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A New Mathematical Model to Investigate Drug Treatment on the Overall Process of Wound Healing

Mohammad H. Pipelzadeh[*1], Abolghasem Jouyban[2], Ali Khodadadi[3] and Ian L. Naylor[2]

[1]Dept. of Pharmacology, Ahwaz University of Medical Sciences, Ahwaz; [2]*School of Pharmacy, University of Bradford, Bradford, W. Yorkshire, U.K. BD7 1DP;* [3]Dept. of Immunology, Ahwaz University of Medical Sciences, Ahwaz, Iran

ABSTRACT

The absence of a universally accepted mathematical model for assessment of the effectiveness of drugs in the process of wound healing is one of the problems the health professionals have been faced. In this study, an "empirical" mathematical model is presented to evaluate its reliability from an experimental viewpoint and from a comparative aspect with previous models used to study wound healing, namely the log and square root models. The excisional wounds inflicted were based on suggestion of Cross *et al.*, 1995, using a variety of drugs. The drugs influence the wound healing process such as indomethacin, mepyramine, dexamethasone, acetylsalicylic acid and prednisolone. The proposed model is based on a quadratic equation having two constants: ~ and . The -~/ ratio and the area-under the curve (AUC) are suggested as being valuable and reliable quantitative values. Also, having more precision in predicting and for expressing the effectiveness of drugs on the wound healing process than the two previous models. *Iran. Biomed. J. 5 (2 & 3): 61-67, 2001*

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INTRODUCTION

As early as 1916, Carrel and Hartmann [1] proposed the change in wound surface area could be used as a parameter for both the assessment and prediction of the process of wound healing. Despite this simple idea of sequentially measuring wound area as a quantitative measurement, in practice it is complex because of the integrated factors involved in the overall biological process. As yet no single, simple mathematical formula has been universally accepted as being representative to fully describe the process of change in the wound surface area and can be used routinely in animal experiments, especially to determine if a drug has an effect on this process.

A diverse range of empirical models has been suggested to represent the process of contraction of the wound surface area. In such studies, the data for wound surface area were transformed into logarithmic values [2, 3], expressed as a first-order differential equation [4], or represented by complicated differential equations with 31 parameters [5]. As an alternative, some researchers have calculated the time for a 25 or 50% decrease in wound area to be measured [6] or as square root of the wound surface area versus time [7]. There is a fundamental problem with such methods, since it can be experimentally determined that wounds contract at different rates during the overall process of wound healing, for example the plateau or lag phase, active or exponential phase and post-exponential or consolidation phase. None of the equations suggested seem to involve all these phases. They merely approximate these three phases to linearity, which may itself alter the assessment of the wound healing process. Consequently, these equations seem to apply only to the processes of the active exponential phase of wound healing. Where, the rate of wound contraction was expressed as a coefficient of wound contraction [8], as a rate of wound contraction [3] or as a decay constant [4] implying that wound contracts at a constant rate. This may be a simplification, which makes the assessment of drug treatment of wounds both difficult and inaccurate.

What relevance has this process to pharmacology? It is currently of great interest in the field of wound healing studied, from a pharmacological viewpoint, to assess what effects drugs or growth factors may have on the overall process of wound healing. Currently, direct comparative studies related to this aspect of wound healing are lacking simply because a simple, reproducible and predictive assessment

*Corresponding Author.

procedure does not exist. This paper suggests a simple mathematical method to enable the effects of drugs to be critically assessed.

In this study, we propose a single and simple model that may be used to express the effect of drug treatment on the overall process of wound healing in the rat. The proposed equation has the advantage of being independent of the changes in the rate of wound healing during the different phases of wound contraction, as it quantifies the differences in the area between the control and drug treatments.

Theoretical Background. In order to standardize the analysis of wound area versus time all the measured wound surface areas were converted to the percentage change of the area (%A) using the simple equation:

$$\%A = \frac{A_t}{A_0} \times 100 \qquad (1)$$

where A_t is the area on day t, A_0 is the initial wound surface area on day 0.

