dialyzed fetal bovine serum (dFBS, Gibco) and 0.32 μM MTX.

Hyperosmolar culture media with various osmolalities were prepared by adding 4M NaCl stock solution to the standard medium with physiological osmolality. The osmolalities of standard and hyperosmolar media prepared were 294, 370, 459, and 522mOsm/kg.

Secreted humanized antibody concentration was measured by sandwich enzyme-linked immunosorbent assay (ELISA). The specific growth rate (μ) was based on data collected during the exponential growth phase. Specific antibody productivity (q_{Ab}) was evaluated. In the hyperosmotic cultures where no growth occurred, q_{Ab} was obtained from the slope of the plot of antibody concentration versus time integral of viable cell concentration. Total cytoplasmic RNA was isolated from 5×10^{6}cells using the protocol by Sambrook et al. (1989). After electrophoresis of 2μg of total cytoplasmic RNA on a 0.8% formaldehyde gel, Northern blot analyses of LC and HC mRNA were performed as described previously(5)

RESULTS AND DISCUSSION

Batch cultures with various osmolalities. Figure 1 shows cell growth and antibody production profiles during batch cultures. Cell growth was depressed at higher osmolalities (Fig. 1A). Antibody concentration was increased slightly at elevated osmolality (370-459 mOsm/kg), despite the depressed cell growth (Fig. 1B). These results imply that q_{Ab} was significantly elevated at higher osmolalities. When the medium osmolality was increased from 294 to 459 mOsm/kg, q_{Ab} was enhanced by approximately 390%. Accordingly, hyperosmotic pressure enhanced qAb and depressed cell growth of rCHO cells (SH2-0.32).

Biphasic cultures with various osmolalities. We investigated the feasibility of biphasic culture where cell growth phase and antibody production phase were separated.

When the medium was exchanged after 2 days cultivation (early exponential phase of

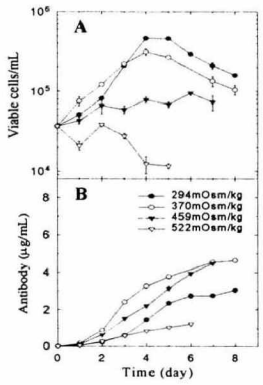

Fig 1. Cell growth (A) and antibody production (B) during a batch culture of rCHO cells(SH2-0.32) at various osmolalities.

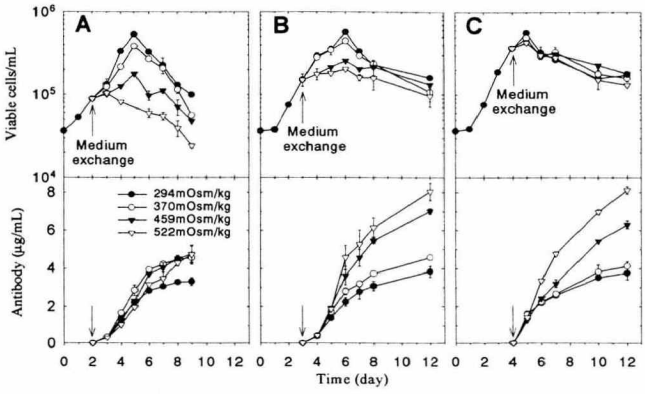

Fig 2. Cell growth and antibody production during biphasic cultures with medium exchange. The spent medium was replaced with the fresh media with various osmolalities after (A) 2 days , (B) 3 days ,and (C) 4 days cultivation, respectively.

growth), no substantial increase in the maximum antibody concentration was achieved regardless of the hyperosmolar media used (Fig. 2A).

The beneficial effect of biphasic culture on antibody production became apparent when the medium was exchanged after 3 or 4 days cultivation.

