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ENHANCED RELEASE OF DRUGS FROM SILICONE ELASTOMERS: (IV) SUBCUTANEOUS CONTROLLED RELEASE OF INDOMETHACIN AND IN VIVO/IN VITRO CORRELATIONS

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ABSTRACT

The subcutaneous controlled release of indomethacin from implants was studied. The subdermal implants were prepared from silicone elastomers containing various levels of glycerol. The *in vitro* and *in vivo* releases of indomethacin were observed to follow a matrix diffusion-control mechanism. The release flux of indomethacin was enhanced when glycerol was incorporated into the silicone elastomers. An *in vivo/in vitro* correlation coefficient of 0.85 ($\pm$ 0.05) was obtained for implants containing up to 20% (w/w) of glycerol. The survival rates on the ninth day post-implantation were determined to be 20, 65, and 100%, respectively, for the mice receiving implants containing 20, 10, and 0% glycerol. An LD_{50} dose of 34 mg/kg was assessed for the subcutaneous controlled administration of indomethacin in CD-1

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mice, which is not significantly different from the intraperitoneal LD_{50} of 28 mg/kg and the intravenous LD_{50} of 40 mg/kg.

INTRODUCTION

Indomethacin, a potent nonsteroidal, anti-inflammatory, antipyretic and analgesic drug, has been widely used for the treatment of inflammatory rheumatic disorders, such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, pseudogout, gouty arthritis, and dysmenorrhea (1). Although its anti-inflammatory effect was being studied as early as the 1940's, it was not realized until the 1960's that it is a strong inhibitor of prostaglandin biosynthesis (2). The blood level of indomethacin required to inhibit prostaglandin synthetase in animals is relatively low compared to the blood level achieved after a therapeutic dose. In man, for example, indomethacin reaches a blood level of 2mcg/ml during a steady dosing with therapeutic quantities. However, because the drug is 90% bound to plasma proteins, the actual free drug concentration in the plasma is only 0.2mcg/ml. This is still higher than the 0.05 mcg/ml concentration found to be inhibitory to prostaglandin synthetase in dog spleen (1). In addition, indomethacin and other non-steroidal anti-inflammatory agents are known to be involved in a number of different body functions, including smooth muscle tone maintenance, control of uterine activity, normal ovary function, fat cell metabolism of lipids, sympathetic nerve activity (in vitro), and renal blood flow regulation (1).

Due to the severe side effects associated with their oral systemic therapy, most nonsteroidal anti-inflammatory drugs (NSAID's) are recommended for only short-term therapy when used

in the treatment of arthritis or related pain. Ulceration of the gastric mucosa, gastric upset, peripheral edema, and severe hematological disturbances, such as leukopenia and agranulocytopenia, are frequently reported as toxic side effects of systemic therapy. Central nervous system effects, such as dizziness, headache, nervousness, anorexia and depression, are also common. Occasional abnormalities in liver function tests have been reported. Also, the NSAID's effect on platelet aggregation may interact with a systemic anti-coagulant taken by the patient (3). Considering the broad spectrum of biological activity caused by indomethacin, its relatively high potency, and the undesirable side effects associated with conventional systemic therapy, development of a controlled-release formulation for the administration of indomethacin would be desirable.

There have been several attempts to overcome the toxic effects of indomethacin. The manufacturer of indomethacin, Merck Sharp & Dome, has developed Indocin® SR capsules (4) based on microencapsulation technology. Seventy-five mg of indomethacin are released in a sustained manner over a period of 12 hours to produce the bioavailability equivalence of a t.i.d. conventional regimen at the same total dose (75 mg). The Indocin® SR capsule formulation is designed to release one third of the dose immediately, while the remaining two thirds is gradually released over a period of 12 hours.

A second attempt to overcome the toxic effects of the drug is the application of the osmotic pump mechanism to develop a controlled release tablet formulation for oral administration (5). This device provides a total daily dose of 85 mg. When marketed in England under the tradename of Indosmos® , an unexpectedly high incidence and degree of severity of toxic effects was reported (6), resulting in the withdrawal of the product from the market in 1983. A consensus has not yet been

reached concerning the cause of the toxicity, whether it was drug-related, potassium bicarbonate (osmotic agent)-induced, or simply due to the high local osmotic pressure created in the gastrointestinal tract. It thus becomes important to investigate the possible minimization of the toxic effects of indomethacin via controlled administration through routes other than oral.