In order to evaluate the effects of a drug on the wound healing process, it is necessary to have a concurrent control group so that the dependent variable (Y) was defined as the difference between the percentage of the surface area of the control and the treated group during the course of the experiment. The following equation was used:

$$Y = \%A_c - \%A_d \qquad (2)$$

where Y is the dependent variable, A_c is the area for the control and A_d is the area of the drug treated group on day t. We assumed a second-degree relationship to be obeyed between Y and t. In addition, because the Y value is zero at time 0 (t = 0), the model must be a no intercept equation. The proposed quadratic equation [9] that is found to be most representatives is:

$$Y = \alpha t + \beta t^2 \qquad (3)$$

where Y is dependent variable and defined by equation 2 above, α and β are the model constants which were calculated by the use of least squares analysis. The positive or negative signs for α and ß are important factors to describe the effect of any drug which is used. Using this equation we may obtain the values for the model constants α and ß when Y = 0, equation 3 is modified to:

$$\alpha t + ßt^2 = 0 \qquad (4)$$

Rearrangement gives:

$$t\,(\alpha + ßt) = 0 \qquad (5)$$

Therefore, equation 5 has two answers; either t = 0, that is in the initial stage of wounding, showing no difference in the relative wound surface area between control and treated groups, or: α + ßt = 0, this may be rearranged as:

$$t = \frac{-\alpha}{\beta} \qquad (6)$$

This ratio gives the duration necessary for the wound to heal to theoretical completion.

In order to compare the accuracy and predictability of the proposed model, the same experimental data were compared using this new proposed method against the log and square root methods which were previously used [2, 7]. From each of these methods the model constants were calculated. Once these methods were known, the next step of the calculations involved back-calculation of the predicted values at time t, and the mean percentage error (MPE) [9] i.e. the capability of prediction, the smaller the MPE the greater the precision, for each model, and treatment was determined and compared using the following equation:

$$MPE = \sum_{i=1}^{i=N} \frac{\%A - \%Acal}{\%A} \frac{1}{N} \qquad (7)$$

where %A is the experimental area, N is the number of experimental data points for each treatment group and $\%A_{cal}$ is the back-calculated %A. The $\%A_{cal}$ had to be derived by using the following relationship:

$$\%A_{cal} = \%A_c - Y_{cal} \qquad (8)$$

where Y_{cal} denotes the back-calculated value of Y based on the calculated values of α and β in equation 3.

MATERIALS AND METHODS

Animals and drugs. All drugs used in this study were purchased from Sigma, (UK.). Male Hooded Lister rats (Bradford strain, 8 animals in each group) weighed between 250 to 300 g were used

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throughout this study. The animals were randomly divided in the following groups:

1- Control 1; excisionally wounded, air-dried and no treatment was given.
2- Dexamethasone-treated; (2 mg/kg p.o.) daily for seven days initiated from the day of excisional wounding.
3- Indomethacin-treated; (2 mg/kg p.o.) daily for seven days initiated from the day of excisional wounding.
4- Mepyramine-treated; (10 mg/kg p.o.) daily for seven days initiated from the day of excisional wounding.

In a separate series of experiments, 18 rats were divided into 3 groups, 6 rats in each group. These experiments were performed at a different time of the year and used the subcutaneous route and hence required their own control values. The first group was therefore used as control 2 (1 ml/kg s.c. of normal saline containing 10% sodium bicarbonate, in the intrascapular region), the other two groups were randomly divided into the following treatment groups:

1- Prednisolone (10 mg/kg s.c.) daily for seven days, administered from the day of wounding.
2- Acetylsalicylic acid (ASA) (200 mg/kg s.c.) daily for seven days, administered from the day of wounding.

Wounding Procedure. One excisional wound of 15 × 15 mm was prepared at the left lower dorsal flank of each animal. Daily tracings of the wounds were made by use of acetate transparencies. Ten daily tracings were recorded for each wound surface area and this was quantified using a computer-linked digitizer (BBC, model Summagraphics, UK, with Digit software). The technique employed was as those reported by Cross *et al.* [8]. Basically, the animals were anaesthetized with a mixture of oxygen and halothane, and with the animal in recumbent position. Then, an excisional wound (15 × 15 mm) was inflicted with size 15 surgical blade. The wounds were not covered and the animals were housed individually throughout the period of the study and had free access to water and food.

The mean of the actual wound surface area is calculated for each animal and then the percentage of wound surface area relative to day 0 were calculated for each animal. The mean percentage for each group was then derived for each group for each time point.

