A linear kinetic approach and near equilibrium thermodynamics of serum parameters from atherosclerosis-induced animals and treated with drugs

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1 Introduction

Atherosclerosis is the major cause of morbidity and mortality throughout the world. Atherogenesis is a complex process in which the lumen of a blood vessel becomes narrowed by cellular and extra cellular components proliferation to the point of obstruction. Lesions tend to form at the branch points of arterial blood vessels and then progress through different stages.

To identify the cells and molecules involved in each stage of the atherosclerotic process, as well as the environmental and genetic factors that promote lesion formation is an important task.

The multi-factorial origin of coronary heart disease (CHD) is now well established. Levels of the major risk factors are changing and understanding of the joint effects of these changes on possible future trends in CHD is vital for treatment of the disease.

Among the major families of hypolipidemic drugs are statins. The statins have been found to have beneficial anti-atherosclerotic effects, as inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. These compounds (statins) decrease cellular cholesterol synthesis, reduce the progression and may induce the regression of atherosclerotic lesions.

There are numerous experimental data indicating that statins can also interfere with some other events involved in the formation or reduction of atherosclerotic lesions, independently or not of their hypo-cholesterolemic potential [1],[2].

To this purpose, we studied the effect of hypolipidemic drugs (i.e.statins) on the evolution of atherosclerotic lesions and of specific serum parameters (cholesterol [C], low density lipoproteins [LDL], high density lipoproteins [HDL], lipid peroxides [TBARS], antioxidant potential [TRAP], angiotensin converting enzyme [ACE], triglycerides [TG] for a group of hyperlipidemic hamsters compared with normal animals.

We used the Golden Syrian hamster model, previously reported as a good atherosclerosis model,[3], [4], to investigate vascular changes along the hyperlipidemic diet. The hamster model shares similarities with human atherosclerosis: 1) the main plasma cholesterol carrier is LDL and Lp-metabolism develops as in man [5]; 2) the hamster LDL receptor gene has common sequences with the human gene [6], 3) atheroma develops in the aortic arch, the aortic aspect of the sigmoid valves and coronary arteries, allowing the sequential following of the atherosclerotic process from its inception.

Hyperlipemic hamsters were fed standard chow supplemented with 3% cholesterol and 15% butter and drug treatment was performed manly by gavage administration of Simvastatin (ZOCOR, MSD). The duration of the hyperlipidemic diet was of three months, both for the control group and for the treated group. For the latter in the last month of diet Simvastatin was administered.

At various time intervals the concentration of serum parameters was measured and at the end of drug administration the animals were sacrificed and the extent of lesions was determined.

The experimental results showed: a) the percentage of lesions is strongly reduced after three months of hyperlipidemic diet combined with one month of treatment, showing that Simvastatin has a very beneficial effect on atherosclerosis progression, b) there are important interactions (correlations) among all measured parameters and also with extent of the lesions, which means the process is of a multi-factorial type, c) the concentration of serum parameters depends of time, along the experimental period, i. e. the stationary state was not reached after one month treatment.

2 The theoretical model

Since the parameters we measured are macroscopic, in order to understand the phenomena (the effects of diet and drug treatment) we imagine a "bio-chemical picture" in the following manner: at time t=0 (initial time) if diet D is administered to the animals there is a "chemical reaction".

$$\sum_i A_i + D \rightleftarrows \sum_i A_i^{(1)} \tag{1}$$

where A_i represents the serum parameter "i", D is the diet and $A_i^{(1)}$ is the same serum parameter "i" with its concentration modified due to the diet D.

At time $t = t_0$ if we administer the same diet D (as a continuation) to the control group and the reaction is:

$$\sum_i A_i + D \rightleftarrows \sum_i A_i^{(2)} \tag{2}$$

Also at time $t = t_0$ if the second group of animals received the drug M along the diet D the reaction is:

$$\sum_i A_i + D + M \rightleftarrows \sum_i A_i^{(3)} \tag{3}$$

The corresponding values $A^{(1)}, A^{(2)}$ depend on the diet D and $A^{(3)}$ on the continuation of diet D and drug M. (We suppose the backward reaction is very unlikely).

2.1 The linear kinetic equation

Because the serum parameters are slowly varying with time, the linear approach (near equilibrium description) was considered adequate.

We note the concentration of the parameters x_i, x_D, x_M respectively and, in the linear approach, we can write the following set of coupled kinetic equations for the variation of concentration with respect time, for all serum parameters, including the diet and drug:

$$\frac{dx_p}{dt} = \sum_q \alpha_{pq} x_q, \quad p, q = i, D, M \tag{4}$$

where in our "bio-chemical model" α_{pq} are coupling coefficients given by:

$$\alpha_{pp} = -\sum_{q \neq p} k_{pq} \quad (diagonal\ terms) \tag{5}$$

$$\alpha_{pq} = k_{qp}, \ q \neq p \quad (off-diagonal\ terms) \tag{6}$$

In (5) and (6) k_{pq} are the rate constants and in general $k_{pq} \neq k_{qp}$

The system of equations (4) satisfies the Onsager's principle.