In previous investigations, it was reported that the release of both hydrophilic and hydrophobic drugs from silicone implants can be substantially increased by incorporating glycerol or other co-solvents into the polymer matrix (7-9). This system was successfully applied to control the subcutaneous administration of melatonin for early onset of estrus cycles in ewes (10) and of insulin for the treatment of diabetes in rats (11). The objective of this study is to extend this technology for the subcutaneous controlled administration of indomethacin. The *in vitro* and *in vivo* release rates of indomethacin from silicone elastomers having various glycerol contents will be determined, and the survival rates of mice receiving indomethacin-releasing subdermal implants will also be reported.

EXPERIMENTAL

A. Preparation of Subdermal Implants Containing Indomethacin

Indomethacin (*1) was incorporated as a powder into medical grade silicone elastomer (*2) containing various concentrations of glycerol (*3). After thorough mixing using a laboratory mixer (*4), the mixture was de-aerated *in vacuo* (28 in. Hg) for ten minutes. A catalyst was added, followed by mixing for another 30 seconds. The mixture was then extruded into sections of Tygon tubing (*5) as the mold, and cured at 25°C for 16-24 hours. After complete curing, the implants formed were removed from the mold and stored until testing.

B. In Vitro Release Study

1. Solubility of indomethacin - An excess amount of indomethacin powder was added to aqueous solutions containing up to 50% (v/v) of PEG 400 (*6). The suspensions were shaken for 48 hours in a shaking waterbath (*7) at 37 C. After filtration through a membrane filter (*8), an aliquot of the filtrate was diluted with methanol (*9) and measured with a spectrophotometer (*10) at 230 nm (7). A standard solution of known indomethacin concentration was also incubated under the same conditions for 72 hours and measured daily for assurance of stability.

2. In vitro release of indomethacin - The drug-containing implants were cut into 1.5 cm lengths and incubated in 10 ml of aqueous solution containing 20% (v/v) of PEG 400 in a 37° C waterbath shaking at 50 cycles per minute. Periodically, the samples were transferred to a new set of culture tubes containing the same eluting medium. The amount of indomethacin released was determined using a spectrophotometer.

C. In Vivo Release Study

1. Subcutaneous release of indomethacin - Sixty CD-1 mice (*11) were divided into three groups with twenty mice in each group. Each of the mice received an indomethacin-containing (1% w/w) implant having up to 20% (w/w) of glycerol. In a separate study, another 15 CD-1 mice received indomethacin-releasing subdermal implants containing 30% (w/w) of glycerol. before implantation, the mice were anesthetized with a dose of 40 mg/kg of pentabarbital sodium solution (*12). The hair on the dorsal surface of each mouse was shaved with an electric clipper (*13) and the shaved area was then sterilized with a 70% isopropanol solution. A pocket was created subcutaneously, using a pair of surgical scissors. The implant was inserted into the pocket and the wound was closed with a Michel wound clip (*14). The

implants were removed from the mice at death or after a predetermined time interval.

2. Preparation of samples - Each implant removed was first cut into at least 25 pieces, then extracted with 10 ml of methanol in a culture tube for 24 hours using a Wrist-action shaker (*15). Extractions were performed three times, rinsing with 5 ml of methanol after each extraction. Both extracts and rinses were collected and diluted to 50 ml volumetrically.

3. Assay Method - The High Performance Liquid Chromatography (HPLC) method used by Skellern and Salole (12) to determine the plasma levels of indomethacin was adapted in this investigation. The HPLC system used consisted of a high pressure pump (*16), a high pressure injector (*17), a U.V. detector (*18) at a fixed wavelength of 254 nm and a recorder (*19). Chromatography was performed using a reverse-phase column (*20) with a reverse-phase precolumn (*21). The mobile phase, consisting of 40 parts of 0.1 M acetic acid (*22) and 60 parts of acetonitrile (*23), was delivered at a flow rate of 1.5 ml/min. Prior to use, the mobile phase was deaerated by sonication for 5 minutes.

A stock solution was prepared in the mobile phase at a concentration of 1 mg/ml. Appropriate dilutions were made with the mobile phase to prepare standard solutions at concentrations of 2-10 mcg/ml. Fifty (50) mcl. aliquots of the standard solution or samples were injected into the HPLC column. The concentration of indomethacin in the samples was determined by comparing their resultant peak height with the peak heights of standard solutions.

D. Survival Rates of Treated Mice

The death of mice resulting from the toxic effects of indomethacin was monitored during the course of the study. Each

morning the dead animals were recorded and their implants were retrieved. The survival rate was expressed as a percentage of the total number of mice in the original population.

RESULTS

A. Equilibrium Solubility of Indomethacin in Aqueous PEG 400 Solutions

The apparent solubility of indomethacin in aqueous PEG 400 solution was observed to increase exponentially as a function of the volume fraction of PEG 400 (Figure 1). Linear regression analysis of the data yields a slope of 0.044 and an intercept of 8.93 mcg/ml, which is reasonably close to the values reported in the literature (13). This linear log solubility vs. PEG concentration has a correlation coefficient of 0.995.

