THE BLOOD PRESSURE AND DERMAL SENSITIVITY EFFECTS OF NYLON HOLLOW FIBRE RELEASING GLYCERIN TRINITRATE IN VIVO

SEYED NASER OSTAD[1], MASUMEH FARHADKHANI[1], BAGHER MINAIEE[2], GOLROKH MALIHI[3] and MOHAMMAD ABDOLLAHI[1]

[1]Department of Toxicology & Pharmacology, Faculty of Pharmacy and [2]Department of Histology, Faculty of Medicine, Tehran University of Medical Sciences, [3]Department of Pharmacology, Faculty of Medicine, Shaheed-Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

In order to improve patient's compliance in taking glycerine trinitrate (GTN) nylon hollow fiber which has been successfully used for release of chlorhexidine diacetate and levonorgestrel was employed to make nylon hollow fiber releasing GTN. Hollow nylon fibres of external diameter 0.63 mm, 75 mm long with an internal capacity of 16 µl, were filled with GTN (190 mg/ml) in 70% ethanol (v/v) or vehicle alone and the ends were heat-sealed. The fibers were then immersed in 10 ml of 0.9% (w/v) saline in a separating funnel. The GTN release pattern from fiber, the effect of the product on blood pressure and its potential dermal toxicity were assessed. The release of GTN from the fibres was approximately 2.7 µg/min when the fibres contained 16 mg of drug. The results showed that the amount of GTN within the single fibre was enough to reduce blood pressure significantly, while it did not show significant dermal toxicity. It is concluded that GTN fiber, if used as monofilament, is not an alternative method for GTN delivery.

Keywords: Glycerin trinitrate, Nylon hollow fibre, Rat, Rabbit, Blood pressure, Draize test

INTRODUCTION

Recent clinical trials demonstrate a wide spread use of organic nitrates in patients with chronic heart failure (1-3). The clinical rationale for their use is based on beneficial effect on hemodynamic profile (4), myocardial ischemia (5), magnitude of mitral regurgitation (6), endothelial function (6,7), cardiac remodeling (8) and exercise capacity (9,10). In addition, when combined with hydralazine nitrates, it improves maximum oxygen consumption, left ventricular ejection fraction and survival (11). GTN ointment has been continuously used in hemorrhoidectomy (12,13), fissure-in-ano (14,15), primary vaginismus (15) and reverse bone loss after oophorectomy (16).

In some cases such as vagismus and fissure-in-ano, oiliness of surface is desirable but in other cases it does not have a clean application area and it is not convenient for patients. Except for oral and parentral administration, it is possible to deliver GTN via sustained release applications such as patches and implant (17-19). Several types of polymeric controlled release systems have been developed and supplied to the market. These products have three basic types of rate controlling mechanisms as described by Langer (23); diffusion, chemical reaction, and solvent activation. Most controlled release systems in use employ one or more of these basic mechanisms for the rate control. Membrane-based controlled release systems have been extensively investigated for a wide range of therapeutic applications, from implants to oral formulation and transmembrane patches (20-22).

However, the patch performances and the success of the transdermal drug delivery can be significantly affected by the quality of contact between the patch and the skin. Implants require an expert for insertion of the product inside of derm, which is relatively invasive.

Since nylon hollow fiber (NHF) releasing system has been successfully used for the release of chlorhexidine diacetate and levonorgestrel (25, 26), it was of our interest to examine the release of GTN from hollow nylon fiber for reducing blood pressure (bp). Furthermore, sustained release feature of this application may help to reduce unnecessary loading dose of GTN due to high

Correspondence: Dr S.N. Ostad, Department of Toxicology & Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14155-6451, Iran. Fax +98 21 6461178, Email: snostad@yahoo.com

capacity of human metabolic system for denitration of nitrate compound. Following study of pharmacological aspect of this application, the primary dermal sensitivity test of NHF were performed as part of safety protocol.

METHODS AND MATERIALS

Materials

Hollow nylon fibres (standard grade nylon 6, internal diameter: 0.5 mm; external diameter: 0.63 mm; Portex, Basingstoke, UK), GTN standard (Shahid Fagiihi Co. LTD, IRAN), absolute ethanol (Sigma Chemical Co. Ltd, UK), and isoflurane (Commercial available in market) were used in this study. Animals were obtained from Pasteur de Institute of Iran.

