

LABELLING OF SUCRALPHATE USING TECHNETIUM LABELLED BOVINE SERUM ALBUMIN- AN ALTERNATE TO Tc99m-HSA.

Pages with reference to book, From 241 To 245

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Abstract

Technetium labelled Sucralphate has been used as an ulcer scanning agent in the gastrointestinal tract. The labelling described in the literature involves incubation of Tc99m-HSA (Human serum albumin) with Sucralphate. Due to difficulty in obtaining HSA we used bovine serum albumin (BSA) to tag radioactivity with Sucralphate.

The radioactive yields (Tagged Sucraiphate and Tc99m-BSA) obtained after purification were more than 99% pure and stable for several hours. Animal and human trials showed good localisation of the agent in ulcer bed (JPMA 38: 241, 1988).

INTRODUcTION

Sucraiphate, a basic aluminium salt of sucrose octasuiphate¹⁻² a cytoprotective agent is used for the treatment of gastric and duodenal ulcer.

When taken orally it coats ulcerated areas due to strong electrostatic action between sucralphate poly anions and proteins concentrated in ulcerated mucosa, which are positively charged. it thus provides a protective covering against acids enzymes and other irritants. This cytoprotective barrier action lasts for as long as 6 hours²⁻³

Technetium labelled sucraiphate may be used to visualise gastrointestinal ulcers⁴. Labelling involves mixing of the suspension of the agent in 0. IN HCl (pH4) with DTPA, HSA or fibrinogen already labelled with Tc99m or lyophilising a suspension of sucraiphate — DTPA — SnCl₂ sucralphate— HSA--SnCl₂, Sucralphate-fibrinogen SnCl₂ with a saline solution of 99m TcO₄⁵. Tc-99m-sucralphate-HSA — SnCl₂ prepared using Tc99m—HSA has highest affinities for ulcerated areas and has been used by others⁴⁻⁶⁻⁷,^{6 7} Due to non-availability of Tc99m—HSA we labelled bovine serum albumin (BSA) with Tc99m and used it to tag radioactivity to the drug. Methodology, studies on normal human subjects and initial clinical data is presented.

MATERIAL AND METHODS

1. Labelling of Sucraiphate in Vitro

Tc99m-HSA was prepared by first reducing 5-10mCi (200-400 MBq) pertechnetate (TcO₄) with stannous chloride in acidic medium and then adding this mixture to 0.1% solution of BSA. The pH was then adjusted to 6 with dilute NaOH. The unreacted radioactivity was removed by passing the mixture through Dowax-1 anion exchange resin (Tc99m-BSA appears in eluate and free 99m leO₄ is retained by the resin)⁸.

200 MBq of Tc99m-BSA was added to 0.6 g powdered sucraiphate suspended in 0. IN HCl (pH4). The mixture was shaken, and then centrifuged for ten minutes. The supernatant was removed, measured and discarded. The residue was resuspended in 5ml of HCl (pH4) and centrifuged, the supernatant

discarded. The same procedure of washing was repeated twice and final yield of tagged sucralphate was measured.

Stability and Radiochemical purity of Tc99m-BSA was checked by paper chromatography (Whatman No. 1, Acetone 90%: Water 10%). For checking stability, radiochromatograms of final yield of Tc99m-BSA were obtained at different hours after preparation. Radiochemical purity of final yield of labelled sucraiphate was also checked by paper chromatography. The stability of tagged drug was checked by suspending the drug in HCl solution at low pH (approximately 1) and incubating the mixture in different tubes at 37°C for different time intervals. After each incubation the mixture was centrifuged and washed. Activity in the residue and supernatant was measured to assess bound and floating fractions.

The labelling yield of Tc99m-BSA obtained after purification by anion exchange chromatography was about 90% ($X = 89.4$ SD = 0.42 n = 10), whereas about 10% ($X = 10.17$ SD = 0.18 n = 10) activity was retained by the resin. Chromatographic results with purified Tc99m—BSA showed more than 99% of the label bound to the albumin ($X_f - 0.65$ SD = 0.02 n = 10) (Figure 1).

