Wound healing, anti-ulcerogenic, anti-inflammatory and anti-proliferative properties of chitosan

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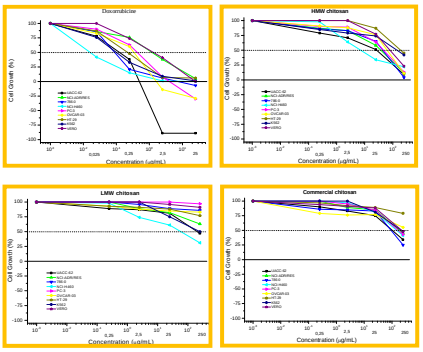
Rationale

Chitosan, a copolymer consisting of β-(1→4)-2-acetamido-D-glucose and β-(1→4)-2-amino-D-glucose units derived from chitin by deacetylation, is known to possess several biological activities including anti-microbial, anti-fungal, anti-inflammatory, hemostatic, wound healing, anti-carcinogenic, etc. However, the relationship between these activities and the molecular weight and degree of deacetylation of chitosan is not yet well established. In this research effort we have tested some of these activities '*in vivo*' and '*in vitro*' by using three different chitosans with different molecular weights and degree of deacetylation.
The aim of this work was to assess the wound healing, anti-ulcerative and anti-inflammatory properties of these chitosans '*in vivo*', and the anti-proliferative activity '*in vitro*'.

Anti-proliferative activity

Chitosans: Low molecular weight (107 kDa, 75-85% DD, Sigma, USA); High molecular weight (624 kDa, 75% DD, Sigma, USA); Commercial (300-350 kDa, 76% DD, Primex, Iceland).

Anti-proliferative activity: The antiproliferative activity of chitosan was evaluated '*in vitro*' against eight human tumor cell lines: melanoma (UACC-62), lung (NCI-H460), leukemia (K-562), ovarian (OVCAR-03 and NCI-ADR/RES), prostate (PC-3), colon (HT-29), kidney (786-0) and VERO cells (normal epithelial kidney cells from green monkey). The Total Growth Inhibition (cytostatic activity) was determined for each compound.



High molecular weight chitosan showed a moderate cytostatic effect. Could chitosan due to its high MW alter the osmotic pressure of the medium leading the cell to change its metabolism?

Figure 1. Cancer cell growth (%) after 48 h-exposure of a)Doxorrubicine (0.025 to 25 μg/mL), b)HMW chitosan (0.25 to 250 μg/mL), c)LMW chitosan (0.25 to 250 μg/mL), and d)Commercial chitosan (0.25 to 250 μg/mL).

Wound healing activity

Wound-healing activity: The wound healing study was performed using diabetic (induced with Streptozotocin, Sigma, 60mg/kg, administered intraperitonially) male Wistar rats (*Rattus norvegicus*) weighing 250–300 g, n=5/group. Seven days prior to the experiment, all animals were housed in polycarbonate cages, under a climate-controlled environment. On day 1, after thiopental anesthesia, excision wounds sized 1.5cm² in average were made. Wounds were treated topically during 10 days by applying 200μL/wound of each sample (0.2mg/mL) and protected by a wound curative. On day 11, animals were sacrificed and tissue samples from wound site of each animal were removed for histopathological analysis and for total collagen determination as described in Jorge et al. (2008).

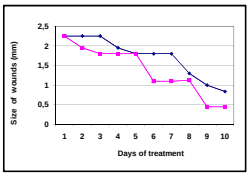


Figure 2. Wound size decrease after treatment with HMW chitosan.

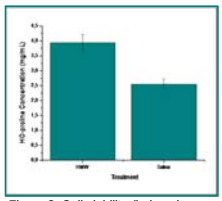


Figure 3. Cell viability (induced fibroblast proliferation) by HMW chitosan.

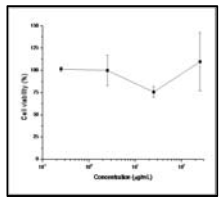
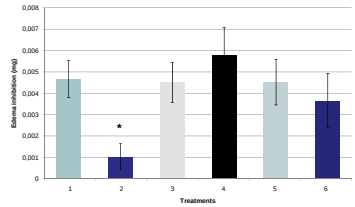


Figure 4. Hydroxyproline (collagen) content induced by HMW chitosan.

As shown in Figure 2, HMW chitosan promoted wound size decrease after day 5 of treatment reaching 98% ± 0.46 of contraction after 10 days. In this period, control animals showed 26.6% ± 0.13 of wound contraction. However, HMW chitosan did not induce cell proliferation and did not interfere with cell viabilibity (>75%) (Figure 3). Induced fibroblast proliferation and collagen production is an efficient way to study the activity of cicatrization drugs (Houghton et al., 2005). We can therefore conclude that HMW chitosan accelerates the wound healing process, probably by stimulating collagen synthesis, as demonstrated in Figure 4.

Anti-inflammatory response

Anti-inflammatory response: Ear edema was used to evaluate the anti-inflammatory effect of chitosans in Swiss adult mice (*Mus musculus*) weighing approximately 30-40g. Croton oil was used as the inflammatory agent and dexametazone as positive control. The edema was measured by subtracting the weight of the ear receiving only acetone (vehicle) by that receiving the irritating agent.



Although HMW chitosan (6), applied after the croton oil, showed a slightly edema inhibition compared to control group (1), this difference was found to be non significant (p>0.05).

Figure 5. Effect of chitosan in ear edema inhibition induced by croton oil; 1-acetone/croton oil, 2-dexamethazone, 3-LMW chitosan, 4-HMW chitosan (1st chitosan, 2nd oil), 5-Primex chitosan and 6-HMW chitosan (1st oil, 2nd chitosan)

Anti-ulcerative activity

Anti-ulcerative activity: Male winstar rats weighing 200-300 g each and fasted for 24 h prior to each experiment were divided into groups (n=5) according to the treatment employed (control animals received 10 mL saline solution/kg body wt.; positive control animals received carbenoxolone 200 mg/kg body wt.; and treatment animals received 1000 mg chitosan (3 types were tested)/kg body wt.). After 30 min of oral treatment each animal received 1 mL of ethanol orally. After 1 h, the animals were sacrificed by cervical displacement and their stomach removed and opened along the greater curvature. The ulcerative lesion index was determined according to Gamberini et al. (1991).

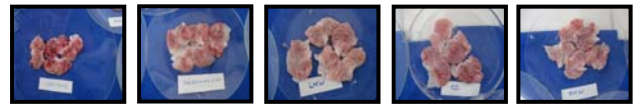


Figure 6. Internal view of the rats' stomachs after opening.

Although LMW chitosan treatment was the most effective in reducing ulcerative wounds induced by ethanol, with HMW chitosan treatment the stomachs had retained the folds rendering a gastroprotective effect. These results suggest that chitosan can potentially be used in sucessful treatment of peptic ulcers.

Conclusions

The '*in vitro*' cytotoxic activity of chitosan was found to be minimal, whereas it showed wound healing ability. The ear edema assay showed no differences among the croton oil group and chitosan groups, demonstrating no anti-inflammatory activity. High molecular weight chitosan seems to render gut protection, but the low molecular weight is the best anti-ulcerative compound.

References

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