B. In Vitro Release of Indomethacin From Silicone Implants

Figure 2 shows the release profiles of indomethacin from the silicone implants containing up to 30% (w/w) of glycerol. At the same drug loading (1% w/w), it was observed that the higher the glycerol content in the silicone implant, the greater the release rate. Similar to the observations made earlier (7, 8, 10), a linear Q vs. $t^{1/2}$ relationship was established for all the glycerol levels studied (Figure 3). It is interesting to note that, for implants containing 10, 20, or 30% (w/w) of glycerol, the curves started to deviate from the linearity when the cumulative percent release reached approximately 70% (Figure 3). The steady-state release fluxes ($Q/t^{1/2}$), calculated from the steady-state slope of Q vs. $t^{1/2}$ plots, are 97.2, 175.3, 214.1, and 266.4 mcg/cm^2 /day$^{1/2}$ for indomethacin implants containing 0, 10, 20, and 30% (w/w) of glycerol, respectively. When the release fluxes obtained from the implants containing glycerol are

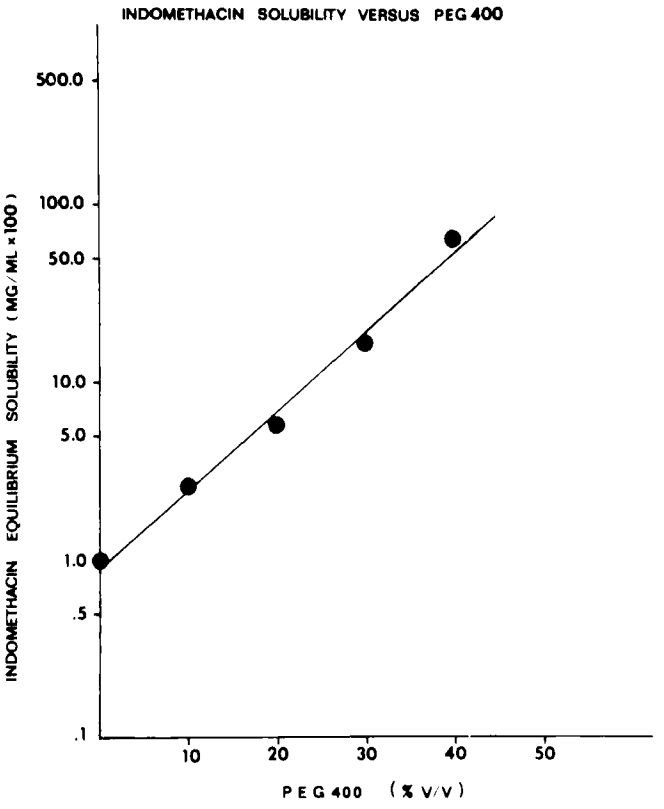

Figure 1. Semilogarithmic relationship between the equilibrium solubility of indomethacin and the volume fraction of PEG 400 in aqueous solutions. Quadruplicate experiments were run for each solution. The standard deviation for all data points was within 5%.

compared with those of the implants containing no glycerol, the release flux was enhanced by 1.8 to 2.74-fold, as 10 to 30% of glycerol was incorporated into the silicone elastomer.

The semilogarithmic relationship between the release flux and the glycerol content reported earlier for other drugs (7, 10) was also observed in this investigation for indomethacin (Figure 4). Linear regression analysis of the data yields a slope of 0.0091 and an intercept of 2.152, which is equivalent to 141.8

