Mathematical Model of the Combined Effect of IL-6, TGF-beta and TNF-alpha on Pancreatic Stellate Cell Activation

Thesis
Presented in Partial Fulfillment of the Requirements for the Masters of Mathematical Sciences in the Graduate School of The Ohio State University

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Abstract

Chronic pancreatitis is a long-term inflammatory disease of the pancreas that renders the pancreas unable to produce enzymes in the amounts needed for the body. Playing a crucial role in the genesis of the disease are the pancreatic stellate cells (PSCs) found in the peracinar region of the organ. Their activation is enhanced by tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), cytokines released by macrophages drawn into the pancreas through inflamed endothelial cells. The PSCS release additional cytokines, including transforming growth factor beta (TGF-β). Together these soluble factors likely have an effect on the activation of PSC, their proliferation rate and subsequent secretion of cytokines into the pancreatic microenvironment.

Using data obtained from a collaborating research laboratory and data found in the literature, I have formulated several differential equations linking the concentrations of these cytokines. In addition, I have formulated one differential equation analyzing the effect of the drug somatostatin on cytokine levels. These equations deserve evaluation for future potential use in diagnosing the course of pancreatic cancer. They may also be useful for the study of interdependency of more cytokines and more drugs on the disease process.
Dedication

This is dedicated to my parents, Erin and Robyn.
Acknowledgments

I would like to thank Dr. Avner Friedman for taking the time to explain the subject matter to me and showing me how to find and interpret the proper literature, as well as Dr. Gregory Lesinski for the data that his lab provided.
Vita

2007 .............................................. Evangelical Christian School

2011 ........................................................ B.S. Biochemistry and Molecular Biology,
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2013 ........................................................ B.S. Mathematics, Florida Gulf Coast University

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Fields of Study

Major Field: Mathematical Sciences
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Introduction

The pancreas is a glandular organ located behind the stomach in human beings, about six inches long, widest at the head and narrowest at the tail. Running through the organ is the pancreatic duct, which connects to the common bile duct and the duodenum (Figure 1). The pancreas is made of exocrine and endocrine cells. Exocrine cells make up most of the pancreatic mass and are responsible for production of pancreatic juice. Endocrine cells are arranged in clusters called the Islets of Langerhans, which release insulin and glucagon to control sugar level in the blood.

Chronic pancreatitis is a long-lasting pancreatic inflammation in which the pancreas progressively undergoes irreversible scarring and function loss, thus rendering enzyme production at levels unsuitable for the body. Symptoms include vomiting, nausea, and abnormal pain. No cure has been found yet, but treatments exist for pain control and artificial pancreatic enzymes. [1,2]

Pancreatic stellate cells (PSCs) are (normally quiescent) cells similar to myofibroblasts that are usually found in the periacinar region of the pancreas [1,2] and are responsible for maintaining tissue structure through regulating the extracellular matrix. PSCs can switch phenotypes from quiescent to activated, by which they migrate to injured tissue sites, partake in repair and excrete ECM proteins. This causes them to have in important role in the beginning of pancreatitis. [2]
Chronic pancreatitis has a multifactorial pathogenesis with several triggers including alcohol and genetic mutations. The disease is characterized by inflammation causing endothelial cells to secrete monocyte chemoattractant protein (MCP) which brings in monocytes from the blood. Once in pancreas, monocytes will differentiate into macrophages and secrete tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), both of which activate the PSCs, which then secrete cytokines that trigger inflammatory cytokines including transforming growth factor beta (TGF-β). All three of these cytokines enhance PSC activation. Activated PSC proliferation is increased by TNF-α and decreased by TGF-β and IL-6. [1,2]

The above processes are visualized in Figure 2. The purpose of this paper is to examine the effects of IL-6, TGF-β, and TNF-α on one another and how they combine to effect PSC activation, as these are the major cytokines that may play a role in the pathogenesis. Because of the focus of this paper, several other cell types, cytokines, and proteins that are involved in this network are not included.