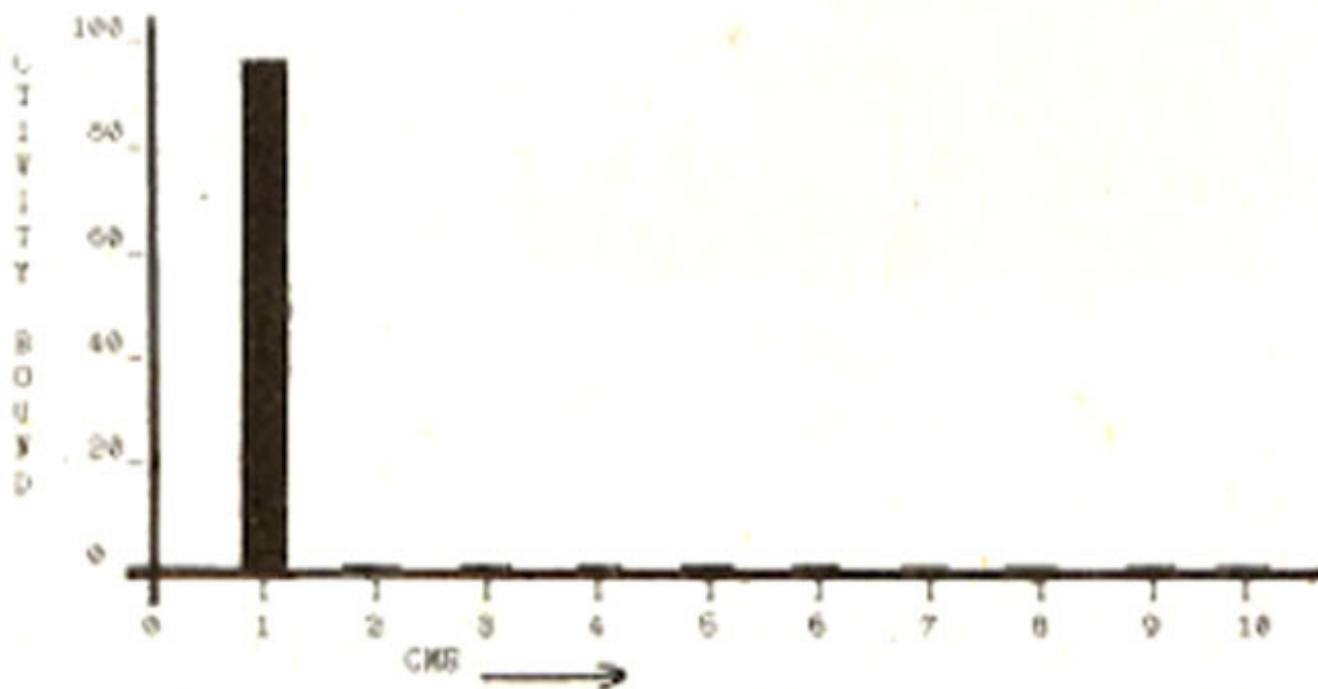


Figure 1. Radiochromatogram of Tc-99m labelled BSA obtained by paper chromatography in Acetone/Water system.

Labelled compound was found stable until 5 hours without significant detachment of the radioactivity from radio yield (Figure 2 and Table. 1).