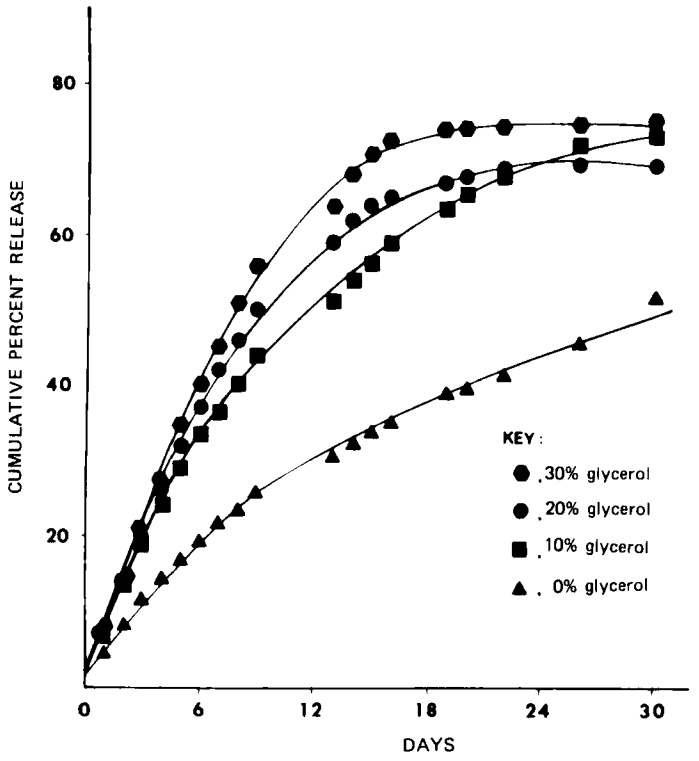

Figure 2. In Vitro release profile (Q vs. t) of indomethacin from silicone implants having various glycerol concentrations. The loading dose of indomethacin in the silicone implants was 1% (w/w).

Key: ● 0% w/w glycerol
▲ 10% w/w glycerol
■ 20% w/w glycerol
⬢ 30% w/w glycerol

$mcg/cm^2/day^{1/2}$. This extrapolated value is about 46% higher than the experimental value of 97.2 $mcg/cm^2/day^{1/2}$. The linear relationship of log (release flux) and glycerol concentration has a correlation coefficient of 0.9997.

C. Correlation Between Swelling and Cumulative Percent Release

The volume change in the indomethacin-containing implants observed earlier (8) was correlated with the cumulative percent

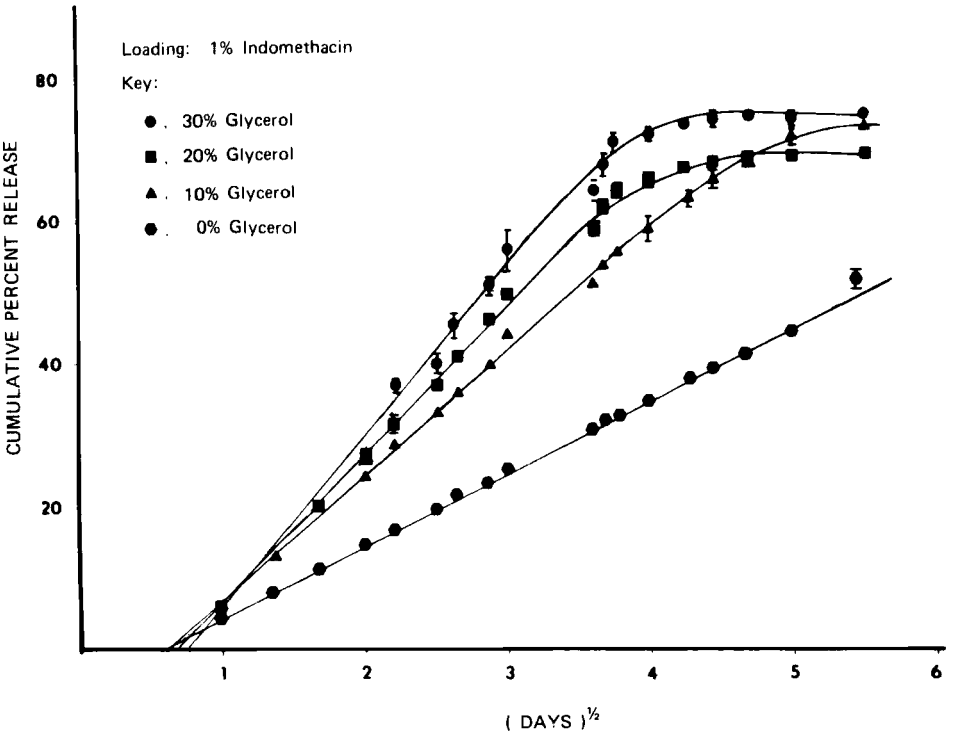

Figure 3. Linear Q vs. $t^{1/2}$ plots of the In Vitro release profiles of indomethacin from silicone implants having various glycerol concentrations. The figure was replotted from the data presented in Figure 2.

release of indomethacin from the same type of implants (Figure 5). A linear correlation between the relative volume change and the cumulative percent release was observed for indomethacin implants having up to 20% (w/w) of glycerol. The slope for the implants containing 20% (w/w) of glycerol is 2.000, which is higher than the slope of 1.212 for implants having 10% (w/w) of glycerol. However, this linearity was not observed for the implants having 30% (w/w) glycerol. The curve fell off eventually, due to the shrinkage of the implants at the latter phase of release.

