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Probabilistic Analysis of Data Dealing With Modeling of Analgesic Effect Of Naproxen Loaded Nanoparticles

VENKATA S. KUCHUBHOTLA
Principal
Sarojini Naidu Vanita Pharmacy Maha Vidyalaya (Pharmacy College),
Hyderabad, A.P.,
India

CHANDRA S. PUTCHA
Professor
Department of Civil & Environmental Engineering
California State University, Fullerton
Fullerton, CA 92834
U.S.A.

JESA H KREINER
Professor
Department of Mechanical Engineering
California State University, Fullerton
Fullerton, CA 92802
U.S.A.

Web Site: http://www.fullerton.edu/ecs/cee/index.htm

Abstract

This paper is based on interactive work between disciplines of engineering and pharmacy. Authors used well known reliability techniques [1] to study the probabilistic effect of pain relieving medicines such as naproxen on animals subjected to pain causing chemical such as acetic acid.

Nanoparticles are the first and foremost representative frontiers of colloidal drug delivery systems and have a solid polymer matrix. Nanoparticles are often defined as particulate dispersions having a size range between 30nm and 500 nm. Due to their extreme small size, nanoparticles may be administered through the normal routes of administration and can be used to target a particular organ. This is contrary to regular drugs administered orally which go through gastro-intestinal tract, liver and finally to the heart. From the heart the drug is distributed to all the organs of the body including the particular organ that is being targeted.

This approach was used to study the analgesic effect of naproxen loaded nanoparticles. Three types of such nanoparticles containing different matrix materials (1) ethyl cellulose (2) cholesterol and (3) a combination of ethylcellulose and cholesterol are prepared. Naproxen, one of the most commonly used analgesic drugs, is loaded into the above mentioned formulations. The analgesic effect of naproxen was tested by comparing the activity of suspension of a commercial product of naproxen with that of naproxen loaded nanoparticles.

The analgesic activity of naproxen-loaded nanoparticles (Dose-2.5mg/kg-bodyweight) following intra-peritoneal administration was assessed by measuring the reduction in number of writhings produced in the albino mice in 15-min. upon injecting 0.3ml of 1%-acetic acid intra-peritoneally. To assess the rate and extent of analgesia, the writhings were measured at 1, 12 and 24-hours after the treatment with the control and test preparations. For each of the group, the statistical average of number of writhings is calculated along with standard deviation. The idea is to study the effect of pain relieving effect of naproxen dose (commercial and non commercial) on the animal induced with pain- causing acetic acid. In the end, probability of the animal having normal writhings is calculated. This work is based on the Ph.D. thesis of the first author, Kuchibhotla [2].

Key Words: Naproxen, Drug, Writhings, Statistical, Mean Value, Standard Deviation

1. Introduction:

In recent years it has become that more more and more evident that the development of new drug alone is not sufficient to ensure progress in drug therapy. Disappointing results in vivo very often follow excellent experimental data obtained in vitro. The main reasons for therapy failure are:

1. Insufficient drug concentration due to poor absorption, rapid metabolism, and elimination

2. Drug distribution to undesired tissues combined with high drug toxicity

3. Poor drug solubility

4. High fluctuations of plasma levels due to unpredictable bioavailability after peroral administration

The promising strategy to overcome these problems involves the development of suitable drug carrier systems. It is not necessary that the properties of the drug should dictate its *in vivo* fate. The carrier systems can modify and control the release rate of the drug and also the localized release of the drug as per needs of therapy. This concept has been realized to a certain extent in some of the commercial products like implants. Already commercially available systems have been developed for the treatment of prostrate cancer and other related diseases [3].

The literature on controlled -release medications has recently been extended to new drug-delivery systems employing polymeric matrix materials and is a class of colloidal aqueous polymer dispersions. These therapeutic systems in drug-delivery combine the physical and chemical properties of the interactive systems (properties of the exciepients and the drug) to design an dispensing device that meets the required release rate for drug delivery in order to maintain the precise serum blood level.

The investigated systems include nanoparticles nanosuspensions, nanoemulsions, liposomes, niosomes micelles, soluble polymer conjugates.