RESULTS

The percentage change in the wound surface area was found to reduce in a time-dependent manner in all groups. However, the extent at which these changes occurred varied with the treatments. Table 1 summarizes these findings. Briefly, the decrease in wound surface area in dexamethasone and prednisolone treated groups were dramatically reduced as compared to the controls; whilst those of indomethacin and ASA treatment were only moderately reduced. In contrast, mepyramine treatment induced an increase in the percentage contraction in the wound surface area showing an enhancement of wound contraction.

Mathematical Analysis of the Data. Using the data in Table 1 and equation 3, the respective α and ß values, together with $-\alpha/\beta$ ratio of the lines were derived by use of the commercially available statistical software SPSS package. Table 2 summarizes these values. As noted in this table, α values for dexamethasone, prednisolone, ASA and indomethacin are all negative, while the value for mepyramine treated group is positive. In contrast, the ß values for the four drugs listed above are positive and for mepyramine is negative. This observation is in agreement with the apparent effects of these agents on the percentage change in the wound surface area measured experimentally.

In order to correlate the experimental results with those derived from the proposed equation the MPE values were calculated using equation 7. Similarly, MPE for logarithmic and square root models were computed and compared with those obtained for the proposed models. Table 3 summarizes the results obtained.

From equation 3, Y values can be back calculated as α and β were previously computed using the same equation. Figure 1 is a graphic representation of the data analyzed showing the "quantitative" influence of each drug treatment on the overall process of wound healing in the rat model used in this study.

For example, the computed mean values for α and β for the dexamethasone-treated group were –18.38 and 1.82 respectively. These values can also be calculated using commercially available calculators. Squaring the equation 3 [$(Y^2 = \alpha t + \beta t^2)^2$], and substituting the experimental Y together with their

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Table 1. Summary of the experimentally measured percentage change in wound surface area in control and treatment groups (mean wound surface area ± S.E.M.)

Treatment	*% of original wound area on day*							
	0	1	2	3	4	5	6	7
Control 1	100	81 ± 2	69 ± 2	46 ± 1	38 ± 1	34 ± 1	32 ± 2	31± 1
Dexamethasone	100	101 ± 1[c]	105 ± 3[c]	85 ± 1[c]	80 ± 1[c]	76 ± 1[c]	73 ± 1[c]	75 ± 1[c]
Indomethacin	100	81 ± 2	70 ± 2	56 ± 2[a]	50 ± 3[c]	39 ± 2[a]	36 ± 1	34 ± 1
Mepyramine	100	64 ± 2[c]	55 ± 1[a]	42 ± 1	25 ± 1[c]	24 ± 1[c]	26 ± 1[b]	28 ± 1
Control 2	100	90 ± 3	83±2	63 ± 2	58 ± 1	50 ± 1	44 ± 1	39 ± 1
ASA	100	100 ± 1[c]	94±1[c]	73 ± 1[c]	66 ± 1[b]	55 ± 1[a]	43 ± 1	25 ± 1[a]
Prednisolone	100	97 ± 1[c]	88 ± 1[b]	72 ± 1[b]	64 ± 1[b]	59 ± 1[c]	55 ± 2[c]	49 ± 1[b]

Control 1 (air dried, no treatment) relates to dexamethasone (2 mg/kg p.o.), indomethacin (2 mg/kg p.o.) and mepyramine (10 mg/kg p.o.); Control 2 (1 ml/kg s.c. of normal saline containing $NaHCO_3$ in the intrascapular region) relates to ASA (200 mg/kg s.c.) and prednisolone (10 mg/kg s.c.). Experiments were conducted at different times of the year. [a]*P*<0.05, [b]*P*<0.01 and [c]*P*<0.001 from their relative control. Unpaired Student's *t*-test, n = 8 for the first and n = 6 for the second control.