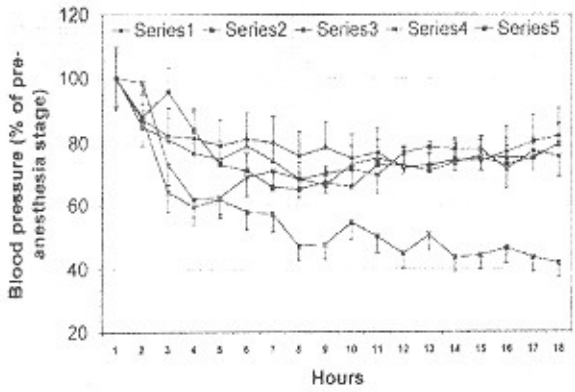


Fig .1. The effect of nylon hollow fiber releasing GTN and GTN ointment in appropriate doses as mentioned in the text (±SD, n=6).

Series 1: empty Nylon hollow fiber
2: Nylon hollow fiber contains 70% ethanol
3: Nylon hollow fiber contains 3.04 mg GTN in 70% ethanol
4: 7.5 mm of 0.2% GTN topical ointment
5: 75 mm of 0.2% GTN topical ointment

Drug release

Hollow nylon fibres with 75 mm length and an internal capacity of 16 µl, were filled with GTN (190 mg/ml) in 70% v/v ethanol or ethanol only and the ends were heat sealed.

The fibres were immersed in 100 ml of 0.9% w/v saline in a separatory funnel. At various times 4 ml of saline solution were removed from the funnel for determination of GTN and were replaced by fresh saline. UV absorption of the GTN was determined at 271 nm using 70% ethanol for background corrections. The concentration of GTN released from NHF was measured by UV absorption spectrophotometry method (27).

Blood pressure effect of nylon hollow fibre releasing GTN

The blood pressure effects of 75 mm hollow fibres containing 3.04 mg of GTN in 16 µl ethanol (70% v/v) or vehicle were assessed using rat as a mammalian model. Animals were divided into 5 groups which received nylon hollow fibre containing GTN (190 mg/ml), ethanol, and empty fibre and in 7.5 mm and 75 mm (containing GTN 10 times more than those of hollow fibres) of 0.2% W/V GTN ointment (as positive control). Each group consisted of 6 male Wistar-Albino rats. Arterial pressure was measured by insertion a catheter of heat-stretched PE-50 tubing into the carotid artery before and 0.5 hour after that animals were anesthetized by isoflurane. The surface area in the back of head of animals were shaved and cleaned by 70% ethanol. Nylon fibres alone or containing GTN and alcohol were attached directly to the skin. When stabilization was achieved, arterial pressure was recorded every half an hour for 8 hours with a pressure transducer (Cobe III Transducer) (28).

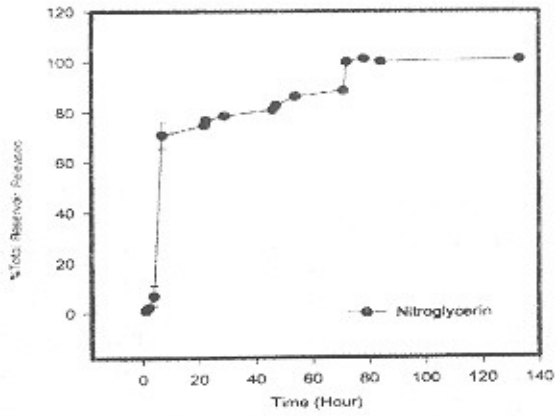


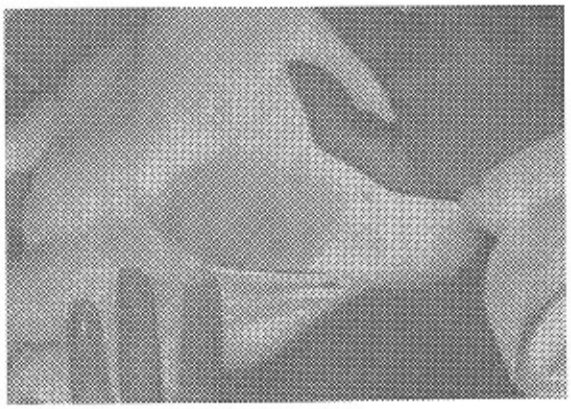
Fig .2. The release of GTN from hollow nylon fiber (±SD, n=6).

