Effect of Isonicotinic Acid Hydrazide and Polyvinylpyrrolidone
On Experimental Peritoneal Adhesions in the Rabbit

By
A. H. SAID, A. H. SHANAIN and TALIB A. MAKKAWI

With one tables

(Received for publication January 16, 1973)

The question of persistent fibrous adhesions which may develop after an abdominal operation presents an important and as yet incompletely solved problem. It is apparent from a review of the literature that up to the present time only two means of prophylaxis have gained general acceptance: (1) Reducing to the minimum the initial peritoneal injury at the time of the operation and (2) Preventing microorganisms from gaining entrance to the peritoneal cavity. It is clear that these ideals cannot be fully realized in practice and so the need for an additional method of prophylaxis remains urgent.

In the past, work had been carried out on physical methods of preventing adhesions by using increased intestinal motility, intraperitoneal instillation of air, grafting and oversewing. Among other methods which have received attention are: i/p administration of anticoagulants such as heparin (CHANDY, 1950; ZAHIR, 1965) or dicoumarol (WHITE, 1949); and the induction of artificial ascites by the use of polyvinylpyrrolidone (PVP) (SCHULTZE, 1959; KALLIGIANIS, 1961; KALAMBAHETI and PAWAR, 1964; BOSTED and BRUMMER, 1969). The use of the proteolytic enzyme fibrinolysin to help in destroying the fibrinous exudate was advocated by SPAGNA and PESKIN (1963), BENZER, BLUMEL and PIZA (1963) and BRYANT (1963). The effect of hyaluronidase was investigated by SCHLOSSER and STAIB (1960). JACQUemain and SHUMAKER (1962) reported on the effect of antihistaminic drugs. A group of adrenal preparations were tried by BEAMER, FREIBERG and CHU (1953), HASSE and SPETH (1956), DIRECKSEN and HOUSHI (1962, 1963) and BOSTEDT (1968).

Each of the methods previously mentioned has its specific limitations which will be discussed later.

SAID (1960) has shown that isonicotinic acid hydrazide (INH), a drug used in the treatment of human tuberculosis, has an inhibitory effect on the healing of bone fractures in rabbits and rats. The same observation was confirmed by MORCOS (1962) in connection with tendon healing. LEVENE (1961)
and Said and Ahmed (1967) showed what this drug increased the fragility of the tissues of 14 day-old chick embryos. It also increased the amount of neutral salt soluble collagen, indicating that it prevents maturation of new collagen fibres.

Fouad, Houwshi and Hosni (1964) showed that the intraperitoneal administration of streptomycin powder favours the formation of adhesions. Since it is sometimes necessary to apply antibiotics intraperitoneally to guard against infection therefore, it was decided to induce adhesions with streptomycin and see if a combination of INH and streptomycin would change the picture.

The main object was to gain further information about the possibility of preventing peritoneal adhesions by the use of INH and PVP.

Material and Methods

Experimental animals. A total of 55 domestic rabbits ranging in weight between 1.750—2.250 kg. were used. The animals were operated on under light ether anaesthesia.

Operative technique. The skin was shaved and thoroughly washed, swabbed several times with alcohol and then covered with a sterile split towel. The instruments were sterilised but strict aseptic conditions could not be maintained.

The abdomen was opened through a low midline incision about 3 cm. in length. The caecum was withdrawn through the incisions with toothless forceps.

At first one gramme of sterilised talcum powder was used to provoke adhesions but the mortality, even in the control group, was relatively high (during an observation period of one week). When the quantity was reduced to 500 mg. there were no deaths. Only 10—30 mg. of talcum powder were used by Mazuji, Kalambaheiti and Pawar (1964). The powder was applied to the surface of the caecum and the adjacent omentum and intestine. The surfaces were gently rubbed against each other for about 15 seconds and the caecum was subsequently returned to the peritoneal cavity. The peritoneum and overlying muscle were closed with a single row of continuous suture of No. 3—0 chromic catgut. The skin incision was closed with interrupted sutures of No. 2—0 black silk. The operating time was about 10 minutes.

Immediately before the peritoneal suture was pulled taut, the agent under investigation (contained in a volume of 20 ml.) was administered i/p through a blunt cannula, care being taken not to spill any of the liquid outside the abdominal cavity.