Table 2. The derived values for both α and ß following treatment with dexamethasone, indomethacin, mepyramine, ASA and prednisolone

Drug	α(S.E.M.)	β (S.E.M.)	$^{-\alpha}/_{\beta}$	*AUC*
Indomethacin (2 mg/kg)	-3.78 (1.15)	0.48 (0.20)	7.82	39.14
Mepyramine (10 mg/kg)	7.03 (2.01)	-0.98 (0.35)	7.17	+ 60.41
Dexamethasone(2mg/kg)	-18.36 (1.5)	1.82 (0.26)	10.09	-311.39
ASA (200 mg/kg)	-8.13 (0.62)	1.43 (0.11)	5.68	-43.88
Prednisolone (10 mg/kg)	-3.34 (0.74)	0.28 (0.13)	11.92	-79.36

Note negative β value indicates an increase in the percentage change while positive values indicate a decrease relative to control. AUC= area under the curve, and calculated using the integration equation: $\int_0^{\alpha/\beta} \alpha t + \beta t^2$, the negative sign for AUC indicates reduction in wound surface area relative to control. $\frac{-\alpha}{\beta}$ is the theoretical period for completion of closure of the wound.

Table 3. The mean percentage errors (MPE) ± S.E.M. between the proposed, the log and square root models

Drug	proposed	log model	square root
Indomethacin (2 mg/kg p.o.)	4.27 (1.06)	3.87 (1.34)	5.99 (1.94)
Mepyramine (10 mg/kg p.o.)	7.34 (2.55)	16.60 (5.47)	21.14 (6.16)
Dexamethasone (2 mg/kg p.o.)	3.71 (0.89)	4.81 (0.96)	4.94 (0.94)
ASA (200 mg/kg s.c.)	1.95 (0.54)	12.84 (3.58)	9.31 (3.24)
Prednisolone (10 mg/kg s.c)	1.86 (0.58)	3.35 (0.51)	3.92 (0.53)
Average MPE	3.82	8.29	9.06

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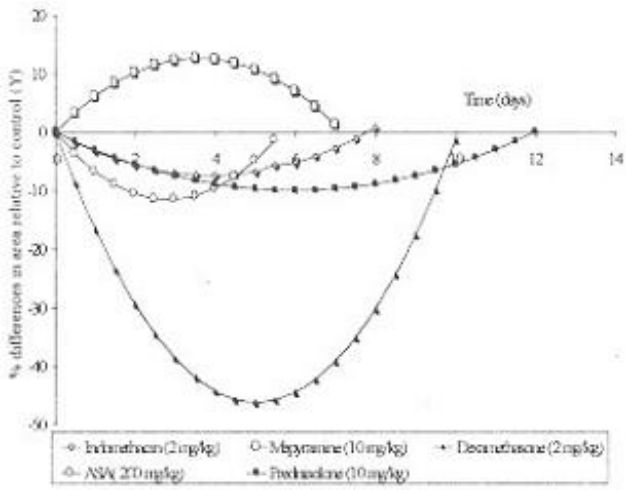

Fig. 1. A graphical representation of the calculated differences in the rate of wound healing using the proposed mathematical model following various drug treatments.

corresponding t values in Table 1, we now have seven equations which are numerically summed. Now we have an equation with two unknowns (α and β), which can be found by use of least square analysis [9]. The negative α value indicates a decrease in the wound healing relative to control. Substitution of these values in equation 3, using Excel software, the daily back-calculated Y values for this group within and including the two time limits where Y was zero (t = 0 and t = $\frac{-\alpha}{\beta}$) were found to be: 0, -16.53, -29.42, -38.66, -44.26, -46.21, -44.52, -39.18, -30.19, and –1.28. The theoretical completion of wound closure period, calculated from the $\frac{-\alpha}{\beta}$ ratio, was 10.09 days. These data were used to construct the graphic representation of the effect of dexamethasone on the overall process of wound healing as shown in Figure 1. Integration of these data gives the area-under the curve (AUC):
$(-18.36/2)\ (10.09)^2 + (1.82/3)\ (10.09)^3 = 311.39$ (Table 2). Similar calculations were made for other treatment groups.

DISCUSSION

Mathematical modeling is a useful tool by which a phenomenon may be described or predicated. In situations where the phenomenon is a simple physical one such as solubility of solutes, the theoretical modeling has been very simplified to describe the process [10, 11]. However, for a complicated biological process this mathematical modeling may not be simple. The researcher needs to use a form of an empirical equation in order to mathematically represent the experimentally obtained data.