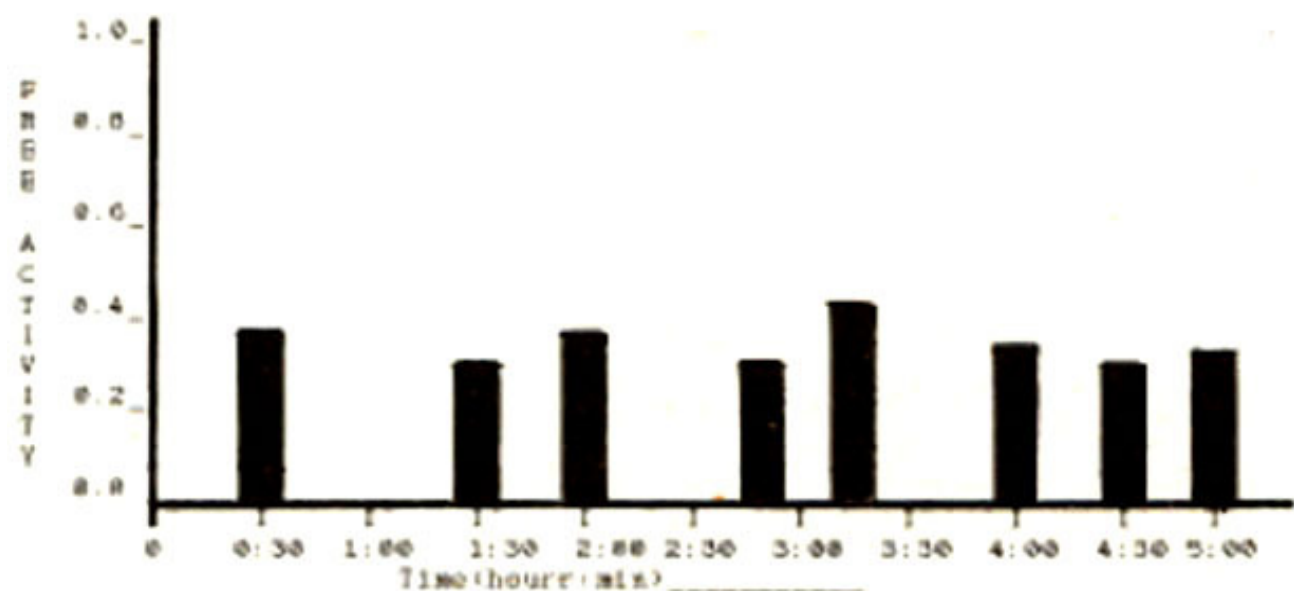


Figure 2. Percentage Free activities vs Time after preparation of Tc99m-BSA, showing no significant change in free activity upto 5 hours.

TABLE -I. Bound activity determined at various Time Intervals (at and after preparation) using Paper Chromatography to find the stability of Tc-99m labelled BSA (purified Radiochemically).

Sr. No.	Time (Hr:Min)	* Bound Activity (%)
1.	0:00	99.30
2.	0:30	99.83
3.	1:30	99.67
4.	2:45	99.65
5.	3:15	99.50
6.	4:00	99.93
7.	4:30	99.65
8.	5:00	99.61

* Percentage of the total added activity.

Radiochemical purity of tagged sucraiphate checked by washing procedure showed 98% of the added activity bound to the drug (\bar{X} 98.08 SD = 1.15 n 11), whereas paper chromatography showed more than 99% of the activity bound to the drug (Figure 3).



Figure 3. Radiochromatogram of Tc-99m-sucralphate-BSA-SnCl₂ obtained by paper chromatography in Acetone/Water system.

The tagged drug was found stable in low pH medium at 37°C with practically no floating activity.

Results of washing the sucraiphate at different time intervals are

Animal studies using labelled sucraiphate(i) Normal Rabbits Two normal domestic rabbits were kept without food and water for 48 hours, followed by administration of 1 mCi (37MBq) of Tc99m-sucraiphate orally by a gastric tube. Serial analogue images were taken for upto 5 hours using a gamma camera. One picture was taken at 24 hours.

Animals were kept in specially designed portable cages with holes at the bottom for delivery of faeces. This was done to prevent the reingestion of faeces causing overestimation of stomach activity at 24 hours.

