Mathematical Model of IL-6 signal transduction in Hepatocytes

Abhay K. Singh, Arul Jayaraman and Juergen Hahn
Department of Chemical Engineering
Texas A&M University
3122 TAMU
College Station, Texas, 77843-3122, U.S.A.

Keywords: Interleukin-6, Hepatocytes, Mathematical modeling, Signal transduction.

1.0 Introduction

This work focuses on the role of IL-6 in the hepatic acute phase response. A kinetic model of the signal transduction network in hepatocytes stimulated by IL-6 has been developed. Signaling induced by the IL-6 family of cytokines involves the activation of two major pathways: the JAK (Janus-associated kinases)/ STAT (signal transducers and transcription factors) and the MAPK (mitogen-activated protein kinases) pathways [2, 3]. While the first pathway involves the activation of gp130 (glycoprotein 130), JAK and transcription factor STAT3, the MAPK pathway is linked to JAK/STAT by SHP-2 (domain containing tyrosine phosphatase 2) through gp130. The activation of SHP-2 is a crucial event for signaling through the MAPK pathway. In addition to acting as an adaptor protein for the MAPK pathway, SHP-2 acts as a phosphatase for the JAK/STAT pathway [4]. The dual role of SHP-2 suggests possible interactions between the two signaling pathways. However, the involvement of both JAK/STAT and MAPK pathways in IL-6 signaling has been studied only in few cases [5] and the interactions between the two pathways and the role played by SHP-2 in IL-6 signal transduction are not clearly understood. In this work, a kinetic model has been developed that considers signaling through both, i.e. the JAK/STAT and the MAPK, pathways. The developed model is used to analyze the interactions between the two pathways and to investigate the role of SHP-2 in signal transduction. In addition to down regulation by SHP2, SOCS3 (suppressor of cytokine signaling 3) also acts as inhibitor for signal transduction through the JAK/STAT pathway [1, 2, 3]. It can be determined that the influence of SOCS3 and SHP-2 on signaling induced by IL-6 is not independent of each other. SOCS3 influences the phosphorylation of SHP2 and through it has an effect on signaling through the MAPK pathway. The influence of SOCS3 on signal transduction has been evaluated in this work by simulation of the developed model.
The results obtained through simulations have been compared with available results from the literature and were found to predict behavior inline with the experimental studies.

2.0 Model description

The kinetic model developed in this work is based on the IL-6 cytokine signaling pathway presented in Heinrich et al. (2003). The JAK/STAT part of the signal transduction pathway and

the model parameters have been adopted from Yamada et al. (2003) while the MAPK signaling pathway is mainly based on the work presented by Schoeberl et al. (2002). In the presented model IL-6 attaches to its receptor gp80, which is referred to as the non-signaling part of the receptor. The signaling part of the receptor gp130 binds to protein tyrosine kinases of the JAK family. The IL-6/gp80 complex then binds to the gp130/JAK compound and as a result the IL6-gp80-gp130-JAK complex is formed. This binding results in the dimerization of the IL6-gp80-gp130-JAK complex forming $(IL6\text{-}gp80\text{-}gp130\text{-}JAK)_2$. gp130 becomes tyrosine phosphorylated as a result of dimerization and recruits the transcription factor STAT3 which is also tyrosine phosphorylated. The phosphorylated STAT3 dissociates from the receptor complex $(IL6\text{-}gp80\text{-}gp130\text{-}JAK)_2$ and undergoes dimerization. The dimerized STAT3 complex translocates to the nucleus where it binds to a specific DNA element in the promoter of IL-6 target genes. In addition to activating the STAT3 transcription factor, the phosphorylated gp130 also recruits protein tyrosine phosphate SHP2 which subsequently undergoes phosphorylation. The activated SHP2 is essential for signaling through the MAPK pathway (Heinrich et al. (2003); Schmitz et. al (2000); Schaper et al. (1998)). The phosphorylated SHP2 interacts with Grb2 (growth factor receptor bound protein) and SOS (son of sevenless). The binding of Grb2 and SOS to the receptor complex leads to the activation of RAS, which further leads to the activation of the Ras-Raf-MAPK cascade.

SOCS3 is induced by IL-6 and inhibits JAK activity by binding to the same site as JAK in gp130. Thus SOCS3 acts as a feedback inhibitor for the JAK/STAT3 pathway. In addition to the phosphatase SHP2, PP1 and PP2 are two other phosphatases considered for the JAK/STAT part of the signal transduction pathway. These phosphatases influence the activation of STAT in the cytosol and the nucleus. Three phosphatases Phosp1, Phosp2 and Phosp3 are considered in the MAPK pathway that influence the activation of Raf, MEK, and ERK, respectively.