Considering the fact that the process of wound healing is a complicated phenomenon of interactions of many factors, the role of each is still awaiting to be fully assessed. On the other hand, in this study, only a limited number of drugs, one animal model (rat) and a square-shaped (15 × 15 mm) wound have been used to test this mathematical model, perhaps more data need to be gathered in order to test and generalize on the suggestions presented in this model. However, this is a merely an inroad towards solving one of the mysteries of this phenomenon.

Two previously published empirical equations [2, 7] to describe the wound healing process, namely the logarithmic and square root models, suggested to be representative of this biological phenomenon. The logarithmic model is based on the following relationship:

$$\text{Log\%A} = \log\%\text{A0} + K.t \quad \textbf{(9)}$$

or

$$\text{Log\%A} = \text{intercept} + K.t \quad \textbf{(10)}$$

where K represents the wound healing rate constant. As seen, this equation assumes a log-linear relationship between A and t.

Similarly, in the square root model, the assumption is based on that the square root of wound surface area (length of wound) is linearly related to time and the following expression was provided:

$$\sqrt{\%A} = \sqrt{\%A0} + R.t \quad \textbf{(11)}$$

or

$$\sqrt{\%A} = \text{intercept} + R.t \quad \textbf{(12)}$$

where R represents the rate of wound contraction.

In the proposed model in this study, an $-\alpha/\beta$ ratio is defined as the time period during which acceleration or delay in the wound surface area may be modified by a drug treatment. The positive sign for β is an indication for a drug delaying wound healing, while a negative sign indicates an enhancement of the process.

The equation proposed for the evaluation of the effect of a drug on the healing process has been

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tested both from experimental viewpoint using real rather than hypothetical data, and from comparative representation with other previously suggested models (log and square root models). In both cases the proposed quadratic model has shown to be capable of representing the experimental data more closely than when compared to the previously suggested models. The results shown in Table 3 clearly show this model, where the new proposed model is capable of a higher degree of accuracy**,** than is shown by the other two models. The proposed -α/β ratio is a useful and easily quantifiable indicator for the comparison between treatments and assessment of the effectiveness of a drug on the course of the wound healing process. The equation also has the advantage of being applicable when longer periods are employed, as it is not influenced by the change in the rate of the healing process, as can be found in the different phases of wound healing.

The proposed equation model has been shown to be more "suitable" in identifying the effectiveness of a drug treatment when using the change in the wound surface area as a parameter of evaluation. The average MPE for the proposed model is shown to be 3.82, as compared to 8.29 and 9.06 for log and square root models, respectively (Table 3). In addition, it has the advantage of providing a numerical value (-α/β ratio) as an index for the effectiveness of a drug relative to other treatment of similar or of different mechanism of action. Therefore, a more constructive conclusion as to the significant role of a given factor in the process of wound healing can be suggested. The α and β values can vary and this depends on two main factors: Firstly, the nature of the control wound for example the wound size/shape or even the animal model. Secondly, the treatment protocol was used in the study. Furthermore, the ease of representing graphically the effectiveness of a drug treatment is another advantage which can clearly illustrate the influence of a pharmacological agent on the overall process of wound healing as compared with other treatments (Fig. 1). This model enables for the first time a comparative assessment to be made. In addition, this equation can also provide quantitative data as found by the AUC. This value when taken in conjunction with α and β can be a very useful indictor as to the overall pharmacological effect and may in fact give an indication of the degree of importance of "factor(s)" or "mediator(s)" involved in wound repair.

The data presented showed that if β had a positive sign this would indicate an inhibitory effect of the drug on the normal course of healing process. This effect was observed clearly with dexamethasone and prednisolone, followed by indomethacin and least with ASA. The negative β was observed with mepyramine, which reflects an increase in the overall process of wound healing. The mechanisms by which these drugs produce these effects are under investigation.

For practical purposes, once we find these parameters for each drug tested, we may be able to quantify and compare its effectiveness on the overall process of wound healing. Finally, the ease of representing graphically the effectiveness of a drug treatment is another advantage which can clearly illustrate the influence of a pharmacological agent on the overall process of wound healing as compared to other treatments (Fig. 1). On the other hand, the values of -α/β ratio and AUC have been shown to represent the extent of these effects. The greater these values, the more effective are the agent to either delay or accelerate the healing process. We propose that this simple mathematical formula may be a very useful guide to the evaluation of drugs on the process of wound healing.

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