Figure 2: General interaction of cytokines on activated PSCs in chronic pancreatitis. Blue arrow represents secretion, orange arrow means that one cytokine is proposed to affect the production of another, red arrow represents decreased proliferation, and green arrow represents increased proliferation.
Calculating rate of IL-6 while independent of TGF-beta

The mathematical model for cytokine action on activated PSCs, based on the network shown in Figure 2, will include the following variables:

- $P$: density of activated PSCs (cells/ml)
- $T_\beta$: concentration of TGF-$\beta$ (g/ml)
- $T_\alpha$: concentration of TNF-$\alpha$ (g/ml)
- $I_6$: concentration of IL-6 (g/ml)

Next, we introduce the general equation for IL-6:

$$\frac{dI_6}{dt} = (a_{PSC}) \times P - \mu_I \times I \quad (1)$$

For the sake of simplicity, IL-6 is assumed to be produced at a constant rate and undergoes a natural decay at constant rate $\mu_I = 0.173$ day$^{-1}$ [5]. The other variables to be determined are as follows:

- $a_{PSC}$: activation rate of IL-6 due to PSC
- $b_{T\beta}$: activation rate of IL-6 due to TGF-beta

The ‘activation rate’ is defined as the amount of IL-6 produced by an activated PSC. To determine the value for ‘$a_{PSC}$’, I have obtained data from Dr. Gregory Lesinski of the Cancer Department of The Ohio State University.
In obtaining the above data, immortilized human PSCs were plated in a 96-well plate (2ml per well) and used enzyme-linked immunosorbent assay (ELISA) to measure cell number at specific time points over a 48 hour period.

Using Berkely-Madonna, solving for \( aP \) from (1) at each time gives (using the same known parameters as described above):

<table>
<thead>
<tr>
<th>Time</th>
<th>( aP ) (g/ml*day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4hrs</td>
<td>0</td>
</tr>
<tr>
<td>8hrs</td>
<td>( 1.38 \times 10^{-10} )</td>
</tr>
<tr>
<td>12hrs</td>
<td>( 2.085 \times 10^{-10} )</td>
</tr>
<tr>
<td>24hrs</td>
<td>( 2.112 \times 10^{-10} )</td>
</tr>
<tr>
<td>48hrs</td>
<td>( 1.212 \times 10^{-10} )</td>
</tr>
</tbody>
</table>

Table 1 - values of \( aP \) calculated at each time interval for data in Figure 3

The ELISA also produced results for the average cell count per well at each time point:

<table>
<thead>
<tr>
<th>Hours</th>
<th>Series1</th>
<th>Series2</th>
<th>48h</th>
<th>12h</th>
<th>24h</th>
<th>48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>45</td>
<td>134.0625</td>
<td>193.4375</td>
<td>204.375</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3 - IL-6 production from PSCs, measured by ELISA over 48 hours using ELISA.
Figure 4 - average cell count per well at each time point for data in Figure 3.

Now we can calculate an ‘a_PSC’ value for each time (excluding the outlier at 24 hours) using Berkeley-Madonna.

<table>
<thead>
<tr>
<th>Time</th>
<th>aP (g/ml*day)</th>
<th>P (cells/ml)</th>
<th>a (g/cells*day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 hrs</td>
<td>0</td>
<td>40,000</td>
<td>0</td>
</tr>
<tr>
<td>8 hrs</td>
<td>1.38x10⁻¹⁰</td>
<td>45,000</td>
<td>3.07x10⁻¹⁵</td>
</tr>
<tr>
<td>12 hrs</td>
<td>2.085x10⁻¹⁰</td>
<td>57,500</td>
<td>3.626x10⁻¹⁵</td>
</tr>
<tr>
<td>24 hrs</td>
<td>2.112x10⁻¹⁰</td>
<td>45,000</td>
<td>4.716x10⁻¹⁵</td>
</tr>
<tr>
<td>48 hrs</td>
<td>1.212x10⁻¹⁰</td>
<td>70,000</td>
<td>1.73x10⁻¹⁵</td>
</tr>
</tbody>
</table>

Table 2 – calculating the value of the activation rate of IL-6 at each time point in Figure 3.
This gives an average \( a_{\text{PSC}} = 3.2855 \times 10^{-15} \text{ g/cells*day}^{-1} \). We can use this data to plot the secretion amounts of IL-6 at different cell concentrations (Figure 5).