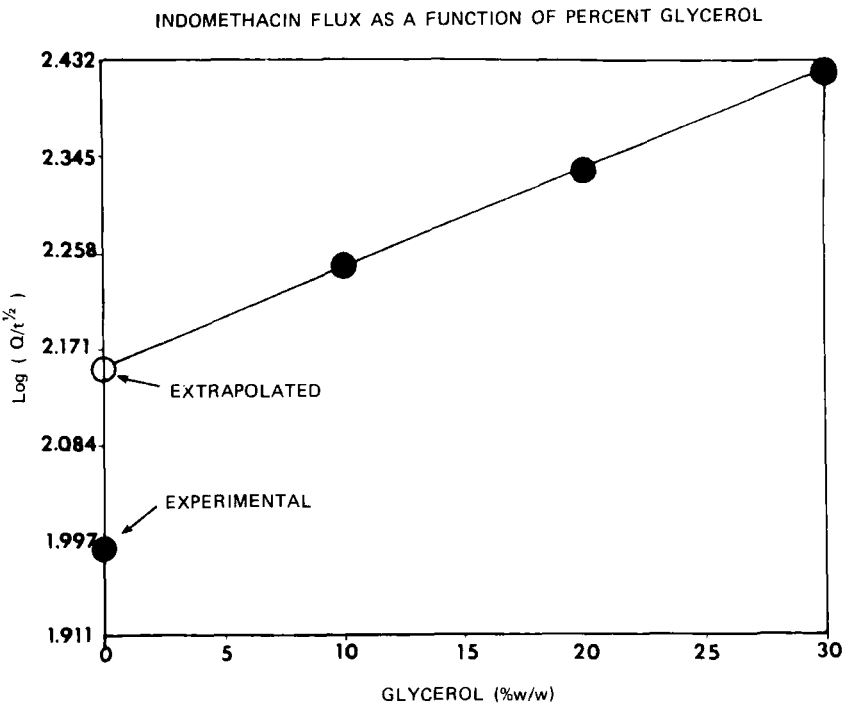

Figure 4. Semilogarithmic relationship between the release flux of indomethacin and glycerol content in silicone implants.

D. In Vivo Release Studies of Indomethacin Implants

After 32 days of subcutaneous implantation, the indomethacin containing implants (without glycerol) released 48.1% of the indomethacin loading dose, while 99.4% of the loading dose was released from the implants having 10% (w/w) of glycerol. It took only 18 days for the implants containing 20% (w/w) of glycerol to release 96.3% of the indomethacin dose. When the normalized in vivo release data (mcg/cm^2) was plotted versus the square root of time, the linear Q vs. $t^{\frac{1}{2}}$ relationship was observed for implants containing 0, 10, and 20% (w/w) of glycerol (Figure 6). The release fluxes ($Q/t^{\frac{1}{2}}$), calculated from the slope of the curve, are 84.1, 156.1 and 169.3 mcg/cm^2/day$^{\frac{1}{2}}$ for the