2. Problem Formulation

2.1 Experimental Part

2.1.1 Materials

Naproxen was gifted by M/s Divi's Laboratories, Hyderabad. . Hydroxypropyl methylcellulose phthalate (HPMCP), ethylcellulose (EC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), Polyvinyl pyrrolidine (PVP) were gifted by M/s Biological E.Ltd. Hyderabad. Toluene, ether, isoproponol, lauryl alcohol, cetyl alcohol, stearic acid, lauric acid, glyceryl monostearate, cholesterol (Extra pure), polysorbate 20, polysorbate 40, polysorbate 80, sodium lauryl sulphate, polysorbitan 20, polysorbitan 40, polysorbate 60, methanol and ethanol, were purchased from M/s Loba Cheme, Mumbai, India. The dialysis tubing and the clips were purchased from M/s Himedia, Mumbai, India.

2.1.2 Preparation of nanoparticles of naproxen

Three types of formulations of nanoparticles were prepared with naproxen as drug in each case with the following matrix materials.

Ethylcellulose (Ec -(N) 250)
Cholesterol (Chol- (N) 250)
Ethylcellulose with Cholesterol (Ec-Chol-(N) 250)

The naproxen-loaded nanoparticles of ethylcellulose and / or cholesterol were prepared by the precipitation of nanoparticles from organic solvent- in – water by removal of solvent using vacuum evaporation. The nanoparticle production involves three stages viz. emulsification, homogenization, and flash evaporation. Ethylcellulose or cholesterol or ethylcellulose and cholesterol as matrix materials, naproxen as drug and polysorbate 80 as o/w emulsion stabilizers were dissolved in specified quantity of toluene (40 ml.). The hot (80 ° C) organic phase was dispersed in 70 ml of demineralized water (maintained at 80°) with the help of emulsification equipment (Remi make, India). Emulsification was carried out for 10 minutes. The emulsion thus obtained was homogenized for 5 minutes using homogenizer.

The organic solvent was removed from the emulsion under reduced pressure by rota evaporation equipment (Super-fit flash evaporator, Mumbai, India). The final nanoparticle dispersion volume was made up to 100 ml with demineralized water. Appropriate amounts of

parabens were added to final preparation to prevent fungal contamination. The composition of various nanoparticle systems was shown in Table 1 (see Appendix).

All the ingredients, except Naproxen, were used as received.

The following characterizations were studied:

1. Scanning Electron Microscopy
2. Particle size
3. Zeta Potential
4. Differential Scanning Calorimetry (DSC)
5. Naproxen content in the nanoparticles
6. Release Profile of naproxen from the nanoparticles

3. Problem Solution

3.1 Comparision of dose and pharmacodynamic effect

The analgesic effect of naproxen suspension, EC-N-250, E-CHOL-N 250, CHOL-N 250 was compared by writhing test in albino mice. This test was previously used by Koster et al [4]. All the three test preparations were injected intraperitonially to mice and (after 1hour, 12 hours, and 24 hours) 0.3 ml of acetic acid was injected intraperitonially.

The average number of writhings produced and percentage of writhings when compared with control in various gropes of animals is tabulated and shown in Table 2 (see Appendix).

3.2 Analgesic effect on the reduction of dose

The second part of the test pertains to the study of analgesic effect on reduction of dose. An effort was made to evaluate the analgesic effect of naproxen in the nanoparticulate due to the reduction of dose. For this purpose similar number of albino mice were prepared as described under part-I.

The test was carried out by comparing the analgesic activity of naproxen present in different systems. However the dose of nanoparticulate form of naproxen was reduced to half (1.25 mg/Kg body weight),while maintaining the same dose of naproxen in suspension form (2.5 mg/Kg body weight).

Different forms of naproxen was injected at a specific times and acetic acid (1%) was injected intra-peritonially in three time intervals, i.e., 1 hour, 12 hours, 24 hours. The writhings produced in the first 15 minutes after injecting acetic acid (1%)were noted. The results were represented in Table 3 (see Appendix).

