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*Articles: </cgi-bin/articles.cgi> Biodegradable Chitosan for Topical
Delivery of Retinoic Acid*

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ABSTRACT

*Purpose: Topical All-Trans-Retinoic Acid (ATRA) is a promising chemopreventive agent for skin cancers, but irritation precludes its
use. A controlled delivery system has the potential to decrease the
level of irritation. Therefore, the objective of this study was to
develop topical chitosan (CH) systems capable of controlling the release
of ATRA. *

*Methods: ATRA/CH formulations were prepared by gel entrapment techniques. Gel formulations included variations in CH content and
molecular weight. These formulations were then tested in in vitro assays, ie, skin penetration and recovery studies using radiolabeled

[3H]ATRA and long-term stability studies at 200 C and 400 C. Human subjects (n=15) were then exposed to the formulations (/in vivo/) in both volar and paraspinal applications to determine the safety as part of a longer-term clinical trial. *

Results: CH gels act as drug delivery systems to sustain the release of ATRA through the skin in in vitro studies. The release rate was largely dependent on the viscosity of the gel, which is a function of both molecular weight and concentration of the CH biopolymer. The topical ATRA-CH gels caused minimal erythema level (EL) comparable to control (PARASPINAL CH EL = 0.67-1, control EL=0.5-1, F_df=5, 55 = 0.95, p=0.46; VOLAR CH EL = 1.17-1.5; control EL = 0.83 F_df=5,20 = 1.38, p=0.28).

*Conclusions: Topical chitosan gels, permitting the sustained release of ATRA through the skin, were successfully prepared using a gel-entrapment technique. *

he incidence of malignant melanoma of the skin, the most serious form of skin cancer, is increasing faster than that of any other cancer in the United States.^1 Chemoprevention as a strategy for the management of malignant melanoma entails the use of specific agents to block, reverse, or suppress carcinogenesis and thereby prevent the development of primary or secondary cancers.^2,3

Retinoids are among the most promising chemopreventive agents with clinical effects of retinoid chemoprevention having been demonstrated in cancers of the head and neck, lung, cervix, ovaries, and skin.^4-6

However, topical tretinoin (ATRA) induces irritation in 90% of patients
and was the main reason for discontinuation of treatment for almost 50%
of patients.^7,8 This high incidence of irritation, leading to poor
compliance, has precluded its use. Irritation has been attributed, in
part, by an overload of the ATRA-dependent pathways with nonphysiologic
amounts of exogenous ATRA in the skin.^9 A topical sustained delivery
system might prevent this overload, thus reducing skin irritation,
making topical ATRA therapy a viable chemopreventive agent.

Natural or synthetic polymers that are biodegradable may offer
advantages over nonbiodegradable polymers for topical drug delivery
applications. In one study, hyaluronic acid biopolymer was shown to
penetrate and disseminate through all layers of intact skin in mice and
humans, reaching the dermis within 30 minutes of application in mice.^10
Cellular uptake of [3H- hyaluronan] was also observed in the lymphatic
endothelium. Whether nonbio- degradable polymers can also penetrate the
skin is not known. Furthermore, no mammalian enzymes capable of
degrading them exist. Possibly, some nonbiodegradable polymers could be
retained by the reticuloendothelial system, with uncertain long-term
consequences. A biodegradable polymer would be advantageous for topical
drug delivery. Chitosan is a fully biodegradable natural biopolymer and
a good substitute for hyaluronic acid.^11 Chitosan is degraded
internally by enzymes, such as lysozyme, glucosaminidases, and lipases
into glucosamine and N-acetylglu-cosamine fragments.^12-14 In addition,
there is a history of using chitosan for drug delivery applications.^15

The objective of this study was to determine whether a chitosan topical

gel formulation would act as a sustained delivery system for retinoic
acid and to determine whether the gel formulation would cause significant irritation in preliminary clinical studies.

MATERIALS AND METHODS

/Materials/
Chitosan (BioChitosan, IVREA Biomedical) with a viscosity ranging from 7
to 552 cps (1% solutions in 1% acetic acid as measured on a Brookfield
LVT viscometer, 25^o C with appropriate spindle at 30 rpm) was employed.
Glacial Acetic Acid USP, Ethanol USP, and BHT USP were obtained from
Spectrum Laboratory Products. Retinoic Acid USP and Cremophor RH40 NF
were a gift from BASF. 3H-ATRA was purchased from NEN Life Sciences.
Carbopol 940 NF was obtained from BF Goodrich Corp.

/Gel Formulation Development/
Exactly 0.1% ATRA was dissolved in 15% ethanol containing 1 g of
Cremophor RH40 NF 0.04% BHT. Solutions up to 5% CH were prepared by
dissolving CH in 1% glacial acetic acid. Higher CH concentrations were
obtained by first heating the CH slurry to 90^o C followed by addition
of 1% acetic acid. The ATRA solution was then mixed with the CH gel or a
standard Carbopol Gel (CG) by mixing and stored in an amber container to
minimize light exposure.