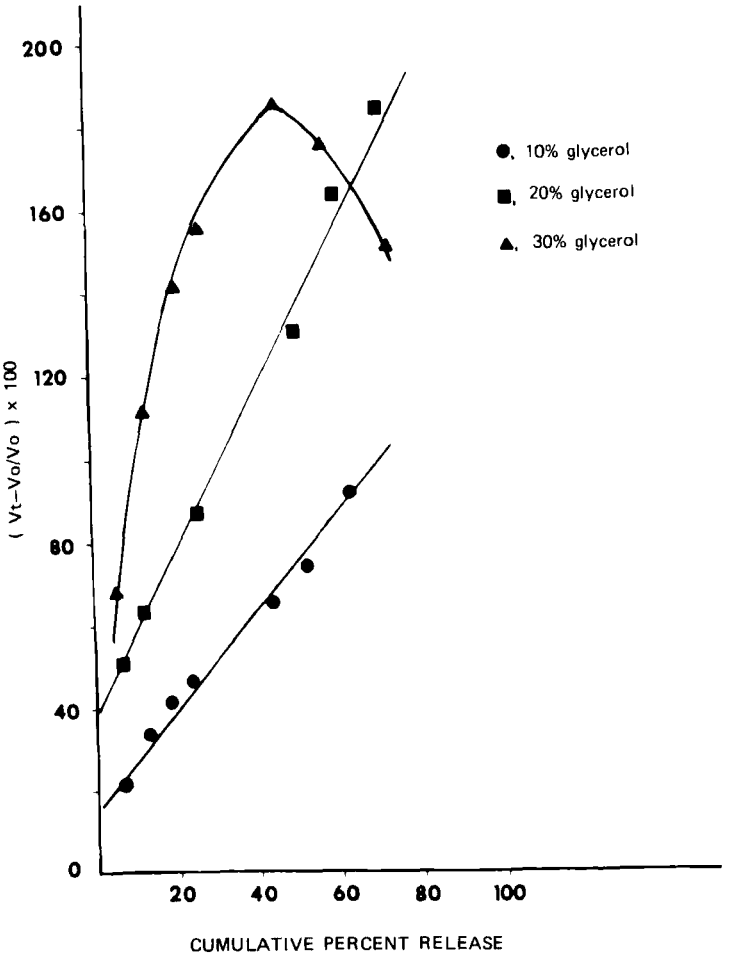

Figure 5. Correlation between the swelling of silicone implant and the cumulative percent release of indomethacin from implants. The relative volume change of silicone implants having 10, 20, or 30% (w/w) of glycerol was obtained previously (8).

Key: ● 10% w/w of glycerol
■ 20% w/w of glycerol
▲ 30% w/w of glycerol

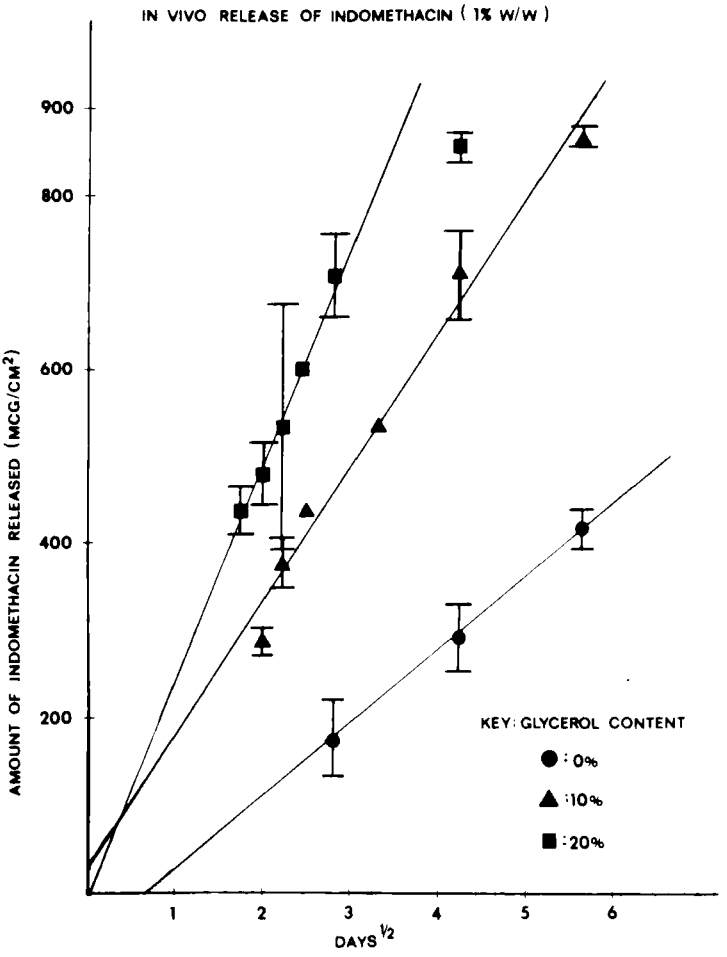

Figure 6. Linear Q vs. $t^{\frac{1}{2}}$ plots of the In Vivo release profiles of indomethacin from subdermal implants containing different concentrations of glycerol. The indomethacin loading in the silicone implants was 1% (w/w).

Key: ● 0% w/w of glycerol
▲ 10% w/w of glycerol
■ 20% w/w of glycerol

implants containing 0, 10, and 20% (w/w) of glycerol, respectively.

E. Correlation of In Vivo and In Vitro Release Fluxes

In Table I, the in vitro and in vivo release fluxes of indomethacin from various silicone implants having different glycerol contents are compared. An in vivo/in vitro correlation of 0.79 to 0.89 was achieved for implants having up to 20% (w/w) of glycerol.

F. Survival Rates of Indomethacin Implant-treated Mice

The survival rate of mice receiving indomethacin-releasing implants having up to 20% (w/w) of glycerol is shown in Figure 7. For mice receiving the implants containing no glycerol, the survival rate was 100% throughout the course of 32-day observation (Only nine days of the survival rate was shown on the histogram). The survival rate for mice receiving implants containing 10% (w/w) of glycerol was 100% for the first three days and then dropped to 85% on day 4, 70% on day 5, 65% on day 6 and beyond. For mice receiving implants having 20% (w/w) of glycerol, the survival rate was 100% for the first 2 days, then dropped to 90% on day 3, 65% on day 4, 40% on day 5, 35% on days 6 and 7, and 20% on and after day 8. In a separate study, it was found that all mice receiving indomethacin implants containing 30% (w/w) of glycerol died within two days of implantation.

