

Effects of intraperitoneal injection of phenol, glycerin and acetic acid on neoplastic ascitis in guinea pigs¹

Efeitos da injeção intraperitoneal de fenol, glicerina e ácido acético na ascite neoplásica em cobaias

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ABSTRACT

Purpose: To investigate the histolytic action of a solution composed by phenol, glycerin and acetic acid on neoplastic ascitis in guinea pigs. **Methods:** Thirty-two guinea pigs were used. The animals were randomly distributed in experimental and control groups, and the effects of the peritoneal injection of the testing solution were studied. Saline solution was used for the control groups. Biochemical and anatomopathological (heart, lungs, kidneys, spleen and peritoneal serous membrane) were evaluated at 24 hours and 4 weeks of development. **Results:** It was observed that solution E, when infused into the peritoneal cavity, caused no clinical, histological or laboratory alterations in these animals when compared to those in the control group. **Conclusion:** Given our results, it would be interesting to study the effects of the proposed solution on cases with experimental neoplastic ascites with a later view to treating it in humans. **Key words:** Phenol. Acetic Acid. Glycerol. Peritoneum. Ascites. Guinea Pigs.

RESUMO

Objetivo: Investigar a ação histolítica de uma solução composta de fenol, glicerina e ácido acético na ascite neoplásica em cobaias. **Métodos:** Foram utilizadas 32 cobaias, distribuídas por sorteio, em grupos experimentais e controles e estudados os efeitos da injeção peritoneal da solução teste. Nos grupos controles empregou-se solução fisiológica. Foram estudadas alterações bioquímicas, anatomopatológicas (coração, pulmões, rins, baço e serosa peritoneal), com 24 horas e 4 semanas de evolução. **Resultados:** Verificou-se que a solução E quando instilada na cavidade peritoneal não provocou nenhuma alteração clínica, histológica ou laboratorial nestes animais, quando comparados com o grupo controle. **Conclusão:** Frente aos resultados obtidos, consideramos interessante estudar os efeitos da solução proposta em casos de ascite neoplásica experimental em animais, com posterior estudo em seres humanos. **Descritores:** Fenol. Ácido Acético. Glicerol. Peritônio. Ascite. Cobaias.

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Introduction

It is estimated that 10% of all ascitis cases are of neoplastic origin, and most of such cases result from gastrointestinal and ovarian tumors.

As regards treatment, the major therapeutic modalities elected are paracentesis, use of diuretics and systemic chemotherapy, which has been progressively replaced by the intraperitoneal route. The results, however, are generally unsatisfactory.¹⁻⁶

Hence, various substances have been studied (radioactive colloids, immunostimulators, matrix metalloprotease inhibitors and new chemotherapeutic drugs) with or without hyperthermia, which shows the importance of the development of new methods in order to face the challenge of treating neoplastic ascitis.¹⁻⁹

A literature review indicated that the infusion of a

solution composed of phenol, glycerin, and glacial acetic acid into the prostate of dogs led to reduction in that organ's volume¹⁰, which resulted from tissue necrosis.

The use of the solution was experimentally assayed in Walker's tumor hepatic metastases, leading to suggestive results of necrosis production without alterations in the clinical development of the tumor¹¹.

Hence, experimentally investigating the possibility of using such substance (henceforth referred to as solution E) in the destruction of suspension neoplastic cells (neoplastic ascitis) is considered to be a worthwhile undertaking.

Therefore, the idea of initially studying the effects of such solution on the peritoneum of normal animals has been considered to be opportune with the purpose to seek answers to the following questions:

- Is/does the presence of solution E in the peritoneal cavity
1. followed by clinical alterations? Does mortality occur?
 2. cause biochemical alterations?
 3. lead to the onset of lesions in other organs?

Hence, this study aimed at seeking answers to the formulated questions with the purpose to support the proposal of applicability of that method in the treatment of experimental neoplastic ascitis.

Methods

Thirty-two male and female guinea pigs weighing 150 to 450g were used.

Prior to each experimental time, the guinea pigs were kept in fast for 8 hours with water provision *ad libitum*.

Solution E was injected into the animals' peritoneum and euthanasia occurred after 24 hours (group 1) or 4 weeks (group 2). Saline solution was injected into the animals comprising the control group and their euthanasia took place after 24 hours (group 3) or 4 weeks (group 4).

Eight animals were used in each group, and euthanasia was performed with a lethal dose of anesthetic.

The sclerosing solution was composed of phenol (0.6g), glycerin (1.2g) and glacial acetic acid (0.6g) in distilled-water solution (28.0ml), denominated solution E. Based on a pilot experiment, the use of 0.5ml of the solution was selected. The same volume of saline solution (F) was used for the control groups.

A dose of nembutal, 33mg/kg of weight, was intrapleurally administered.

Median laparotomy was performed in the distal third of the abdomen with 2cm in extension for peritoneal infusion. Injection was accomplished by using a 5-ml syringe coupled to a number-22 butterfly.

Biochemical dosing was performed after the collection of 5ml of blood by heart puncture. Blood was collected immediately prior to anesthesia and euthanasia.

The methodology used was as follows: glycemia - GOP-PAP test - Automation - ABRA - 100 - Reactoclinic Kit; alkaline phosphatase - (Alk. F). Spectrophotometer - Kinetic UV test - Automation - ABRA - 100 - Reactoclinic kit; bilirubins (Bd, Bi, Bt). MALLOY-EVELYN method - Manual - the laboratory's own kit; glutamic oxalacetic transaminase (GOT) - Kinetic UV test - Automation - ABRA - 100 - Reactoclinic Kit; glutamic pyruvic transaminase (GPT) - Kinetic UV test - Automation - ABRA - 100 - Kit and gamma glutamyl transferase (Gamma GT) - Kinetic UV - Automation - ABRA - 100 - Reactoclinic Kit.