/In Vitro Testing of Radiolabeled Retinoids/
Fresh hairless mouse skin samples were obtained from Charles River
Laboratories. The apparatus consisted of 6 Franz diffusion cells
(PermeGear Inc) operating in parallel and maintained at a constant
temperature of 37^o C. Approximately 200 mg/cm^2 of each formulation
containing 0.04 µCi of 3H-ATRA was applied to the epidermal side of the
skin sample (1 cm^2). Each formulation was tested in triplicate. The

dermal surface of the skin was perfused with receptor solution consisting of buffered saline containing 0.5% Volpo (Croda, Inc). At daily intervals, 500 mL of the receptor solution was sampled. Following 2 weeks, a surface wash consisting of 2 X 500 mL of a 1% acetic acid solution in absolute ethanol was applied to the skin surface. The skin sample was digested overnight in 4 mL of Solvable (Packard Instruments). The entire contents of the receptor volume (5 mL), the surface wash, and digested skin layer were then mixed with Ultima Gold scintillation fluid (Packard Instruments) for 3H counting.

/Preliminary Clinical Testing of the Topical Formulations/ IRB approval was obtained from Boston Medical Center to conduct the clinical studies including 15 healthy individuals, aged 28 to 75, without previous allergic reactions to retinoids or shell fish. The irritant potential of the gel formulations was assessed by means of patch test evaluations using the occlusive Hill Top Chamber patch testing system (Hill Top Research, Inc), which incorporates 0.2 mL of test sample.16 Patches were applied for 24 hours to either the volar forearm or the paraspinal area according to a Latin Square Design.

Phase I involved six human subjects. Each subject received the 6 formulations listed in Table 1 applied to the more sensitive volar forearm area. Phase II involved three additional human subjects, each subject exposed to three patches applied to the paraspinal area of the back. Phase III involved six additional human subjects receiving six patches applied to the paraspinal area. For Phase II and III, evaluation was done at 30 minutes and at 24 hours after removal of the patch. The data were evaluated in terms of a Mean Irritation Score (Table 2) and

other adverse skin reactions. Statistical evaluation included both
frequency and severity of erythema seen at treated sites using ANOVA and
paired t-tests.

*RESULTS AND DISCUSSION
*

/In Vitro Studies/
Viscosity was found to be the most important variable for controlling
the release rate of ATRA. The effect of CH concentration on the viscosity of the chitosan solution could be estimated from the Philipof's equation: V= (1 + KC)^8 , where V is the viscosity in centipoises (cps), K is a constant, and C is the concentration expressed
as a fraction. According to this equation, a higher viscosity polymer
can be replaced with a lower viscosity polymer at a higher concentration.

The cumulative ATRA levels in each skin compartment of hairless mouse
skin after 200 hours exposure to different CH formulations is shown in
Figure 1. By increasing the viscosity of CH from 550 cps (1% High Molecular Weight -MW~360,000 daltons) to 3.3x10^6 cps (8% Middle Molecular Weight chitosan MW~120,000 daltons), it was possible to decrease the percutaneous ATRA penetration from 70% to 10%. In addition,
it was possible to increase (Figure 2a) or decrease (Figure 2b) the ATRA
penetration compared to CG control by simply changing the CH concentration.

The time-release profile of the different gel formulations is shown in
Figure 3a. The CG (empty circles) display a percutaneous ATRA sustained
release somewhere in between the 1% and 3% CH formulations (full symbols). Increasing the concentration of the MMW chitosan up to 8%
further decreased the percutaneous release rate (Figure 3b). ATRA gels
made from the HMW chitosan at concentrations greater than 2% were stable
for at least 120 days and comparable in stability to the CG

(Figure 4).

/Preclinical Studies/
As shown in Table 3 and Figures 5 and 6, minimal erythema occurred in all treatments, regardless of delivery vehicle or ATRA content. This minimal erythema was probably a result of the use of ethanol in the formulation, a solvent known to cause irritation. The Analysis of Variance (ANOVA) indicated there were no significant differences in irritation among the treatments in Phase I ($F_{df=5,20} = 1.38$, $p=0.28$), Phase II ($F_{df=2,10} = 1.00$, $p=0.40$), or Phase III ($F_{df=5, 55} = 0.95$, $p=0.46$). For adverse side effects in Phase I, pruritus occurred in all treatments except B (1% CH/0.1% ATRA), and scaling only occurred in treatment A (1% CH). There was only one occurrence of scaling for treatment A in Phase II (3% CH) , and pruritus occurred in all treatments except A (3% CH/0.1% ATRA) and C (3% CH/0.05% ATRA) in Phase III. These results are preliminary, and a larger clinical sample size with extended topical application is necessary to further assess the potential of this technology.

CONCLUSIONS

The in vitro studies showed that CH gels act as sustained delivery vehicles for ATRA, the delivery rate being a function of the viscosity of the gel. The cumulative percutaneous penetration of ATRA could be decreased from 70% to 10% by increasing the viscosity of the topical CH gel. The preliminary clinical studies indicated a minimal erythema in all formulations, including the control gel, without significant difference in irritation or adverse reactions between the gel formulations. Thus, CH gels may offer some advantages over existing topical delivery systems for the administration of irritating substances, such as ATRA for the chemoprevention of skin cancers.

ACKNOWLEDGEMENTS

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BIOGRAPHIES

CENTER

Dr. Maurizio Cattaneo currently serves as President and Chief Scientist of Ivrea Biomedical. As a Research Officer with NRC Canada, he developed fiber-optic biosensors for clinical and environmental applications. Prior to founding his own company, he was Director of Technology Development at Cambridge Scientific, Inc, specializing in tissue engineering for cartilage and bone replacement. Dr. Cattaneo earned his PhD in Chemical Engineering at McGill University, where his studies focused on microencapsulation and artificial organs.

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