Toxicological Evaluation of Film Forming Methacrylic Acid Copolymers

A.S. DARVESH, M.N. SARAF* AND MALA MENON[1]
Dept. of Pharmacology, Dept. of Pharmaceutics[1], Bombay College of Pharmacy, Kalina, Santacruz East, Mumbai-400 098

Accepted 19 March 1999
Received 21 August 1998

Three copolymers of methacrylic acid and 2-ethylhexyl acrylate in the ratios 30:70, 40:60, and 50:50 were synthesized by emulsion polymerization technique in our laboratory. The following acute toxicity and biological reactivity tests were carried out on these polymers : (a) Systemic Injection Test, (b) Intracutaneous Test, (c) Dermal Irritation Test, (d) Implantation Test, (e) RBC Haemolysis Test, (f) Agar Diffusion Test, (g) Direct Contact Test and (h) Elution Test. The results revealed that the copolymers are devoid of any acute toxicity and biological reactivity.

Acrylate polymers comprise an important group of polymers used in pharmaceutical film coating[1]. In an earlier study conducted in our laboratory[2], it was found that a copolymer prepared from methacrylic acid (MA) and 2-ethylhexyl acrylate (EHA) by solution polymerization technique produced flexible, internally plasticized films. In a similar study[3], copolymers of MA and EHA synthesized by emulsion polymerization technique were found to be effective in pharmaceutical film coating.

The present study was initiated with the objective of carrying out the *in vivo* and *in vitro* acute toxicity and biological reactivity tests of the MA-EHA copolymers in order to extend the use of these copolymers in the development of Novel Drug Delivery Systems.

Three copolymers were synthesized by emulsion polymerization technique using 30:70, 40:60 and 50:50 MA:EHA ratios in the monomer feed in our laboratory and were coded as POLY-Q, POLY-R, and POLY-S. Free films of the copolymers were also prepared in our laboratory. Eudragit L 100-55 was used as the standard copolymer. Albino mice of Swiss strain of either sex in the weight range of 17-23 g, albino rabbits of the New Zealand strain of either sex in the weight range of 2.5-2.8 kg, and the L-929 mammalian fibroblast cell line 'ATCC cell line CCL, 1, NCTC clone 929' obtained from the National Centre For Cell Sciences, Pune was used for the biological reactivity tests.

*For Correspondences

Copolymer extracts were prepared using the following method[4,5] : 0.2 g of each copolymer was used per ml of the following four extraction media : sodium chloride injection, 1 in 20 solution of alcohol in sodium chloride injection, polyethylene glycol 400 and vegetable oil. Extraction was carried out by autoclaving at 121° for 60 min. Blanks were prepared by autoclaving the extraction media at 121° for 60 min.

The following pharmacopoeial as well as non pharmacopoeial biological reactivity tests were carried out : For the systemic injection test[5,6,7] mice were injected intraperitoneally with a dose of 25 ml/kg of the copolymer extract while the control groups received the respective blank. Mice were examined immediately, and then 4,24,48 and 72 h after injection, to observe if the mice of the test group showed biological reactivity greater than those of the control and standard groups. While this test appears to be less sensitive, it is carried out with the rationale that it may highlight some subtle systemic toxicity which may otherwise not be evident in the other tests[1,2]. In the intracutaneous test[5,8,9] copolymer extract and blank were injected intracutaneously in a dose of 0.2 ml/site on 5 different sites on depilated skin, each side of the spinal column of the rabbit. The injection sites were examined immediately, and then 4,24, 48 and 72 h after injection for any tissue reaction such as edema, erythema and necrosis. Scores were given as per the Draize and USP scoring system. For dermal irritation test (patch test)[8,9] the copolymer films and standard film measuring