DISCUSSION AND CONCLUSIONS

In view of the increasing importance and clinical use of non-steroidal anti-inflammatory agents (NSAID's), including indomethacin (14), and the public awareness of the adverse effects associated with the long-term oral administration of

Table I.

Comparison of *In Vivo* and *In Vitro* Release Fluxes of Indomethacin

Glycerol content[1] (% w/w)	In Vitro Release Rate[2] ($mcg/cm^2/day^{1/2}$)	In Vivo Release Rate[3] ($mcg/cm^2/day^{1/2}$)	Ratio[4]
0	97.2	84.1	0.87
10	175.3	156.1	0.89
20	214.1	169.3	0.79
30	266.4	-	-

(1) Silicone implants containing 1% (w/w) indomethacin with various concentrations of glycerol.
(2) Calculated from the slope of the linear plots in Figure 3.
(3) Calculated from the slope of the linear plots in Figure 6.
(4) Ratio of in vivo/in vitro release fluxes.

NSAID's, pharmaceutical scientists have shifted their interests to the development of controlled release formulations for the delivery of these drugs (5, 15, 16). In this study, the feasibility of subcutaneous controlled release of indomethacin from silicone elastomers in experimental mice was investigated. In the preformulation studies, a co-solvent system was incorporated to enhance the aqueous solubility of indomethacin, and the equilibrium solubility of indomethacin in aqueous PEG 400 solution was observed to increase exponentially as a function of the volume fraction of PEG 400 in the aqueous solution. Using this relationship, an *in vitro* sink condition, which contains 20% (v/v) of PEG 400 in the aqueous solution, was established for studying the controlled release of indomethacin. This study confirmed the previous observations that incorporation of glycerol into silicone elastomers enhances the release of drugs from the device (7-10). Therefore, even at the same drug loading, a simple manipulation of the glycerol content in the silicone polymer matrix will program the release of indomethacin at different rates.

A fairly good correlation was observed between *in vivo* and *in vitro* release rates of indomethacin from silicone implants