The experiment consisted of the following groups:

Group A (Control) consisted of three subdivisions: i. The animals were sham-operated to test the effect of the course of the operation on inducing abdominal adhesions. ii. Talcum powder was applied in the same manner as in the experimental group to see if it would give rise to adhesions in all animals. iii. Animals received in addition to talcum powder 20 ml. of sterile saline i/p to test the effect of the volume of solution injected in the experimental groups on the development of adhesions.

Group B (PVP) was given as 20 ml. of a sterile 4 % PVP solution i/p.

Group C (INH) was given as 20 ml. of 0.5 % INH (100 mg.) i/p, plus 1 ml. 1 % INH (10 mg.) i/m, starting from the 2nd day and continued during the first week.

Group D (INH + PVP) received 10 ml. of 4 % PVP plus 10 ml. of 0.5 % INH instilled i/p at the time of the operation. On the second day, 1 ml.
of 1% INH was administered i/m and continued till the end of the 2nd week.

*Group E* 0.5 g. of streptomycin sulphate replaced the talcum powder.

*Group F* 0.5 g. of streptomycin sulphate was combined with 100 mg. INH and given i/p.

The cutaneous sutures were removed after a week.

The animals were sacrificed at the end of the 4th week following laparotomy.

Five classifications were used in grading the extent of adhesions:

*Grade 0* No adhesions; the entire abdominal cavity and the abdominal wall were free.

*Grade 1* Minimal adhesions; 1—2 strands extending from the caecum to the adjacent mesentery.

*Grade 2* Moderately dense and scattered adhesions which could be easily broken down.

*Grade 3* Moderately dense but diffuse adhesions presenting no difficulty in separating them.

*Grade 4* Dense and diffuse adhesions between the caecum, extending to the adjacent gut, mesentery and abdominal wall and not easily separable.

The effect of the various treatments under investigation on the strength of healing of the cutaneous wounds was tested by removing a square piece of skin (1.5 × 1.5 cm.) with the wound in its centre. The tensile strength of the wounds was assessed by fixing one end of the specimen and applying gradually increasing weights to the other. The breaking point was determined by observing the weight that caused rupture of the repair tissue.

### Results

All abdominal wounds healed without apparent clinical difference in the control and treated groups. There was no dehiscence or disruption except for 3 cases of stitch abscesses which healed favourably after removal of sutures.

There were no deaths in the control animals following the application of 500 mg. talcum powder. This is an important fact in favour of the procedure used; a significant mortality in the controls would make it difficult to evaluate the risk introduced by the therapeutic measures under investigation.

The intraperitoneal injection of 20 ml. saline did not affect the incidence of development of adhesions, indicating that the volume of solutions used was well tolerated.

Table 1 compares the grades of adhesions in the different groups.

It is remarkable that the operative technique itself was not responsible for the development of any adhesions. On the other hand new accretions were always noticed whenever talcum powder was introduced into the abdominal cavity. Six out of ten control rabbits developed diffuse adhesions, three of which were of the high grade. Moderately dense adhesions were observed in the remaining four animals.

Polyvinylpyrrolidone, when used alone in the concentration and quantity mentioned, did not succeed in preventing the formation of adhesions, but the reaction was milder than in control animals since minimal adhesions were observed in three rabbits, moderate adhesions in five and high grade adhesions in only two.

Isonicotinic acid hydrazide protected three rabbits against the irritant effect of talcum powder. The powder did not disappear completely from the abdominal cavity; being insoluble, its granules became enclosed in small thin-walled granulomas appearing on the serous surface of the caecum and intestine or implanted in the omentum but none of these structures was bound to the
other in any form of new tissue formations. In five rabbits, fibrinous strands which could be easily broken down connected the caecum to the surrounding tissues. In some cases a loop of bowel was coiled over the caecum giving the impression that adhesions were extensive. In fact the adhesion was merely focal and when manually separated the surfaces in between were free from any reaction.

The results were more encouraging when half doses of INH and PVP were combined and administered simultaneously and the injections of INH were continued for two weeks. Four animals developed few, easily separable bands, while moderately dense scattered adhesions were encountered in one animal only. High grade adhesions were entirely absent.