(ii) Rabbits with induced ulceration.

Two rabbits kept without food and water for 48 hours received 600mg acetylesalicylic acid orally by a gastric tube. Eight hours later they received 1mCi (MBq) of tagged sucraiphate. Serial analogue pictures were taken for 5 hours. One picture was taken at 24 hours.

RESULTS

Animal Studies Normal Rabbits:

No area of focally increased activity in any of the rabbits was seen during the entire study. Clearance of radioactivity was however slow and diffuse images of stomach were obtained even after 3 hours of administration of labelled agent. No activity was detected outside GIT until 4 hours of study. Slight radioactivity in the thyroid was seen after 4 hours. Activity was almost completely moved from stomach at 24 hours into the lower gut which was visualized.



Figure 4.



Figure 5.

Figures 4 & 5. Rabbits with induced ulcer. Focal areas of persistent activity (arrows) after stomach has cleared :----- 4 hr image.

Figures 4 & 5. Rabbits with induced ulcer. Focal areas of persistent activity (arrows) after stomach has cleared TABLE II.

TABLE- II. Bound activities of Tagged Sucralphate determined at various Time Intervals (at and after preparation) using washing procedure to find the stability of the Radiopharmaceutical.

Sr.No.	Time (Hr:Min)	* Bound Activity (%)
1.	0:00	98.36
2.	0:30	98.95
3.	1:00	97.52
4.	1:30	98.30
5.	2:00	99.20
6.	2:45	99.50
7.	3:00	97.97
8.	3:30	98.09
9.	4:00	98.07
10.	4:30	98.68
11.	5.00	97.50

***Percentage of the total added activity.**

Bound activities of Tagged Sucraiphate determined at various Time Intervals (at and after preparation) using washing procedure to fmd the stability of the Radiopharmaceutical.

2. Human Studies

Two males under 35 years of age with no history of GI disease volunteered for normal studies. After an overnight fast, they were given 0.5g of sucraiphate labelled with 3mCi of Tc99m-BSA. This was suspended in 200ml of tap water and taken orally. 10 mg of metoclopramide syrup and a large drink of plain water was given after 30 minutes. This was done to hasten gastric emptying. Another large drink of plain water was given after one hour to wash residual activity from the stomach and duodenum. Anterior, right lateral, left lateral and posterior views were taken at 0, 0.5, 1, 1.5,2,2.5,3,4,5,24 and 48 hours respectively. The stomach, the small gut, the large gut were identified at 0 - 0.5, 2.5 and 3.5 hours respectively (Figure 6).



Figure 6 (a)



Figure 6 (d)