1 cm^2 each were placed on 5 different sites on depilated skin of albino rabbits on each side respectively. The films were covered with a gauze patch, and non-medicated adhesive tape was used to hold it in place. The films were removed after 24 h. The application sites were examined immediately, and then 4,24, 48 and 72 h after removal of the films for any tissue reaction such as erythema, edema and necrosis. Scores were given as per the USP scoring system. The Implantation test[5,10,11] was performed on albino rabbits by aseptically implanting 4 copolymer strips measuring 10 mm x 1 mm on one side of the spine into the paravertebral muscle and 2 standard strips on the other side in a similar manner. After 120 hours the animal was sacrificed, and the implant sites were examined macroscopically for presence of haemorrhage, necrosis, discoloration, infection, and encapsulation. The erythrocyte system has been employed as a general indicator of potential toxic leachables, particularly those which demonstrate significant membrane activity or biochemical disruption[12]. Haemolysis less than 5% is considered insignificant under the experimental conditions used[7]. For the RBC haemolysis test[12,13,14], 1 ml fresh rabbit blood was diluted to 10 ml with heparinised saline and placed in a water bath maintained at 37° A negative control was prepared by adding 0.2 ml diluted blood to 10 ml saline maintained at 37° A positive control (100% haemolysis) was prepared by adding 0.2 ml diluted blood to 10 ml distilled water containing 0.02% haemolysing agent. Five grams of the copolymer as well as standard film each were cut into small pieces (<0.5 cm in largest dimension), placed in a test tube and covered with 10 ml of Sodium Chloride Injection, and placed in a water bath maintained at 37° for 30 min and then 0.2 ml of diluted blood was added to test tube containing test sample. Test tubes containing test samples as well as controls were incubated at 37° for 60 min. After the 60 min incubation period all the test tubes were centrifuged for 5 min to sediment the erythrocytes. An aliquot of the supernatant in each test tube was transferred to a cuvette and the optical density (O.D.) at 545 nm was determined. The percent haemolysis was calculated as follows :

$$\% \text{ Haemolysis} = \frac{(\text{O.D. test sample}) - (\text{O.D. negative control})}{(\text{O.D. positive control}) - (\text{O.D. negative control})} \times 100$$

In case of all samples, % haemolysis was found to be <2% indicating lack of haemolytic activity.

The *in vitro* biological reactivity tests have been designed to detect the response of a mammalian monolayer cell culture to readily diffusible components from materials[7]. For these tests, confluent monolayers of the L-929 mammalian fibroblast cell line were prepared in radiation sterilized tissue culture petriplates and incubated at a temperature of 37±1° and a 4±1% carbon dioxide atmosphere. For all the tests the biological reactivity was described and rated as per the USP grading system on a scale of 0 to 4. For the agar diffusion test[5,7,15] medium containing 2% agar was placed on top of the monolayer and 2 pieces of copolymer film measuring 10 mm x 10 mm and one similar piece of standard film were placed on top of the agar surface. The monolayer was microscopically examined after 24 hours. For the direct contact test[5,16], one piece of copolymer film and one piece of standard film were placed directly on top of the monolayer and microscopically examined after 24 hours. The elution test[5] was carried out by extracting 2 g of copolymer per ml of tissue culture medium for 24 hours at 37±1°. The medium in petriplates having monolayer was replaced by the extract and monolayer was microscopically examined after 48 hours.

The results of the biological reactivity tests revealed that the methacrylic acid copolymers are devoid of any acute toxicity and biological reactivity. The absence of biological reactivity for all the copolymers can be attributed to their high degree of curing and excellent polymerization, as it is the unpolymerized monomer, which is responsible for the biological reactivity[17]. However further long term toxicity studies on the copolymers are warranted to ensure absence of other toxicological manifestations such as carcinogenicity, mutagenicity, and teratogenicity.

REFERENCES

1. Lehmann, K.O.R., In; McGinty, J.W. Ed., **Aqueous Polymeric coatings for Pharmaceutical Dosage Forms,** Marcel Dekker, Inc., New York, 1989, 161.
2. Puthli, S.P. and Shrivastava, R., **Indian J. Pharm. Sci.** 1996, 58, 184.
3. Cardoza, R.M., **M. Pharm. Sci. Thesis,** University of Mumbai, 1997, 91.
4. Crompton, T.R., **Additive Migration from Plastics into Food,** Pergamon Press, New York, 1979, 20.
5. **United States Pharmacopoeia/National Formulary, USP 23/NF 18,** Asian edition, United States Pharmacopoeial Convention, Inc., 1260', Twinbrook Parkway, Rockville, MD 20852, 1995, 1695.