Streptomycin sulphate powder was shown to provoke variable degrees of intra-abdominal reactions in all members of the group. Adding INH to it protected four out of five animals against adhesions and low grade reactions were observed in the 5th rabbit.

The tensile strength of the cutaneous wounds (Table 1) shows that the drugs used had no ill effect on the repair of skin injuries.

**Table 1**

<table>
<thead>
<tr>
<th>Group and treatment</th>
<th>No of animals</th>
<th>Grades of adhesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of adhesions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Group A (control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Sham operation</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>ii. Laparotomy &amp; talcum</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>iii. Laparotomy, talcum &amp; saline</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Group B (PVP)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Group C (INH)</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>D (INH + PVP)</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>E (Streptomycin)</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>F (Streptomycin + INH)</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

± Standard deviation from the mean. No significant differences could be detected in the

**Discussion**

The surgical complications of peritoneal adhesions present a challenging problem from the standpoint of both aetiology and prophylaxis. A review of the literature shows that present methods lack one or the other of the two essential requirements, efficiency and safety. In no instance has the effectiveness of any method been shown conclusively.

There is much discrepancy between the opinions of investigators using the same drug. For example, Mazuji, Kalambaheti and Pawar (1964) achieved good results with the use of PVP, whereas in the hands of Nagata (1964) the same drug failed to control the formation of adhesions. Moreover, the use of different species of animals, different methods of induction of adhesions, types of drugs, their dosage and routes of administration as well as the duration of treatment seem to be largely responsible for the present state of confusion. It is, therefore, hard to make comparisons or draw solid conclusions.

In recent years two preparations, corticosteroids and fibrinolysin, have achieved a good reputation for being able to minimize or even prevent peri-
Effect of Isonicotinic Acid Hydrazide and Polyvinylpyrrolidone

The formation of peritoneal adhesions after the primary trauma than against the reformation of surgically separated adhesions. When the drugs were used in doses large enough to check new tissue formations this led to an increased incidence of infection and ventral hernia. When intestinal anastomosis was performed 15 out of 20 animals died from peritonitis due to deficient healing of the anastomosis. The treated animals also showed an increased tendency to haemorrhage, probably due to reduced fibrin formation. When oxytetracycline was given conjointly with hydrocortisone acetate, the incidence of coligranulomatosis could be reduced after the mechanical injury to the caecum of albino rats (Thomasbeek, 1958). The simultaneous use of broad spectrum antibiotics and corticosteroids adds to the already high expenses of cortisone therapy.

Spagna and Peskin (1961) demonstrated unequivocally that fibrinolysins given i/p diminished or prevented adhesions. If infection was present fibrinolysin should be combined with chlortetracycline to eliminate this hazard. Benzzer, Blumel and Piza (1963) warned that the enzyme should exert its action only locally, otherwise an all-round increase of fibrinolytic activity would run the risk of producing haemorrhage or would interrupt the process of wound healing. Bryant (1963) reported that the enzyme was capable of reducing post-operative peritoneal adhesions in dogs but failed to prevent them completely.

Roberts (1971) stated that INH has been employed in the treatment of non-tuberculous diseases in man, such as actinomycosis. Roberts further added that the exact mechanism of the drug was unknown, but it is probably an antimetabolite.

The catabolic effect of INH has been proved by the work of Said (1960), Levene (1961), Morcos (1962) and Said and Ahmed (1967). The promise of this drug led us to investigate its action on the prevention of peritoneal adhesions. The results in Table 1 indicate that it possesses an inhibitory effect on the development of new tissue formation after i/p application of talcum powder.

Table 1: Grades of adhesions in different groups of rabbits at four weeks following laparotomy

<table>
<thead>
<tr>
<th>Grades of adhesions</th>
<th>Tensile strength in kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>moderately dense and scattered</td>
<td>2</td>
</tr>
<tr>
<td>moderately dense and diffuse</td>
<td>3</td>
</tr>
<tr>
<td>dense and diffuse</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Tensile strength of the skin wounds of various animal groups at 95% confidence level.
powder. When used alone, the drug was able to protect 30% of the animals against adhesions. Minimal reactions appeared in half of the rabbits used, and moderately dense adhesions in the remaining 20%. Better still were the findings that a combination of INH and PVP could protect 50% of the animals against adhesions. PVP on its own helped to reduce the intensity of adhesions but did not completely prevent their formation. PVP is believed to cause temporary artificial ascites. Thus, it dilutes the exudate, lubricates the intestinal serosa and possibly binds histamine-like tissue substances leading to low absorption and a favourable influence on coagulation of the exudate (Mazuji et al., 1964; Kalligianis, 1961). Schultze (1959), reporting on eight and a half years experience in man and in animal experiments, mentioned that PVP should not be used in peritonitis even when the infection is only local. On the other hand, INH is known for its antimicrobial effect (Gibbons, Catcoll and Smithcors, 1970).

