OTR12 - Effects of L-alanyl-L-glutamine media supplementation on batch hybridoma growth and monoclonal antibody production

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Introduction:
As the demand for monoclonal antibodies (mAb) is increasing, there is a significant interest in developing optimized cell culture processes for hybridoma. Optimization process comprises a number of variables, including the selection of better producer hybridomas, culture media and bioreactor culture conditions. L-glutamine is an unstable essential amino acid involved in hybridoma energy production, cell growth and antibody synthesis. However, L-glutamine breaks down to ammonium that can, at least, lower hybridoma growth and mAb production. Dipeptides of L-glutamine with L-alanine or L-glycine are stable forms, which can be used in cell culture media to avoid its negative effects.

Objective:
Evaluate the effects of L-alanyl-L-glutamine on hybridoma growth kinetics and mAb productivity.

Methodology:
The murine hybridoma cell line 90DA5/CB5/AA3, which produces mouse immunoglobulin (Ig) G1κ against PBP2a protein, was used in a series of batch experiments performed in roller bottles during 7 days. The medium utilized was DMEM high glucose (4.5g/L; LONZA) supplemented with glutamine or L-alanyl-L-glutamine (6.4mM; Gibco) and 10% v/v fetal calf serum (FCS). Cell counts were performed in Neubauer chamber, under optical microscope, after dilution in Trypan Blue 0,4%. After cell counting, each sample was centrifuged (200g, 10min) and the supernatant frozen for further analysis. Murine IgG (Mouse-IgG ELISA, Roche), L-glutamine (YSI2700 analyzer) concentrations were determined. Specific cell growth rate (μ) and doubling time (dt) were calculated using the differential method, during the exponential growth phase. Specific L-glutamine consumption rate (qSglu) and IgG production rate [qP(I-
were estimated by plotting total cell concentration, cumulative substrate consumption or production, versus the integral of viable cells (IVC) and fitting the plots with a regression coefficient of close to one.

**Results:**

L-alanyl-L-glutamine compared to L-glutamin- -supplemented media increased hybridoma cell growth, after 7 days, as measured by IVC (212390000 and 169267500 cell.h/mL, respectively) and extended the stationary phase (2.05±0.13 and 0.87±0.08 x106 cell/mL at 96h, respectively). Interestingly, it did not affect the maximum viable cell concentration (2.70±0.17 and 2.60±0.06 x106 cells/mL at 72h, respectively), μ (0.023 and 0.023h⁻¹, respectively) and dt (30 and 30h, respectively). In addition, free glutamine concentration during hybridoma cultivation with L-alanylL-glutamine-supplemented medium differed from glutaminesupplemented medium since it started with low levels (0.035 versus 1.180g/L), peaked at 24h (0.844 versus 0.853g/L) remained above control (0.523 versus 0.320g/L) and both returned to basal levels at 72h. Of note, spontaneous release of glutamine from L-alanyl-L-glutamine was observed in cellfree medium supplemented with FBS at 4 and 37°C. After 7 days of hybridoma cultivation in batch mode, L-alanylL-glutamine-supplemented medium presented an increase of 55% in antibody volumetric productivity when compared to glutamine-supplemented medium (70.7 and 45.4µg/mL, respectively) and an increased qP(IgG) at exponential phase (6.0 versus 4.5 x10⁻⁷µg/cell.h).

**Conclusion:**

L-alanyl-L-glutamine supplementation increased the hybridoma cell growth and significantly increased antibody volumetric productivity.

**Keywords:** Hybridoma, IgG Production, L-alanyl-L-glutamine supplementation