After euthanasia using a lethal dose of anesthetic, the animal's abdomen was opened in the median line, which was followed by thorax opening with resection of the costal arches at 2cm from the median line and continuing to the neck

Next, smear from the peritoneal and thoracic cavity fluid was prepared and macroscopic examination of the viscera was performed by removing the liver, spleen, kidneys (with the adrenal glands) lungs, heart and fragments of the peritoneal membrane.

Slides were prepared from each organ and stained by hematoxylin for histopathological examination. Once standardization of slide reading was performed, examination was carried out without previous knowledge of the group to which the slides belonged.

For the descriptive analysis of laboratory dosing, the mean, standard deviation and amplitude (smallest and largest value) were calculated.

Results

Biochemical dosing

All the dosage amounts for glycemia, gamma-t, alkaline phosphatase, bilirubins, aspartate aminotransferase and alanine aminotransferase were within reference values, thus showing no statistically significant difference in any of the dosages at the different studied moments.

When the individual values for each animal in the experimental group were compared, it was not possible to find alterations pointing to group tendency. Also, no alterations were found between the groups receiving solution E and those receiving saline solution (F).

Clinical alterations

During the infusion of both solution E and saline solution into the peritoneal cavity of all animals, no topical alterations or vital signs alterations were observed. Neither were any abnormal alterations noted after 24 hours or after 4 weeks

Anatomopathological examination

During macroscopic examination, no noteworthy alterations were observed in any of the groups as regards the heart, lungs, kidneys, spleen or peritoneal serous membrane.

There were few, discreet lesions in the examined organs, most of which were non-specific and could be found in both groups: animals receiving solution E and those receiving saline solution.

No differences were found in the alterations observed in the treated animals as compared to controls.

As regards peritoneum macroscopy of the animals treated with solution E, no differences were found between the animals sacrificed after 24 hours and those sacrificed after 4 weeks.

Discussion

Guinea pigs were used due to the fact that such animals are frequently utilized in experimental studies. As regards anesthesia, since the venous route was not feasible, and considering that ether anesthesia had led to complications as well as that the peritoneal route was not indicated due to occasional interference with the drug being tested, the use of the intrapleural route was standardized with good results. No references to the use of such route in guinea pigs were found in the literature.

A dose of 0.5ml of solution E was standardized for the peritoneal infusion based on a pilot experiment which demonstrated that such volume was compatible with the size of the peritoneal cavity. As regards body weight, the dose corresponded to 1.0 to 1.5 ml/Kg of the animal's weight.

Phenol, one of the solution's components, features antiseptic (shown by Lister in the 19th century) and anesthetic action. Phenol's germicidal and histotoxic action results from protein denaturation.¹²

According to Goodman and Gilman,¹² the solution of phenol in glycerin is less active than the aqueous solution as they report that its oral ingestion may cause mucocutaneous and gastrointestinal corrosion.

In humans, phenol, at a dose of 4g, causes intoxication, and a dose of 15 to 20g may lead to death.⁶ Systemic toxicity⁵ is shown by the transitory stimulation of the CNS, and the carcinogenic potential of phenol is disputable, according to those authors.¹²

Phenol is mainly eliminated by the kidneys, and most of it is excreted in the first 24 hours.¹²

Glacial acetic acid also features bactericidal action when the 5% solution in smaller concentrations is bacteriostatic.¹² In mice, the DL50 dose is 5g/Kg of weight. In humans, a lethal dose is of 15 to 30g of pure acid or 300 ml of 5% solution.¹²

Glycerin also has diuretic action (osmotic diuretics) and is quickly metabolized. The dose for adults is of 1 to 1.5g/Kg of weight. A daily dose must not exceed 120g.¹² In mice, its lethal dose is 31.58g/kg of weight when orally administered or 7.56g/Kg, if it is venously injected. It is known that glycerin has renal vasoconstricting action (in higher doses) and may produce renal insufficiency.

In view of this fact and by taking into account that phenol is also excreted by the kidneys, the histological study of that organ as well as the analysis of the liver, lungs and spleen were performed.

Solution E, containing such components, was empirically used, as previously mentioned, for prostatic patients in the early 21st century in India¹. Since then, it has been used by other authors, but always in the prostate.^{10,13-15}

No other similar studies to ours were found in the literature and neither were references to biochemical dosages in animals noted so as to enable comparison to our findings. We studied the action of this solution in liver of guinea pig and observed that solution produced necrosis limited to the injected area and that hepatic tissue recovery occurred after four weeks with the formation of a small necrosis area¹⁶. No biochemical parameters were altered either in the experimental or in the control group.

Our results showed that the infusion of solution E into the peritoneal cavity of guinea pigs at the used dose did not lead to noteworthy biochemical or histopathological alterations. Differences between the animals receiving the testing solution and those receiving saline solution were not observed.

Conclusion

Given our results, it would be interesting to study the effects of the proposed solution on cases with experimental neoplastic ascites with a later view to treating it in humans.

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Conflict of interest: none
Financial source: none

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Received: March 18, 2008
Review: May 14, 2008
Accepted: June 17, 2008

How to cite this article

Saad-Hossne R, Saad Hossne W, Montenegro MRG. Effects of intraperitoneal injection of phenol, glycerin and acetic acid on neoplastic ascitis in guinea pigs. *Acta Cir Bras.* [serial on the Internet] 2008 Nov-Dec;23(6). Available from URL: <http://www.scielo.br/acb>