**Figure 5 – IL-6 production at different PSC concentrations**

**Calculating rate of IL-6 while dependent upon TGF-beta1**

When considering the effect of incubation within a constant TGF-\( \beta \) concentration we must also consider the effect of the TGF-\( \beta \) saturation \( K_{T\beta} = 1 \times 10^{-9} \text{ g/ml} \) [4]. This leaves one final variable to find - \( b_{T\beta} \): activation rate of IL-6 due to TGF-beta, or the amount of IL-6 produced due to the effect of TGF-\( \beta \).

With PSCs incubated in a fixed concentration of TGF-\( \beta \), the general equation for IL-6 is rewritten as:
\[
\frac{dl_6}{dt} = \left( a_{PSC} + b_{T\beta} \times \frac{T_{\beta}}{K_{T\beta} + T_{\beta}} \right) \times P - \mu_l \times l \quad (2)
\]

In 2006, Aoki et al. published evidence for the existence of interdependence between IL-6 and TGF-beta1 in activated PSCs in rats [3]. Included in their research is an experiment by which they examined the stimulating effect of TGF-beta1 on the PSC secretion of IL-6, which was done by examining the effect of TGF-beta1 on IL-6 expression and secretion from PSCs [3].

<table>
<thead>
<tr>
<th>TGF-beta1 (ng/ml)</th>
<th>IL-6 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.39</td>
</tr>
<tr>
<td>0.02</td>
<td>6.67</td>
</tr>
<tr>
<td>0.2</td>
<td>11.4</td>
</tr>
<tr>
<td>0.6</td>
<td>11.9</td>
</tr>
<tr>
<td>1.2</td>
<td>12.5</td>
</tr>
<tr>
<td>2</td>
<td>13.1</td>
</tr>
</tbody>
</table>

Table 3 – concentration of IL-6 secreted after 48 hours as effected by varying levels of TGF-\(\beta\)

Using this data and equation (1), we can determine average values for the cross products of the IL-6 activation rates and the PSC density:

- \(a_{PSC}\): 3.78 \(x\) 10\(^{-9}\) day\(^{-1}\)
- \(b_{T\beta}\): 7.41 \(x\) 10\(^{-9}\) day\(^{-1}\)

Now using the value of \(a_{PSC}\) as determined from Dr. Lesinski’s data, the value of \(P\) can be determined for this experiment using Berkeley-Madonna.

<table>
<thead>
<tr>
<th>TGF-beta1 (ng/ml)</th>
<th>(aP) (g/ml*day(^{-1}))</th>
<th>(P) (cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.78(x)10(^{-9})</td>
<td>1,150,500</td>
</tr>
</tbody>
</table>

Table 4 – calculating the concentration of cells for the Aoki data.
Assuming this P stays consistent for every trial, we can calculate the value of ‘b_{\beta}’

This gives an average $b_{\beta} = 6.8521 \times 10^{-15}$ g/cells*day$^{-1}$ not including the outlier at T=0.2. Now that all the coefficients are known, equations (1) and (2) can be plotted using the given concentration of TGF-β (Figure 6).