The answer to the question why a combination of INH and PVP was more effective than either substance independently cannot be deduced from the results of the present investigation on a limited number of animals. It may be that PVP acts as a dispersing vehicle producing a uniform local distribution of INH.

Further support for the present results is given by the finding that whereas streptomycin sulphate powder applied i/p caused adhesions in all animals, those receiving a combination of streptomycin and INH were free from any undesirable reaction except for one with weak fibrinous bands.

The variability in results among animals given the same treatment indicates that the infliction of a standardized trauma could not be completely achieved. Moreover, individual variation in reaction to external stimuli and the possible presence of a varying incidence of low grade infection should be taken into consideration.

The application of sterile talcum powder i/p resulted in adhesions consisting of numerous granulomata unlike the adhesions ordinarily seen after certain cases of abdominal surgery.

The results of tensile strength measurements reported in Table 1 support the view that INH and PVP do not interfere with wound healing in contrast to the known (Howes et al., 1950) inhibitory effect of corticosteroids on the process of tissue repair.

It is not claimed that the problem of prevention of peritoneal adhesions has been totally solved. Attention is merely directed to a drug that deserves further consideration in view of the fact that it is safe and cheap. It is hoped that this work may stimulate interest and that INH will be tried in other species of animals.

Work is in progress to test the effect of INH on re-formation of peritoneal adhesions after their lysis and on the healing of wounds after intestinal resection and establishment of anastomosis.

**Summary**

A review of the literature shows that an effective and safe drug for the prevention of peritoneal adhesions is still lacking.

The use of isonicotinic acid hydrazide (INH) was tried because of its proven effect in preventing the synthesis of new collagen fibres in the developing chick embryos. Polyvinylpyrrolidene (PVP) was used for comparison.

Peritoneal adhesions were induced in rabbits by the use of sterile talcum powder. Adhesions were prevented in 3 out of 10 animals, and low to moderate grade reactions were observed in the other 7 when 100 mg. INH were
administered i/p, followed by the intramuscular injection of 10 mg. INH for a week. Better results were obtained when 50 mg. INH, combined with 10 ml. 4% PVP, were instilled into the abdominal cavity and systemic injections of INH were continued for two weeks. PVP alone did not completely protect against adhesions.

Further evidence of the effect of INH was the finding that i/p application of 500 mg. streptomycin sulphate powder initiated new tissue formations which could be completely revented in 4 out of 5 rabbits when combined with INH, while only weak fibrinous adhesions were detected in the 5th animal.

INH in the doses used had no ill-effect on the healing of the cutaneous wounds over a period of 4 weeks as shown by normal tensile strength at the site of incision.

The results suggest that INH may prove of value in human and animal abdominal surgery but further work is needed.

Zusammenfassung

Der Einfluß von Isonikotinsäure-Hydrazid und Polyvinylpyrrolidon auf experimentell erzeugte Peritonealadhäsionen bei Kaninchen


Peritonealadhäsionen wurden durch steriles Talkumpulver erzeugt. Bei einer Dosierung von 100 mg INH i. p. gefolgt von täglichen intramuskulären Injektionen von 10 mg INH während einer Woche wurden bei 3 von 10 Tieren Adhäsionen verhindert, während sich dieselben bei den 7 anderen Tieren nur gering bis mittelgradig entwickelten. Bessere Resultate wurden mit folgender Methodik erzielt: Kombinierte Instillation von 50 mg INH und 10 ml 4% PVP in die Bauchhöhle und tägliche Injektionen von 1 ml 1% INH i. m. während zwei Wochen. PVP allein vermochte Adhäsionen nicht vollständig zu unterdrücken. Die Verabreichung von 500 mg Streptomycinsulfat in Pulverform i. p. induzierte Gewebsneubildungen. Diese konnten bei 4 von 5 Kaninchen mittels INH vollständig verhüet werden, während beim 5. Tier sich nur leichte fibrinöse Adhäsionen entwickelten.