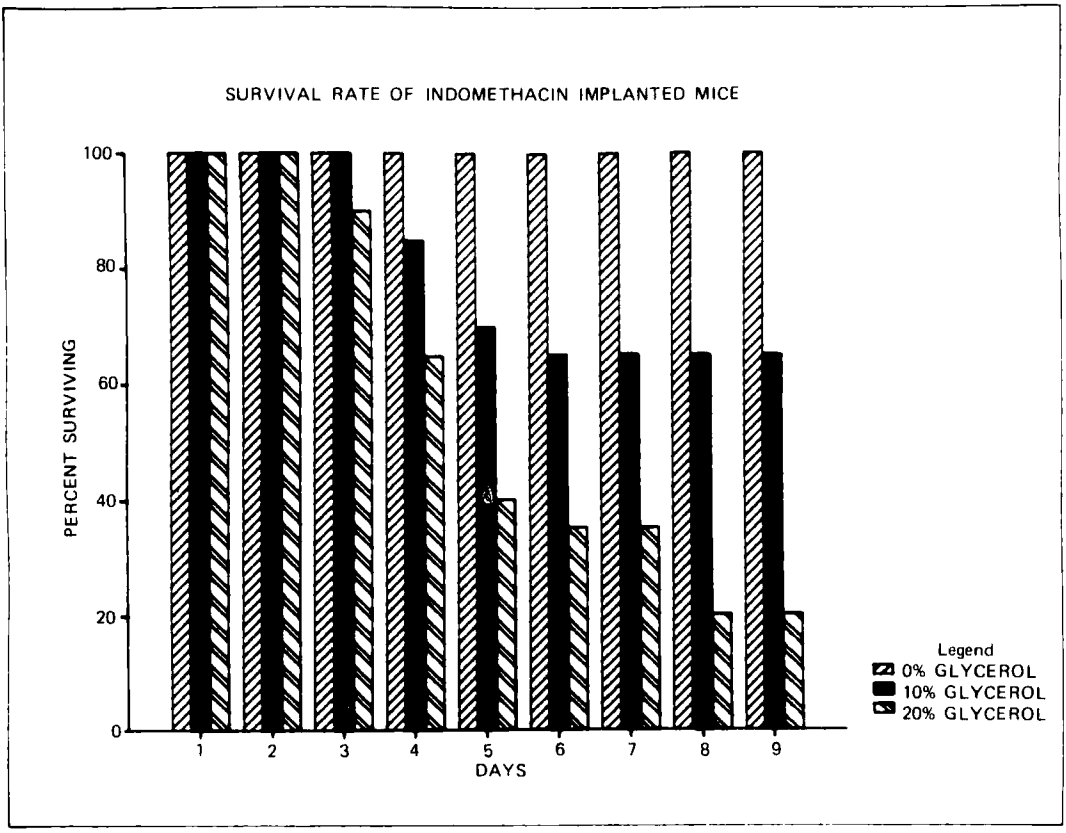

Figure 7. Survival rate of mice receiving indomethacin-releasing subdermal implants having different glycerol contents.

having various levels of glycerol. A correlation coefficient of 0.85 (± 0.05) was obtained (Table I). Therefore, the *in vivo* release rates of indomethacin from silicone implants in experimental mice can be predicted fairly well from the *in vitro* release rates.

According to the literature (13), the acute oral LD_{50} dose for indomethacin is 50 mg/kg in mice, while the intraperitoneal and intravenous LD_{50}'s are 28 mg/kg and 40 mg/kg, respectively. Death usually occurs within 3-7 days after the administration of a single dose. Pathological studies revealed ulceration of the gastrointestinal tract in mice.

In this chronic toxicity study, the results in Figure 7 show that half of the mice had died within five days of receiving indomethacin implants containing 20% (w/w) of glycerol. The cumulative amount of indomethacin released from the silicone implants for five days was 0.848 (± 0.222) mg. Therefore, the subcutaneous LD_{50} for mice receiving indomethacin-releasing subdermal implants having 20% (w/w) of glycerol was calculated to be about 34 (± 9) mg/kg, based upon the average body weight of 25 grams. On the other hand, due to a slower rate of indomethacin release, the mice receiving indomethacin implants containing 10% (w/w) of glycerol showed a survival rate of 70% on day 5 and 65% on days 6-9. The toxic effects of indomethacin appear to be cumulative. A gross examination of the dead mice revealed abdominal bloating. The viscera were greenish in appearance. When the visceral sacs were opened, a highly putrid odor was emitted. From these observations, it appears that the toxic effects of indomethacin are systemic.

Indomethacin is an effective inhibitor for prostaglandin synthetase at 0.06 - 0.50 mcg/ml (2). Since prostaglandins are involved in a number of biological functions including immunity (17), silicone implants capable of delivering indomethacin continuously at controlled release rates seem well suited for *in vivo* investigations of immunological reactions in experimental animals. These immunological reactions include macrophage activity (phagocytosis and chemotaxis) and antibody formation (IgG, IgM, etc.). A better understanding of the effects of indomethacin on the immune system may lead to a reduction of toxicity in the case of NSAID's, as well as an improved use of these drugs in other disease states, such as cancer (18).

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Footnotes

*1. Sigma Chemical Company, St. Louis, MO.

*2. Medical Grade Silicone Elastomer 382, Dow Corning, Midland, MI.

*3. Fisher Scientific, Springfield, NJ.

*4. Model 4380-00, Cole-Parmer, Chicago, IL.

*5. 0.125 inch,(i.d.), Fisher Scientific, Springfield, NJ.

*6. Polyethylene Glycol 400, Fisher Scientific, Springfield, NJ.

*7. Model 127, Fisher Scientific, Springfield, NJ.

*8. Type HAWP, Millipore Corp., Bedford, MA.

*9. HPLC Grade, Fisher Scientific, Springfield, NJ.

*10. UV/Vis Spectrophotometer, Model 559, Perkin-Elmer, Norwalk, CT.

*11. Charles River Breeding Laboratories, Wilmington, MA.

*12. Nembutal sodium soln., Abbott Laboratories,North Chicago, IL.

*13. Fisher Scientific, Springfield, NJ.

*14. Fisher Scientific, Springfield, NJ.

*15. Model 75, Burrell Corp., Pittsburgh, PA.

*16. Model 6000A, Waters Associates, Milford, MA.

*17. Model U6K, Waters Associates, Milford, MA.

*18. Model 440, Waters Associates, Milford, MA.

*19. Model D5117, Houston Instrument, Houston, TX.

*20. Bondapack-C (250 x 4.6 mm i.d.), Waters Associates, Milford, MA.

*21. C (37-50 um, 40 x 3.2 mm i.d.), Waters Associates, Milford, MA.

*22. Certified ACS, J.T. Baker Chemical Co., Phillipsburg, NJ.

*23. HPLC Grade, Fisher Chemical, Fair Lawn, NJ.

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