The solutions $x_p(t)$ of (4), may explain, in principle, both qualitatively and quantitatively the experimental data obtained when drugs are given to animals.

We are looking for solutions of (4) under the form [7]:

$$x_p = A_p e^{rt} \tag{7}$$

where r values are obtained, as functions of α_{pq} from the characteristic determinant:

$$det|r\delta_{pq} - \alpha_{pq}| = 0 \tag{8}$$

For some values of α_{pq} the roots of (8) can be complex conjugate numbers: $r = \alpha + i\beta$, $r^* = \alpha - i\beta$ and $x_p(t)$ becomes [7].

$$x_p(t) = (A_p \cos\beta t + B_p \sin\beta t)e^{-\alpha t} + C_p \tag{9}$$

When the product βt is very small, the solution (9) becomes:

$$x_p(t) \simeq (a_p + b_p t)e^{-\alpha t} + c_p \tag{10}$$

where a_p, b_p and c_p are constant which can be determined from the limit conditions. For example: at $t = 0$ we have $a_p + c_p = x_p(0)$ and for t going to infinite and $\alpha_p > 0$, $c_p = x(\infty)$. The constants b_p can be determined when $x_p(t)$ of (10) is introduced in (4) or by fitting our solutions with the experimental data. In the last case, depending of the experimental values of x_p, from (10) we obtain curves with maximum or minimum peaks, and these extremum points help us to determine α. These types of solutions, of equations (4), explain quite well the variation of serum parameters concentrations.

In the case of our experiment, for the time interval of three months, the average values of [C], [TG], [LDL], [HDL], [TBARS] and [ACE] correspond to curves having a maximum and [TRAP] a minimum. Similar results has been obtained recently for human [LDL], [8] and [TG], [9]. Also for rabbits platelet-derived growth factor (PDGF), [10] and mices cholesterol [11].

In addition, from equation (4) we deduce the condition of conservation of matter in the process of parameters interaction i.e.

$$\sum_p \frac{dx_p}{dt} = 0 \tag{11}$$

When the equation (11) is integrated both for the control and treated group, by using the limit conditions to determine the integration constants, we obtained an expression for the "consumed" drug concentration

$$X_M(t) = \Big[X_i^{(D)}(t) - \sum_i X_i^{(D+M)}(t)\Big] + \Big[X_D^{(D)}(t) - X_D^{(D+M)}(t)\Big] +$$
$$\Big[\sum_i X_i^{(D+M)}(t_0) - \sum_i X_i^{(D)}(t_0)\Big] + \Big[X_D^{(D+M)}(t_0) - X_D^{(D)}(t_0)\Big] + X_M(t_0) \quad (12)$$

where the symbol (D) is used for the control group, $(D+M)$ for the treated one and t_o is the time when the treatment with the drug begins. The expression (12) for X_M gives us the possibility: a) to calculate, at any given time point, the "consumed" drug concentration (in the process of interaction) which is time dependent even if the given drug is constant, b) to increase or decrease the dose of the drug in order to optimize the treatment.

To estimate $X_M(t)$, via (12), we can use either the solutions (9), and (10) or the experimental values. The relation (12)under some conditions, may be simplified. For example, if the control and treated groups are homogeneous enough (which is an important requirement in such experiments)and the direct interaction between diet and drug is very weak (a realistic assumption) then instead of (12) one obtains the relation.

$$X_M(t) \simeq \Big[\sum_i X_i^{(D)}(t) - \sum_i X_i^{(D+M)}(t)\Big] \quad (13)$$

To obtain the relation (13) in addition to the above assumption, we supposed that the effect of the drug is manifested after a time longer than t_0 which is equivalent to:

$$X_M(t_0) \simeq \Big[\sum_i X_i^{(D)}(t_0) - \sum_i X_i^{(D+M)}(t_0)\Big] \quad (14)$$

When the average values of the measured serum parameters (both for control and treated groups of hamsters) at the end of experiment are used, via (13), we get:

$$X_M(t) \simeq 0.48 mmol/L \quad (15)$$

which is about 72% of the administered Simvastatin dose of one day. Also, the value of estimated X_M can be compared with the corresponding dose of the drug at least or higher than 0.55 m mol/L that can be found in the hamster liver.

2.2 The production of entropy

In the frame of the same "bio-chemical picture" and near thermodynamic equilibrium the production of entropy can be written as

$$\frac{dS_{ext}^{(D)}}{dt} + \frac{dS_{int}}{dt} \rightleftarrows \frac{dS_{int}^{(D)}}{dt} \quad (16)$$

$$\frac{dS^{(D)}}{dt} + \frac{dS_{ext}^{(M)}}{dt} \rightleftarrows \frac{dS_{int}^{(D+M)}}{dt} \quad (17)$$

where "ext" refers to the administered diet D and drug M and "int" to hamsters parameters.