<table>
<thead>
<tr>
<th>TGF-beta1 (ng/ml)</th>
<th>bP (g/ml*day$^{-1}$)</th>
<th>b (g/cells*day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>8.25x10$^{-9}$</td>
<td>7.1708x10$^{-15}$</td>
</tr>
<tr>
<td>0.2</td>
<td>1.78x10$^{-8}$</td>
<td>1.5472x10$^{-14}$</td>
</tr>
<tr>
<td>0.6</td>
<td>8.7x10$^{-9}$</td>
<td>7.5619x10$^{-15}$</td>
</tr>
<tr>
<td>1.2</td>
<td>6.7x10$^{-9}$</td>
<td>5.8236x10$^{-15}$</td>
</tr>
<tr>
<td>2</td>
<td>6x10$^{-9}$</td>
<td>2.2291x10$^{-15}$</td>
</tr>
</tbody>
</table>

Table 5 – calculating the average activation rate of IL-6 due to TGF-β
Calculating rate of TGF-beta while dependent upon IL-6

The differential equation for TGF-beta1 is given as:

\[ \frac{dT_\beta}{dt} = \alpha \cdot P - \mu_T \cdot T_\beta \quad (3) \]

Here ‘\( \alpha \)' is the activation rate of TGF-beta and ‘\( \mu_T \)' is the degradation rate of TGF-beta. However, if TGF-\( \beta \) concentration is indeed dependent on the constant amount of IL-6 that the cells are incubated in, then we can add to the equation:

\[ \frac{dT_\beta}{dt} = \left( \alpha + \beta \cdot \frac{I_6}{K_I + I_6} \right) \cdot P - \mu_T \cdot T_\beta \quad (4) \]
Here ‘$K_i$’ is the IL-6 saturation. For this equation, the following are known:

- $K_i = 3 \times 10^{-11}$ g/ml [6]
- $\mu_T = 333 \text{ day}^{-1}$ [7]

To determine the validity of this model, refer to Figure 6 of the Aoki paper, in which PSCs are incubated for 48 hours with or without 100ng/ml IL-6.

<table>
<thead>
<tr>
<th>IL-6</th>
<th>Secreted (pg/ml)</th>
<th>TGF-beta1</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td>100 ng/ml</td>
<td>125</td>
<td></td>
</tr>
</tbody>
</table>

Table 6 – Data from Aoki et. al. showing the effect of IL-6 on TGF-β secretion

Substituting the constants found above into equation (4) and setting $t=2$ days, the following can be determined for the estimated TGF-beta1 values:

- $\alpha P = 2.07 \times 10^{-8}$ day$^{-1}$
- $\beta P = 2.1 \times 10^{-8}$ day$^{-1}$

Assuming the same cell density as before, $P = 1,150,000$ cells/ml, we get:

- $\alpha = 1.8 \times 10^{-14}$ g/cells*day$^{-1}$
- $\beta = 1.8261 \times 10^{-14}$ g/cells*day$^{-1}$

When (4) is plotted (Figure 7), it is found that regardless of the effect of IL-6, the concentration of TGF-β reaches its maximum point at approximately 0.01 days (~14.4 minutes), which coincides with the Aoki paper reporting TGF-β to reach its maximum secretion level at 10 minutes.
Figure 7 – secretion of TGF-β from PSCs with and without the presence of IL-6.

**Calculating Rate of IL-6 dependent upon TNF-alpha in Pancreatic Periacinar Myofibroblasts**

Pancreatic periacinar myofibroblasts (PPM), found in the periacinar region of the human pancreas, has been demonstrated to secrete large amounts of IL-6 and IL-8 when activated by IL-1 and TNF-α. In [8], we are told the effects of somatostatin on IL-6 mRNA expression in PPM that had been incubated for four hours with 50 ng/ml of TNF-alpha with or without somatostatin.
The resulting data is used for determining the coefficients for IL-6 concentration change while dependent upon TNF-alpha levels and the drug Somatostatin:

<table>
<thead>
<tr>
<th>TNF-alpha (ng/ml)</th>
<th>Somatostatin (nM)</th>
<th>IL-6 (ng/10^5 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>13.6364</td>
</tr>
<tr>
<td>50</td>
<td>None</td>
<td>109.0909</td>
</tr>
<tr>
<td>50</td>
<td>0.01</td>
<td>95.4545</td>
</tr>
<tr>
<td>50</td>
<td>0.1</td>
<td>72.7273</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
<td>59.0909</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 7 - combined effect of the drug Somatostatin and TNF-α on IL-6 secretion