Primary dermal sensitivity test

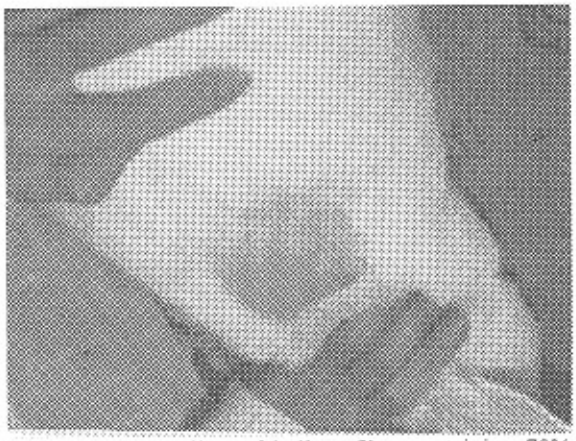
7.5 cm of GTN ointment, 70% alcohol, and nylon hollow fibre containing 190 mg/ml GTN were applied to the clipped, intact skin of eight albino rabbits under semi-occlusive dressings for a 4 hrs exposure period. Application sites were evaluated in accordance with the method of Draize (29) at 30-60 min and 24, 48 and 72 hrs after bandage removal and once daily thereafter through day 12 if irritation persisted. The primary irritation index was calculated to ensure presence of non-irritating state for none of groups.

Statistical Analysis

All data are expressed as mean±SEM. Comparisons of control with other groups were analyzed using factorial ANOVA. A value of $p<0.05$ was considered statistically significant.



Picture 1. The effect of hollow fiber releasing GTN on the rabbit skin after 24 hrs. No reaction was observed.



Picture 2. The effect of hollow fiber containing 70% ethanol as vehicle on the rabbit skin after 24 hrs. No reaction was observed.

RESULTS

Drug release

By having a solution of the agent in the reservoir of the membrane-based controlled release device, the loading of the agent in the reservoir is limited by its solubility in the reservoir solvent. Under the constant sink conditions of the experiment, the release of GTN was maintained for 7 hours at a release rate of 2.7 µg/min, after which, it decreased to zero. The release pattern was biphasic with negligible lag time. The first phase of release was very fast and within 7 hours more than 70% of reservoir was released. In the second phase of release, the rest of reservoir was released within 110 hours (figure 1).

Blood pressure effect of nylon hollow fibre releasing GTN

Under resting conditions, there were no significant changes in either heart rate or mean arterial blood pressure (MAP) in experimental groups after anesthesia. Except the group that received 75 mm GTN ointment and their mean arterial pressure was significantly different with sham during the time of experiment, map of other groups were not noticeably different. In group using 7.5 mm GTN ointment the mean arterial pressure noticeably deceased for the first 5.5 hours ($p<0.05$) but after this time probably due to the compensation reflex did not change significantly. There was a slight but insignificant decrease in MAP with GTN nylon fibre group between 6 to 9 hours after treatment. The time-dependent changes in MAP with either nitroglycerin products or vehicle are illustrated in Figure 2.

Primary dermal sensitivity Test

Pictures 1 and 2 show the Draize skin test results in rabbits in which, there are no significant difference between GTN fibre treated and control groups.