In (16) and (17) $\frac{dS_{int}}{dt} \geq 0$ (required by Prigogine principle) while $\frac{dS_{ext}}{dt} \gtrless 0$ for the diet and drug.

Following [12] - [14] and in agreement with our model the production of entropy of the hamster (with diet or/and drug) can be written as:

$$\frac{dS_{int}^{(f)}}{dt} = \frac{AJ}{T} = R\left[k_{12}\prod_k (x_k)^{\nu_k} - k_{21}\prod_l (x_l)^{\nu_l}\right] \cdot \left[lnK\frac{\prod_k (x_k)^{\nu_k}}{\prod_l (x_l^{\nu_l}}\right] \tag{18}$$

where A is the chemical affinity, J is current flux, T-the temperature, R-the ideal gases constant, k_{12}, k_{21} the rate constants, K-the chemical equilibrium constant, ν_k and ν_l the stochiometric coefficients for reactants and products respectively and f=D or D+M.

The form (18) of entropy production satisfied the Prigogine principle, i. e. $\frac{dS_{int}^{(f)}}{dt} \geq 0$

For us the most interesting case is when

$$\frac{d}{dt}\frac{dS_{int}^{(D+M)}}{dt} = 0, \ \frac{dS_{int}^{D+M}}{dt} = constant \tag{19}$$

i.e. the stationary state,because in that case the treatment is supposed to be the most efficient.

The relation (19) tells us that in a stationary state the production of entropy is minimum (Prigogine principle).

To see if relations (19) are satisfied, via (18) there are two possibilities: a) to use the solutions of (4) for x_p or b) to use the corresponding experimental values after a time $t \geq t_s$ when x_p are almost constant (t_s is the moment when stationary state is reached).

Considering t_s, the moment when animals are sacrificed, in a good approximation, the condition of stationarity is fulfilled, which means the experimental interval of three months is relevant.

In general, from our calculations results that, to satisfy (19), via (18), it is necessary to have interactions between at least two components. This is in agreement with our approach[eqs.(4)] and also with the experimental data. In addition, from the minimum of entropy production it can be determined the time t_s(the critical time) in order to obtain the stationary state.

As an application we consider the following case, for the terms of left side in (17):

$$\frac{dS_{int}}{dt} = 0, \frac{dS_D}{dt} = C_D = constant, \frac{dS_M}{dt} = C_M = constant \tag{20}$$

We considered that this approximation is justified because we started with an initial state of equilibrium, the given dose of the drug and diet were constant during the experimental period and the "consumed" drug is slowly varying with time.

For homogeneous groups of subjects, by integration, via (16), (17) and (20), for a stationary state, we obtain.

$$S_{int}^{(D+M)}(t) - S_{int}^{(D)}(t) = C_M(t - t_s) \tag{21}$$

for $C_M = -C_D, \ t > t_s$

The equality $C_M = -C_D < 0$ is imposed by the requirement to have a stationary state (i.e. $\frac{dS_D}{dt} > 0$ and $\frac{dS_M}{dt} < 0$).

From (21) results that the entropy of the treated hamsters is smaller than the one of the control group, the theoretic result being very well correlated with the extension of lesions found in the two cases.

2.3 Results of the theoretical model

a) The solutions of the set of linearly coupled equations (4) (both in general and a semi-empirical version) can explain the variation in time of the experimental serum parameters concentration (the relations (9) and (10)).

b) The quantity of the drug actually used by the animals can be derived from (12) and (13) as a function of time, even if the given dose of drug was constant. The same relations (9) - (13) give the possibility to get the time limit after which the treatment can be stopped or kept constant.

c) The stationary state, obtained from the production of entropy, requires coupling between different serum parameters, a fact which is very well satisfied by our experimental results.

d) For constant production of entropy, both for diet and drug (i. e. considering the same diet and drug throughout the whole experimental period), the entropy of the treated animals is lower than for the non-treated ones, resulting in a degree of order for healthy animals higher than for atherosclerotic ones. This result is very well correlated with the different extension of the lesions for the two groups of animals.

f) the proposed model is not limited to the presently measured parameters and drug used (Simvastatin) but it gives the possibility to include in the set of equations (4) all relevant parameters which may interfere with the atherosclerotic process. If only a few parameters are taken into account, then the meaning of X_M from (12) and (13) is to calculate how much drug is used in the process of interaction with those parameters.

We can conclude that our "bio-chemical picture" and near equilibrium approach are a satisfactory phenomenological description of the atherosclerotic process in hyperlipidemic hamsters treated with statins. However for a more realistic description of physiological processes the administration of diet and treatment with a drug it is necessary to consider far from equilibrium approaches.

3 References

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