3.3 Discussions

3.3.1 Case study (Part I)

(Assumption: same amount dose of naproxen in conventional suspension and nanoparticulate forms)

3.3.1.1 Comparision of dose and pharmacodynamic effect

One-hour Study: 70% of writhings (pain) was felt in the group of mice, which were administered, with a suspension of naproxen. However the extent of pain (writhings) experienced by the groups of mice into which different forms nanoparticles of naproxen were administered, was appreciably. The nanoparticles of ethylcellulose brought down the pain to 15%, while in the case of the nanoparticles of ethylcellulose with cholesterol the writhings were only 18% of the control group. The nanoparticles of cholesterol brought down the pain to 29%.

Twelve-hour Study: There was no difference in the pain of the pure naproxen group of mice and was 70%, i.e., the same extent felt after one hour. Similarly there was no change of analgesic activity in the case of nanoparticles of ethylcellulose. The writhings (pain feeling) in the other two groups have slightly increased (to 23% in the case of the system with ethylcellulose and cholesterol and to 37% in the case of cholesterol system).

Twenty-four-hour Study:. In this case, each animal in the respective groups was administered with the drug from the relevant system and the study was carried out after 24 hours. The object was to assess the duration of analgesic effect of naproxen in each group. The animals in which naproxen suspension was administered experienced pain 84% writhings i.e. nearly equal to those of control group (100%). It indicates that the analgesic effect of naproxen was not available in this group. after 24 hours after its administration.
However the nanoparticles of the polymer with/ or lipid produced appreciable analgesic activity after 24 hours also. In fact the pain has come down further in the case

of ethylcellulose nanoparticles to a minimum 9 % (from 15% experienced after 1 hour and 12 hour studies) and in the nanoparticles of ethylcellulose with cholesterol to13% (from 18% and 23% in 1 hour and 12 hours), after 24 hors of administration. Even in the case of cholesterol nanoparticles also the writhings have come down from 37% in 12- hour test to 29% in the 24– hour study.

3.3.1.2 Statistical analysis

The concept of safety index (β) has been used to compare the safety margin of the results of Analesic effect of Naproxen loaded Nanoparticles of choloestrol in acetic acid included writhings in Albino Mice after i.p. administration. Is defined as:

$$\beta = \bar{g} / \sigma_g \qquad (1)$$

where,

g represents the failure surface. $\bar{g}$ represents the mean value and σ represents the standard deviation. The values of β for all the groups studies are well above the accepted normal value of 3.0 [5].

3.3.1.3 Conclusion

The analgesic activity of naproxen varied with the type of system. The analgesic activity of naproxen in the suspension form was minimal (less than 70 %) even after one after its administration. But in the case of nanoparticles, the analgesic activity has come down appreciably. In fact the best analgesic activity was noted after 24 hours only in every system. Among the systems, the nanoparticles of ethylcellulose showed the best analgesic activity after 24 hours reducing the pain to mere 9 % followed by nanoparticles of ethylcellulose with cholesterol 13 % and nanoparticles of cholesterol 29%. Thus nanoparticles of ethylcellulose showed most effective analgesic activity on the albino mice even after 24 hours after its administration.

3.3.2 Case study (Part II)

(Dose of naproxen in nanoparticulate forms(1.25mg/kg body weight) is reduced to half of naproxen in the conventional suspension (2.5/kg body weight))

One-hour Study: The effect of naproxen (1.25mg/kg body weight) after one hour after its administration in different forms indicated that

i. Almost no effect for suspension form of naproxen (94.8%)
ii. Maximum analgesic effect in the case of nanoparticles of ethylcellulose with cholesterol (41.6%) followed by those of cholesterol (56%) and ethylcellulose (65.9%). It indicatesd that release of naproxen from ethylcellulose complex is slowest.

Twelve-hour Study:

i. Very little analgesic activity as noted by the reduction in the number of writhings in the case of naproxen suspension .
ii. Increased effect after 12 hours of administration of nanoparticles has been observed. The order of reduction in the writhings is ethylcellulose nanoparticles (45.5%),cholesterol (44.5%), and nanoparticles of ethylcellulose with cholesterol (37.5%).