In the presented model all the reactions are represented by mass-action kinetics. There are 68 state variables in the presented model, resulting in as many ordinary differential equations. The differential equation for a particular component (A) is written as:

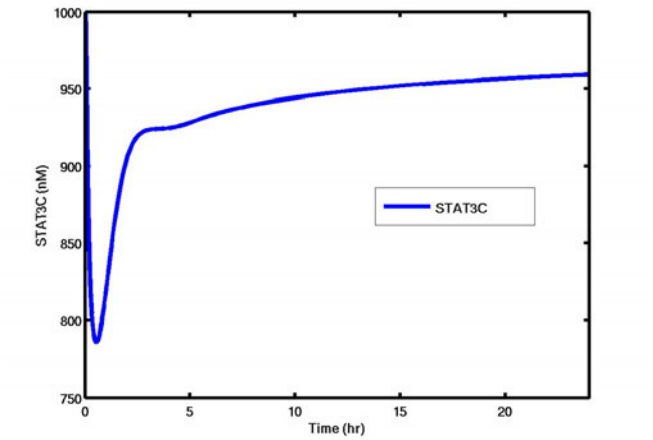
$$\begin{pmatrix}\text{Rate of change}\\ \text{of component (A)}\end{pmatrix} = \begin{pmatrix}\text{Amount of (A)}\\ \text{formed in all reactions}\end{pmatrix} - \begin{pmatrix}\text{Amount of (A)}\\ \text{consumed in all reactions}\end{pmatrix}$$

$$\frac{dN_A}{dt} = v_{A,produced} - v_{A,consumed}$$

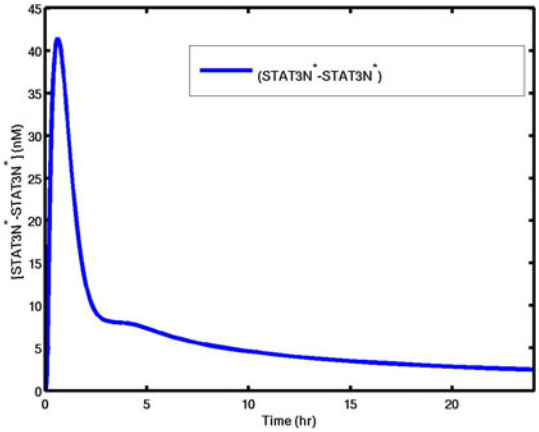
where v_A represents the rate of formation/consumption of species A in a particular reaction.
The presented model is solved using the routine ODE15s implemented in MATLAB.

3.0 Results

IL-6 binds to the receptor gp80 with low affinity (K_d=500pM). The IL-6-gp80 complex further binds with the gp130 with high affinity (K_d=15pM). IL-6 binding to the receptor eventually results in dimerization and phosphorylation of the IL6-gp80-gp130-JAK complex. Subsequently, the STAT3C (STAT3 in the cytosol) transcription factors undergo phosphorylation (Figure 1a) and dimerization. The dimerized STAT3 translocates to the nucleus and the nuclear STAT3 complex ($STAT3N^*\text{-}STAT3N^*$) reaches a maximal concentration in about 1 hour, rapidly decays, and attains a new steady state after about 5 hours (Figure 1b).



(a)

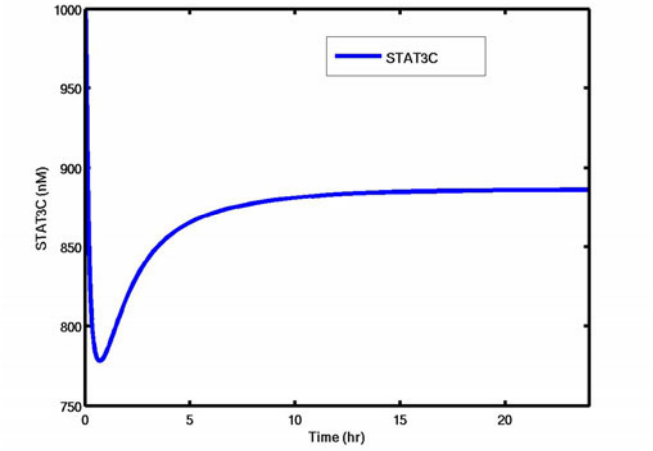


(b)