DISCUSSION

Using microporous membranes such as nylon hollow monofilament as controlled release devices in aqueous-organic partition based systems, release of a drug can be extended considerably by introducing a dispersed phase into the reservoir. This process results in a hybrid system which may exhibit diffusion limited or dissolution limited rate control. Following vehicle diffusion, the drug in the reservoir at concentrations above its solubility exist as dispersion. The presence of a dispersed drug phase in the reservoir results in the release of drug for an extended time (24,25). If the dissolution relative to diffusion is faster, the disposed drug is immediately available to replace drug that has been released by diffusion. The drug concentration in the reservoir phase is maintained at the solubility level until dispersion has been depleted. However if dissolution is slow relative to diffusion, the drug in the reservoir solution is maintained at a very low concentration because it is not immediately replaced by drug from the dispersed phase. The results of this study in which 71% of a 16 µl reservoir of GTN released from nylon hollow fiber within the first 7 hrs show that

the release is concentration dependent (first order) during this period (24,25). These results also show that all reservoir of GTN will be available for 5 days while neither nylon hollow fibre containing GTN nor 7.5 mm GTN ointment had a significant effect in rat blood pressure after 6 hours. The latter had significant effect during 2-5 hours after administration. Applying 75 mm ointment (10 times of NHF reservoir) could reduce blood pressure significantly during experiment.

The reason that nylon releasing GTN had negligible effect could be attributed low capacity of each fibre for GTN loading. Using the sheet of nylon hollow fibre containing GTN in the adhesive bases could possibly solve this problem. The release of GTN from nylon hollow fibre obeys Fick's low (passive diffusion) and storing the nylon hollow fiber at room temperature results in lose of its capacity to release GTN. Our previous study showed that release of chlorhexidine diacetate is negligible if it is kept at 4°C (24). A primary irritant is a substance that as the result of a direct cytotoxic effect produces inflammatory changes in the skin characterized by the presence of inflammation, vesiculation and necrosis. The modified Draize irritation test showed that NHF releasing GTN is totally safe for dermal use is totally safe.

REFERENCES

1. The SOLVD investigators (1991). Effect of enalapril on survival inpatients with reduced left ventricular ejection fraction and congestive heart failure. New. Engl. J. Med., 325: 293-302.
2. Packer, M., O'Connor, C.M., Ghali, J.K., Pressler, M.L., Carson, P.E., Belkin, R.N., Miller, A.B., Neuberg, G.W., Fried, D., Wertheimer, J.H., Cropp, A.B., DeMets, D.L. (1996). Effect of amlodipine on morbidity and mortality in severe chronic heart failure. N Engl. J. Med., 335: 1107-1114.
3. The Digitalis Investigation Group (1997). The effect of digoxin on mortality and morbidity in patients with heart failure. New. Engl. J. Med., 336: 525-533
4. Elkayam, U., Roth, A., Medira, A., Ostrzega, E., Shotan, A., Kulick, D., Jamison, M., Johnson, J.V., Rahimtoola, S.H. (1991) Randomized study to evaluate the relation between oral isosorbide dinitrate dosing interval and the development of early tolerance to its effect on left ventricular filling pressure in patients with chronic heart failure. Circulation., 84: 2040-2048.
5. Abrams, J. (1996). Beneficial action of nitrates in cardiovascular disease. Am.J.Cardiol., 77: 31C-37C.
6. Elkayam, U. (1996). Nitrates in the treatment of congestive heart failure., Am.J. Cardiol., 77: 41C-51C,
7. Schwarz, M., Katz, S.D., Demopoulos, L., Hirsh, H., Yuen, J.L., Yuen, J.L., Jondeau, G., LeJemtel, T.H. (1994) Enhancement of endothelium-dependent vasodilation by low-dose nitroglycerin in patients with congestive heart failure. Circulation 89: 1609-1614.
8. Jugdutt, B.I. (1998) Nitrates and left ventricular remodeling. Am. J. Cardiol., 81: 57A- 67A.
9. Leier, C.V., Huss, P., Magorien, R.D., Unverferth, D.V. (1983) Improved exercise capacity and differing arterial and venous tolerance during chronic isosorbide dinitrate therapy for congestive heart failure. Circulation 67: 817- 822.
10. Franciosa, J.A., Goldsmith, S.R., Cohn, J.N. (1980) Contrasting immediate and long-term effects of isosorbide dinitrate on exercise capacity in congestive heart failure. Am. J. Med., 69: 559-560.
11. Cohn, J.N. et al (1986). Effect of vasodilator therapy on mortality in chronic congestive heart failure: results of a veteran's administration cooperative study (V-HeFT). N Engl. J. Med., 314: 1547-1552.
12. Wasvary, H.J., Hain, J., Mosed-Vogel, M., Bendick, P., Barkel, D.C., Klein, S.N. (2001) Randomized, Prospective, double-blind, placebo-controlled trial of effect of nitroglycerin ointment on pain after hemorridectomy. Dis. Colon. Rectum. 44(8): 1069-1073.
13. Moorthy, K., Chavez, R. (2001) Audit of topical glyceryl trinitrate for treatment of fissure-in-ano. Ann. R. Coll. Surg. Engl., 83(3): 220-221.
14. Bassoti, G., Clementi, M., Ceccarellli, F., Pelli, M.A. (2000) Double-blind manometric assessment of two topical glyceryl trinitrate formulations in patients with chronic anal fissures. Dig. Liver. Dis., 32: 699-702.
15. Peleg, R., Press, Y., Ben-Zion, I.Z. (2001) Glyceryl trinitrate ointment as a potential treatment for primary vaginismus. Eur. J. Obstet. Gynecol. Reprod. Biol. 96: 111-112.
16. Wimalawansa, S.J. (2000) Nitroglycerin therapy is as efficacious as standard estrogen replacement therapy (premarin) in prevention of oophorectomy-induced bone loss: a human pilot clinical study. J. Bone. Miner. Res. 15: 2240-2244.