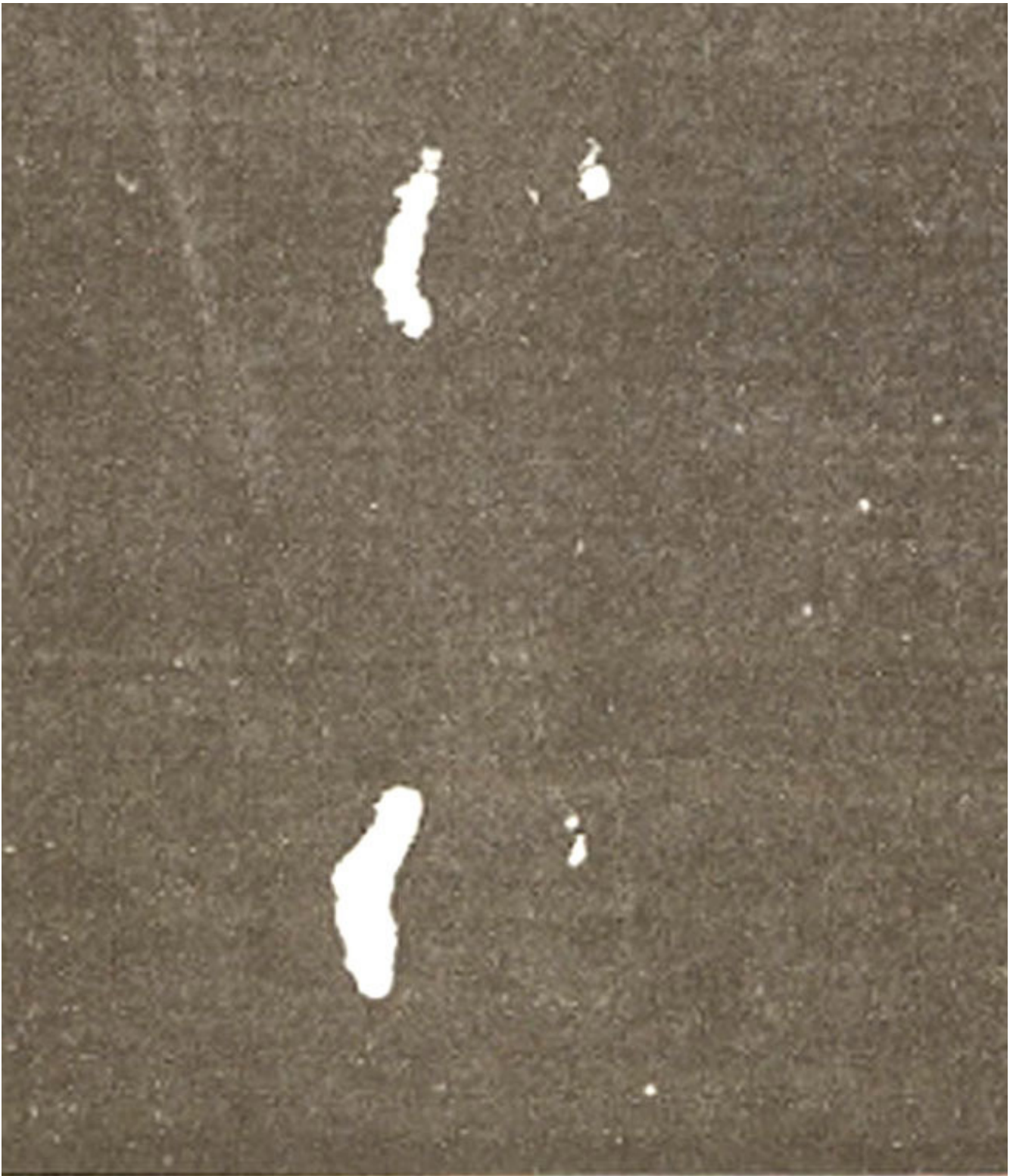


Figure 6 (c)

There was very little activity left in the stomach at 1 .5 hours. There was diffuse activity in the colon and rectum at 24 hours and no measurable activity at 48 hours.

Clinical trials were done in 37 cases of suspected peptic ulcer. All of them underwent either an upper G.I endoscopy⁹ (21 cases) or barium meal (5 cases) or both (11 cases). Eight cases were negative on

endoscopy or barium. Of 29 positive cases, 18 showed positive isotopic scanning, 9 were false negative and 2 scans were un-interpretable. On statistical analysis, the specificity of the test was 100%, sensitivity 76.3% and accuracy 80.4%.

DISCUSSION

Sucraiphate ulcer scanning has a good to excellent correlation with endoscopy for upper and lower GI ulceration. The method of preparation is relatively easy and our experience with this compound has been very satisfying. There were initial difficulties in obtaining HSA in sufficient quantities from the local market therefore BSA was used instead of HSA. The radiochemical tests and early clinical experiences are convincing that the BSA linked compound is as effective as the frequently reported HSA linked agent. Studies in endoscopically confirmed cases of peptic ulcer showed good correlation of focally increased activity with abnormal areas reported on endoscopy⁹. Similar findings were observed in the present study.

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