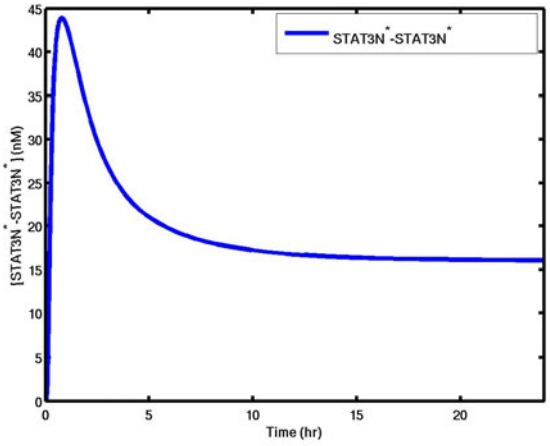
Figure 1. Dynamic response in hepatocyte cells for signal transduction induced by 10 ng/ml of IL-6 for24 hrs. (a) STAT3 in the cytosol (b) nuclear STAT3N-STAT3N**

In order to examine the influence of SOCS3 on signal transduction through the JAK/STAT pathway, dynamic responses of STAT3 (Figure 2a) and translocated nuclear STAT3 (Figure 2b) in SOCS3 knockout cells have been analyzed and compared. In SOCS3 knockout cells, STAT3 in the cytosol and nuclear STAT3 ($STAT3N^*$-$STAT3N^*$) show sustained activation. While the STAT3C concentration attains a steady state value of 875nM in about 5 hours (Figure 2a), STAT3C concentration in normal hepatocytes shows continuous recovery and the level of STAT3C in the cytosol reaches 960nM after 24 hours (Figure 1a).

As SHP-2 is one of the main components for initiating the MAPK pathway, it also acts as an inhibitor for the JAK/STAT pathway. SHP2 dephosphorylates the $(IL6\text{-}gp80\text{-}gp130\text{-}JAK^*)_2$ complex and thereby inhibits the phosphorylation of STAT3C. Enhanced activation of STAT3 in the cytosol and in the nucleus ($STAT3N^*$-$STAT3N^*$) is observed for simulations of cells where

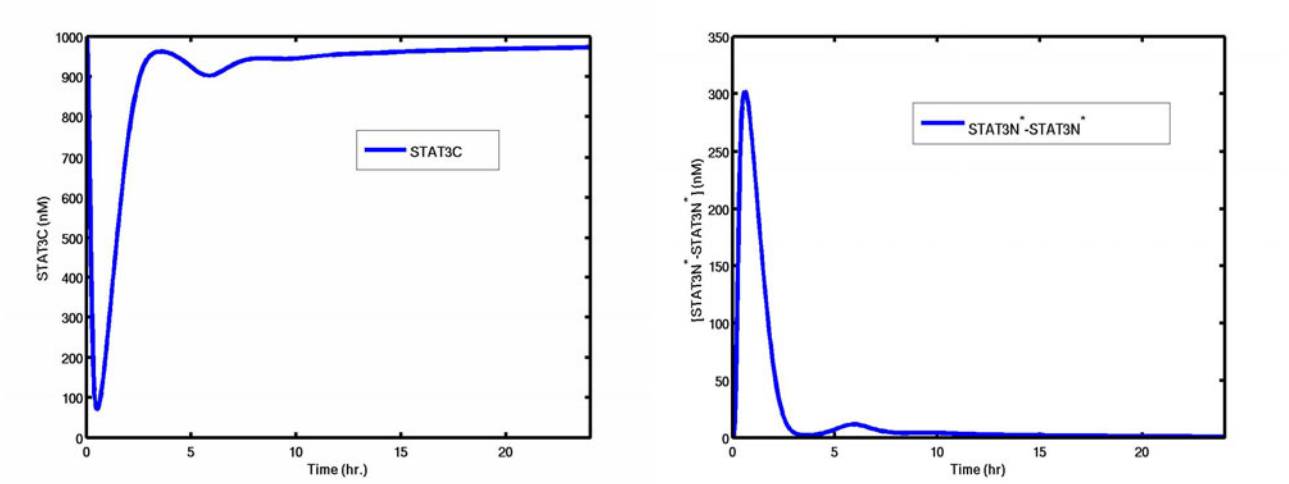


(a)



(b)

Figure 2. Dynamic response in SOCS3 knockout cells for signal transduction induced by 10 ng/ml of IL-6 for24 hrs. (a) STAT3 in the cytosol (b) nuclear STAT3N-STAT3N**



(a) (b)

Figure3.. Dynamic response for signal transduction induced by IL-6 for 24 hrs. with no phosphorylation of SHP-2 (a) STAT3 in the cytosol (b) nuclear STAT3N-STAT3N**

SHP-2 phosphorylation is blocked (Figure 3). The maximal level of $STAT3N^{*}$-$STAT3N^{*}$ in the above case reaches 300nM (Figure 3b) compared to 42nM in normal hepatocytes (Figure 1b). Essentially, signaling through the JAK/STAT pathway is enhanced when the MAPK pathway cannot be activated due to blocking of SHP-2 phosphorylation. Similar conclusions about the effect of SHP-2 on STAT activation have been reported in Schaper et al. (1998).