17. Lund, J.N. (2000) Glyceryl trinitrate for fissures; patch or paste. Int. J. Colorectal. Dis., 15: 246-247
18. Zuben BF, Raiput MR, Abro H, Shaikh SA (2000). A randomized trial of glyceryl trinitrate ointment and nitroglycerin patch in healing of anal fissures. Int. J. Colorectal. Dis. 15: 243-245.
19. Davis, R.E., Wachholz, J.H., Jassir, D., Perlyn, C.A., Agrama, M.H. (1999) Comparison of topical anti-ischemic agents in the salvage of failing random-pattern skin flaps in rats. Arch. Facial. Plast. Surg. 1: 27-32.
20. Borodkin, S., Tucker, F.E. (1975) Linear drug release from laminated hydroxypropyl cellulose-polyvinyl acetate films. J. Pharm. Sci. 64: 1289-1294.
21. Minghetti, P., Casiraghi, A., Cilurzo, F., Montanari, L. (2001) Evaluation of adhesive properties of transdermal therapeutic systems containing nitroglycerin. Bull. Chim. Farm. 140: 63-67.
22. Shaw, J.E. (1984) Pharmacokinetics of nitroglycerin and clonidine delivered by the transdermal route. Am. Heart. J. 108: 217.
23. Langer, R. (1990) New methods of drug delivery. Science. 249: 1527.
24. Ostad, S.N. (1996) In: The teratogenic and toxicological effects of drugs delivered directly to the female genital tract. Ph.D Thesis. Brighton, UK.
25. Ostad. S.N., Malhi, J.S., Gard, P.R. (1998) In vitro cytotoxicity and teratogenicity of norethisterone and levonorgestrel released from hollow nylon monofilaments. J. Con.Rel. 50: 179-186.
26. Ostad, S.N., Gard, P.R. (2000) Cytotoxicity and teratogenicity of chlorhexidine diacetate released from hollow nylon fibres. J. Pharm. Pharmacol. 52: 779-784.
27. Siek. T.J., Osiewicz, R.J.R. (1976) Identification drugs and other toxici compounds from their ultraviolet spectra. Part III: ultraviolet absorption properties of 22 structural groups. J. Forensic.Sciences. 21(3): 525-551.
28. Pfeffer, J.M., Pfeffer, M.A., Frohlich, E.D. (1971) Validity of an indirect tail-cuff method for determining systolic arterial pressure In: Unanesthetized normotensive and spontaneous hypertensive rats. J.Lab.Clin.Med. 78(6): 927-961.
29. Ecobicon, D.J. (1997) Dermal toxicity study. In:The basis of toxicity testing 2nd Eds. CRC Press New York. pp: 61-68.