Fig. 2B shows cell growth and antibody production profiles when the medium was exchanged after 3 days cultivation (mid- to late exponential phase of growth). The maximum antibody concentration obtained in biphasic culture with 522 mOsm/kg medium exchange was 8.1 μg/ml, which is 161% higher than that obtained in batch culture with the standard medium. This increased maximum antibody concentration in biphasic culture with 522 mOsm/kg medium exchange was achieved because of enhanced q_{Ab} and increased cell concentration. The q_{Ab} after 522 mOsm/kg medium exchange was 11.1 $\mu g/10^6$cells/day while the q_{Ab} after 294 mOsm/kg medium exchange was 2.1 $\mu g/10^6$cells/day.

When the medium was exchanged after 4 days cultivation (the maximum cell concentration was achieved on day 4 in batch culture), cells continued to grow regardless of the media used. Despite this increased cell concentration, the maximum antibody concentration obtained with the medium exchange after 4 days cultivation was similar to that obtained with the medium exchange after 3 days cultivation because the q_{Ab} was not enhanced as much as q_{Ab} with the medium exchange after 3 days cultivation.

To understand the effect of hyperosmotic pressure on q_{Ab} of rCHO cells at the transcription level, possible changes in immunoglobulin (Ig) mRNA levels caused by hyperosmotic pressure were examined. Cells on day 4 in Fig. 2B, one day after medium exchange, were sampled from biphasic cultures at 294-522 mOsm/kg. Figure 3 shows the autoradiograms obtained as a result of hybridization of HC and LC probes to the

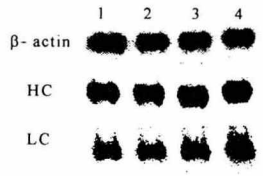

Fig 3. Northern blot analysis of SH2-0.32. Lane 1-4, 294, 370, 459, and 522mOsm/kg, respectively.

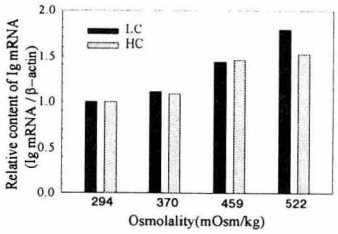

Fig 4. Relative content of Ig mRNA(intensity of Ig mRNA/ intensity of β- actin).

total cytoplasmic RNA. The rCHO cells (SH-0.32) subjected to hyperosmotic pressure displayed a positive relationship between the relative content of Ig mRNA and qAb, indicating that transcriptional regulation was involved in the response of rCHO cells to hyperosmotic pressure.

In conclusion, the feasibility of simple biphasic culture strategy based on hyperosmotic culture for improved foreign protein production from rCHO cells was demonstrated. Due to the enhanced q_{Ab} and increased cell concentration in biphasic culture, the maximum antibody concentration obtained in biphasic culture with 522 mOsm/kg medium exchange was 161% higher than that obtained in batch culture with the standard medium.

ACKNOWLEDGEMENTS

The authors thank Dr. H. J. Hong for providing the humanized antibody expression vector. This work was supported in part by grants from the Ministry of Science and Technology (National Research Laboratory Program) and the Ministry of Education (Brain Korea 21 Program).

REFERENCES

1. Cherlet M, Marc A, "Hybridoma cell behavior in continuous culture under hyperosmotic stress"(1999), *Cytotechnology*, *29*, 71-84.

2. Lee MS, Lee GM, "Hyperosmotic pressure enhances immunoglobulin transcription rates and secretion rates of KR12H-2 transfectoma"(2000), *Biotechnol. Bioeng.*, *68*, 260-268.

3. Oyaas K, Ellingsen TE, Dyrset N, Levine DW, "Hyperosmotic hybridoma cell cultures: Increased monoclonal antibody production with addition of glycine betaine"(1994), *Biotechnol. Bioeng.*, *44*, 991-998.

4. Ozturk SS, Palsson BO, "Effect of medium osmolality on hybridoma growth, metabolism, and antibody production"(1991), *Biotechnol. Bioeng.*, *37*, 989-993.

5. Ryu JS, Kim TK, Chung JY, Lee GM, "Osmoprotective effect of glycine betaine on foreign protein production in hyperosmotic recombinant Chinese hamster ovary cell cultures differs among cell lines"(2000), *Biotechnol. Bioeng.*, *70*, 167-175.