SOCS3 also influences the phosphorylation of SHP-2 and through it down regulates the signal transduction through the MAPK pathway. In order to corroborate these observations simulations have also been carried out in SOCS3 knockout cells. In the absence of SOCS3, phosphorylated SHP-2 shows sustained activation. Such sustained activation of SHP-2 will likely result in sustained signaling through the MAPK pathway as illustrated in Figure 4.

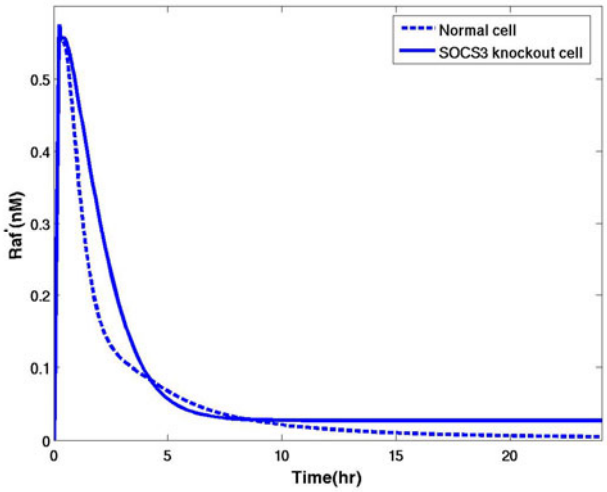


Figure 4. Dynamic response of Raf^{*} in normal cells and in SOCS3 knockout cells.

4.0 Conclusions

In this work a kinetic model for signal tranduction of hepatocytes stimulated by IL-6 has been presented. The model has been shown to predict behavior inline with the experimental studies found in the literature. The model was found to be robust with respect to variations in the initial conditions of proteins and phosphatases in the cells.

The presented model was used to investigate the inhibitory effect of SHP-2 and SOCS3 on signal transduction induced by IL-6. The model shows SOCS3 acts as an inhibitor for the JAK/STAT pathway, it also down regulates the phosphorylation of SHP-2 and inhibits signaling through the MAPK pathway.

References:

(1) Fischer, P.; Lehmann, U.; Sobota, R.M.; Schmitz, J.; Niemand, C.; Linnemann, S.; Haan, S.; Behrmann, I.; Yoshimura, A.; Johnston, J.A.; Muller-Newen, G.; Heinrich, P.C. & Schaper, F. (2004) The role of the inhibitors of interleukin-6 signal transduction SHP2 and SOCS3 for desensitization of interleukin-6 signaling. *Biochemical Journal*, 378, 449-460.
(2) Heinrich, P.C.; Behrmann, I.; Muller-Newen, G.; Schaper, F. & Graeve, L. (1998) Interleukin-6-type cytokine signaling through the gp130/JAK/STAT pathway. *Biochemical Journal*, 334, 297-314.
(3) Heinrich, P.C.; Behrmann, I.; Haan, S.; Hermanns, H.M.; Muller-Newen, G. & Schaper, F. (2003) Principles of interleukin (IL)-6-type cytokine signaling and its regulation. *Biochemical Journal*, 374, 1-20.
(4) Kim, H. & Baumann, H. (1999) Dual signaling role of the protein tyrosine phosphate SHP-2 in regulating expression of acute-phase plasma proteins by interleukin-6 cytokine receptors in hepatic cells. *Molecular and Cellular Biology*, 19, 5326-5338.
(5) Schaper, F.; Gendo, C.; Monika, E.; Schmitz, J.; Grimm, C.; Anhuf, D. & Kerr, I.M. (1998) Activation of the protein tyrosine phosphate SHP2 via the interleukin-6 signal transducing receptor protein gp130 requires tyrosine kinase JAK1 and limits acute-phase protein expression. *Biochemical Journal*, 335, 557-565.
(6) Schmitz, J.; Weissenbach, M.; Haan, S.; Henerich, P.C.; Schaper, F. (2000) SOCS3 exerts its inhibitory function on interleukin-6 signal transduction through the SHP-2 recruitment site of gp130. *The Journal of Biological Chemistry*, 275, 12848-12856.
(7) Schoeberl, B.; Eichler-Jonsson, C.; Gilles, E.D.; Muller, G. (2002) Computational modeling of the dynamics of the MAP kinase cascade activated by surface and internalized EGF receptors. *Nature Biotechnology*, 20, 370-375.
(8) Yamada, S.; Shiono, S.; Joo, A. & Yoshimura, A. (2003) Control mechanism of JAK/STAT signal transduction pathway. *FEBS Letters*, 534, 190-196.