6. Guess, W.L. and Autain J., **Am. J. Hosp. Pharm.,** 1964, 21, 261.
7. Autain, J., **Artif. Organs,** 1977, 1, 53.
8. Draize, J.H. Woodward, G. and Calvery, H.O., **J. Pharmacol. Exp. Ther.,** 1944, 82, 377.
9. Ritter, L. and Franklin, C.A., In; Arnold, D.L., Grice, H.C., Krewski, D.R., Eds. **Handbook of *in vivo* ToxicityTesting,** Academic Press, Inc., San Diego, California, 1990, 247.
10. Turner, J.E., Lawrence, W.H. and Autain, J., **J. Biomed. Mater. Res.** 1973, 7, 39.
11. Lawrence, W.H., Mitchell, J.L., Guess, W.L. and Autain J., **J. Pharm. Sci.,** 1963, 52, 958.
12. Lawrence, W.H., In Bruck, S.D., Ed., **Controlled Drug Delivery-Clinical Applications,** Vol. II, CRC Press, Inc., Florida, 1983, 2.
13. Boyer, M.J., Horn, I., Firestone, R.A., Norwood-Steele, D. and Tannock, I.F., **Br. J. Cancer,** 1993, 67, 81.
14. Keith, H.B., Ginn. E., Williams, G.R. and Campbell, G.S., **J. Thorac. Cardiovasc. Surg.** 1961, 41, 404.
15. Guess, W.L., Rosenbluth, S.A., Schmidt, B. and Autain, J., **J. Pharm. Sci.,** 1965, 54, 1545.
16. Rosenbluth, S.A., Weddington, G.R., Guess, W.L. and Autain, J., **J. Pharm. Sci.,** 1965, 54, 156.
17. Dillingham, E.O., Webb, N., Lawrence, W.H. and Autain, J., **J. Biomed. Mater. Res.,** 1975, 9, 569.

Chemical and Pharmacological Evaluation of *Hygrophila spinosa* Root

U.K. MAZUMDER*, MALAYA GUPTA AND S. MAITI
Divisions of Pharmaceutical Chemistry and Pharmacology,
Department of Pharmaceutical Technology, Jadavpur University, Calcutta-700 032

Accepted 10 April 1999
Received 14 September 1998

Chemical investigation of *Hygrophila spinosa* root exhibited the presence of a greasy mass, lupeol and lupenone in petroleum ether extract. Crude petroleum ether extract, when administered (i.p.) to mice, potentiated the sedative-hypnotic action of chlorpromazine, diazepam, pentobarbitone, chlordiazepoxide and protected against strychine-induced convulsions.

Hygrophila spinosa (Acanthaceae), a small herb, is found to be distributed throughout India[1]. Its roots, seeds and leaves are reported to be used in different diseases[2,3,4]. Chemical investigations on seeds[5], leaves[6], and roots[7] led to the isolation of a sterol, a triterpine alcohol, xylose, and uronic acids. Petroleum ether fraction of the root has been reported to exhibit antitumour activity in mice[8]. During a toxicity study[9], the petroleum ether extract of *H. spinosa* root was found to exhibit mild passivity and decreased touch response. Hence the extract was investigated for CNS depressant action. The present communication deals with chemical evaluation of *Hygrophila spinosa* root and activity of crude petroleum ether extract on central nervous system.

The roots *Hygrophila spinosa* were shade dried and powdered. It was extracted with petroleum ether (60°-80°) in a soxhlet and the extract was concentrated under vacuum. Thin layer chromatography of the crude petroleum ether extract (yellowish white in colour) using chloroform as solvent indicated the presence of four components, I, II, III and IV showing R_f values 0.95, 0.7h, 0.5h and 0.1m respectively. To separate the components, the crude extract was chromatographed over silica gel (60-120 mesh) in a column using petroleum ether:ethyl acetate (90:10) as the eluting system. The first elute after evaporation was found to be a greasy mass (component I). The second fraction was a white mass (component II) which was crystallised from n-hexane (m.p 166°) and analysed spectroscopically. Mass spectra (associated machine model JEOL-JMS AX 500 Spectrometer) of the compound indicated molecular ion peak (M^+) at 424. Its IR spectra (associated machine model 873 Perkin Elmer Spectrometer) exhibited the presence of a keto carbonyl group (γ_{max} at 1770 cm^{-1}). From the mass, IR and

*For Correspondence