Twenty-four hour Study:

i. An increase in the percentage of writhings in the case of suspension of naproxen (87.5%)
ii. Further reduction in the writhings and increased analgesic activity og naproxen of nanoparticlate systems after 24 hours of administration. The order of analgesic activity – nanoparticles of ethylcellulose with cholesterol (25%)>cholesterol (29.2%)> ethylcellulose (33.3%).

4 CONCLUSION

The analgesic affect of naproxen in the nanoparticulate systems is much superior to that of naproxen in conventional suspension form. There is an appreciable increase in the analgesic activity in the nanoparticles forms when compared with suspension form in all the three time periods, i.e., one hour, twelve hours and twenty-four hours. There was further increase in analgesic activity after 24 hours in all the cases.

An interesting observation was that the analgesic activity of nanoparticulate form naproxen was better even when its dose was reduced to half (1.25 mg/Kg body wt.) when compared with suspension form of naproxen (2.5 mg/kg body wt.).

Thus the naproxen in nanoparticulate form is far more effective even in half of the concentration of the naproxen in suspension form and that too for a longer period. The analgesic activity has improved with time period from 1 hour up to 24 hours.

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APPENDIX (Tables)

TABLE 1. Composition of Various Nanoparticle Systems of Ethylcellulose and or Cholesterol Containing Naproxen.

System code	Ethylcellulose	Cholesterol	Polysorbate 80	Naproxen	Organic solvent	Aqueous phase
Ec -(N) 250	1.00 GM	--	9.00 GMS	0.250 mg	40 ml	70 ml
Ec-Chol-(N) 250	1.00 GM	0.500 MG	9.00 GMS	0.250 gms	40 ml	70 ml
Chol- (N) 250	1.00 GM	1.00 GM	9.00 GMS	0.250 gms	40 ml	70 ml

TABLE 2. Average Number of Writhings Produced And Percentage Of Writhings.

the S.No	Time in hours	Dose in mg/kg body wt.	Control (no naproxen)	Naproxen Suspension	Naproxen Nanoparticles of ECNAP	Naproxen nanoparticles of ECHOLNAP	Naproxen nanoparticles of CHOLNAP
1	1	2.5 mg	46.20 (100%)	32.6 (70%)	7.00 (15%)	8.4 (18%)	13.5 (29%)
2	12	2.5	44.00 (100%)	31.00 (70%)	6.5 (15%)	10.00 (23%)	16.2 (37%)
3	24	2.5	48.6 (100%)	40.8 (84%)	4.4 (9%)	6.2 (13%)	14.00 (29%)

TABLE 3. Writhings Produced in First 15 Minutes after Injecting Acetic Acid.

S. No 3	Time in hours	Dose in mg/kg body wt.	Control (with out naproxen)	Naproxen Suspension	Naproxen Nanoparticles of ECNAP	Naproxen nanoparticles of ECHOLNAP	Naproxen nanoparticles of CHOLNAP
1	1	1.25 mg	42.2 (100%)	40.4 (95.7%)	27.8 (65.9%)	17.6 (41.9%)	23.6 (56%)
2	12	1.25	44.2 (100%)	37.4 (84.6%)	20.00 (45.2%)	16.6 (37.6%)	19.6 (44.3%)
3	24	1.25	40.8 (100%)	36.8 (90.2%)	13.6 (33.3%)	10.2 (25%)	12.2 (29.9%)

Table 4. Comparison of Analgesic Activity of Naproxen Suspension (2.5 Mg/Kg Body Wt.) with Half the Dose (1.25%) of Naproxen in Nanoparticulate Form.

S.No	Time lapsed after administering naproxen	Naproxen in suspension 2.5mg/Kg body wt.	Naproxen in ECNAP 1.25 mg/Kg body wt.	Naproxen in ECHOLNAP 1.25 mg/kg body wt.	Naproxen In CHOLNAP 1.25 mg/Kg body wt.
1	1 hour	70%	65.9%	41.9%	56%
2	12 hours	70%	45.2%	37.6%	44.3%
3	24 hours	84%	33.3%	25%	29.9%