INH hatte keine nachteiligen Effekte auf die Heilung der Hautwunden. INH dürfte somit in der Bauchchirurgie bei Mensch und Tier wertvoll sein. Weitere Untersuchungen sind jedoch notwendig.

Résumé

Influence de l'acide isonicotinique-hydrazide et du polyvinylpyrrolidon sur des adhérences du péritoine induites expérimentalement chez le lapin

On n'a pas trouvé jusqu'à ce jour un moyen sûr pour éviter des adhérences péritonéales.

Étant donné que l'acide isonicotinique-hydrazide (INH) inhibe la synthèse de nouvelles fibres collagènes chez l'embryon de poulet, on a choisi de tester cette préparation. On a pris le polyvinylpyrrolidon (PVP) comme élément de comparaison. Les adhérences péritonéales ont été induites au moyen de talc stérile. Une dose de 100 mg INH i/p suivie d'injections journalières i/m de 10 mg INH pendant une semaine ont empêché des adhérences chez 3 animaux sur 10, alors que de faibles à moyennes adhérences se sont développées chez les 7 autres animaux. On a obtenu de meilleurs résultats de la manière suivante une instillation combinée de 50 mg INH et 10 ml
de PVP à 4 % dans la cavité abdominale et des injections i/m de 1 ml de INH à 1 % pendant deux semaines. PVP seul n’a pas permis d’empêcher complètement des adhérences. L’application de 500 mg de sulfate de streptomycine en poudre i/p a provoqué des néoformations tissulaires; ces dernières furent complètement inhibées chez 4 lapins sur 5 au moyen de INH alors que seules de légères adhérences fibrineuses se développèrent chez le 5e lapin. INH n’a pas eu d’effets contre-indiqués sur la guérison des plaies cutanées. INH devrait être valable aussi bien en chirurgie abdominale humaine qu’animal. Une poursuite des recherches s’avère nécessaire.

**Resumen**

Efecto de la hidrazida del ácido isonicotínico y polivinilpirrolidona sobre las adhesiones peritoneales producidas experimentalmente en conejos

Hasta hoy no se conoce un remedio seguro para prevenir las adherencias peritoneales.

Puesto que la hidrazida del ácido isonicotínico (HAI) impide en el embrión de pollo la síntesis de nuevas fibras colágenas, se contrastó la especialidad mencionada. Como fármaco comparativo se eligió la polivinilpirrolidona (PVP).

En conejos se indujeron adherencias peritoneales usando polvo estéril de talco. Con la dosificación de 100 mg HAI i. p. seguida de inyecciones intramusculares diarias de 10 mg HAI durante una semana se impidieron las formaciones de adherencias en 3 de 10 animales, mientras que las mismas solo de desarrollaron en los otros 7 animales de forma escasa hasta media. Se alcanzaron resultados mejores con la técnica siguiente: instilación combinada de 50 mg HAI y 10 ml PVP al 4 % en la cavidad peritoneal e inyecciones diarias i. m. de 1 ml HAI al 1 % durante dos semanas. La PVP sola no era capaz de proteger por completo contra las adherencias. La administración i. p. de 500 mg de sulfato de estreptomicina indujo neoformaciones fistulares. Estas se podían impedir en su totalidad en 4 de 5 conejos por medio de HAI, mientras que en el 5º animal solo se desarrollaban adherencias fibrinosas ligeras.

La HAI no ejercía efectos desfavorables sobre la curación de las heridas cutáneas. Por ello, la HAI debe tener cierto valor en la cirugía abdominal del hombre y animales. Sin embargo, se precisa seguir adelante con estos estudios.

**References**


Author's address: Prof. Dr. A. H. Said, Department of Veterinary Surgery, Faculty of Veterinary Medicine, Giza, Egypt, Dr. A. H. Shanain and Dr. Talib A. Makkawi, Departments of Veterinary Surgery and Veterinary Pathology, Faculty of Veterinary Medicine, Abu-Ghraib, Iraq.