The equation for IL-6 can be written as:

$$ \frac{dI_6}{dt} = a_{PPM} + b_{TNF} \left( \frac{T_\alpha}{1 + \rho D} \right) - \mu_I I_6 \tag{5} $$

Rearranged this comes to:

$$ \frac{dI_6}{dt} = a_{PPM} + \frac{b_{TNF} T_\alpha}{K_{TNF} (1 + \rho D) + T_\alpha} - \mu_I I_6 \tag{6} $$

Here ‘$a_{PPM}$’ is the activation rate of IL-6 due to PMF, ‘$b_{TNF}$’ is the activation rate of IL-6 due to TNF-alpha, $K_{TNF}$ is the TNF-alpha saturation, ‘$\rho$’ is the Somatostatin dependence, ‘$D$’ is the somatostatin concentration, and ‘$\mu_I$’ is the degradation rate of IL-6. (Because of the units of $\mu_I$, we do not need to consider PPF density.)

Using the following known constants:

- $K_{TNF} = 5 \times 10^{-9}$ g/ml [9]
- $\mu_I = 0.173$ day$^{-1}$

We can calculate the following variants:

- $a_{PPM} = 1.49 \times 10^{-13}$ g/cells*day$^{-1}$
- $b_{TNF} = 1.1 \times 10^{-12}$ g/cells*day$^{-1}$

To calculate ‘$\rho$’ we use the following data and Berkeley-Madonna, with the molar mass of Somatostatin as 1638.1 g/ml [10]:

12
The mean value of \( \rho \) is calculated to be \( 1.2563 \times 10^{10} \). When the equation (6) is plotted (Figure 8), it is shown that TNF-\( \alpha \) has a strong positive effect on IL-6 production, but increasing doses of somatostatin bring the secretion levels down, matching the data.

<table>
<thead>
<tr>
<th>Somatostatin (nM)</th>
<th>Somatostatin (ng/ml)</th>
<th>IL-6 (ng/10^5 cell)</th>
<th>( \rho ) (unitless)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>0.016381</td>
<td>95.4545</td>
<td>8.5x10^{10}</td>
</tr>
<tr>
<td>0.1</td>
<td>0.16831</td>
<td>72.7273</td>
<td>3.75x10^{10}</td>
</tr>
<tr>
<td>1</td>
<td>1.6831</td>
<td>59.0909</td>
<td>6.75x10^{9}</td>
</tr>
<tr>
<td>10</td>
<td>16.381</td>
<td>50</td>
<td>1x10^{9}</td>
</tr>
</tbody>
</table>

Table 8 – calculating the Somatostatin dependence.

Figure 8 – IL-6 secretion as affected by TNF-\( \alpha \) and the drug Somatostatin
Conclusion

The production of cytokines IL-6, TNF-α, and TGF-β are all highly deregulated in chronic pancreatitis, and we have shown how high concentrations of one will affect the production rate of another. Because each of these cytokines affects the proliferation rates of activated PSCs, knowing the levels of each may be useful in diagnosis. Furthermore, targeting these cytokines may help to alleviate the disease.

Suggestions for future work are investigating the production of one cytokine while incubated in levels of two or more different cytokines, and in studying the effects other drugs on cytokine production.
Appendix: calculating the degradation rate of IL-6 and TGF-beta

According to [5], IL-6 has a half-life of 3-4 days. Thus the degradation rate ($\mu_I$) is:

$$\mu_I = \frac{\ln 2}{4 \text{ days}} = 0.173 \text{ day}^{-1}$$

According to [7], active TGF-beta1 has an active half-life of 2-3 min. From this, we can calculate the degradation rate ($\mu_T$) as follows:

$$\mu_T = \frac{\ln 2}{3 \text{ min}} \times \frac{60 \text{ min}}{1 \text{ hr}} \times \frac{24 \text{ hr}}{1 \text{ day}} = 333 \text{ day}^{-1}$$
References