Effects of phenol, glycerin and acetic acid on the liver of guinea pigs[1]

Efeitos da soluçao de fenol, glicerina e ácido acético em figado de cobaias

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ABSTRACT

Purpose: To investigate the histolytic action of a solution composed of phenol, glycerin and acetic acid for irresectable hepatic metastasis. **Methods**: Thirty-two (n=32) guinea pigs were randomly distributed into two groups of 16 animals. The animals in group 1 (experimental) and group 2 (control) were redistributed in two subgroups of eight animals each, according to the day of sacrifice (24 hours and four weeks after injection). All the animals were submitted to median laparotomy, which was followed by the injection of solution E and saline into the livers of subjects in both the experimental and control groups, respectively. The animals were evaluated for biochemical and anatomopathological (liver) alterations after 24 hours and four weeks of the experiment. **Results**: It was observed that solution E produced necrosis limited to the injected area and that hepatic tissue recovery occurred after four weeks with the formation of a small necrosis area. No biochemical parameters were altered either in the experimental or in the control group. **Conclusion**: In view of the obtained results, the possibility of using the proposed solution can be considered in cases of irresectable metastasis.

Key words: Acetic Acid. Phenol. Glycerol. Liver. Guinea pigs.

RESUMO

Objetivo: Investigar a ação histolítica da solução composta de fenol, glicerina e ácido acético para os casos de metástases hepáticas não ressecáveis. **Métodos**: Foram utilizadas 32 cobaias, distribuídas, por sorteio, em quatro grupos: experimental (24 horas e quatro semanas) e controle (24 horas e quatro semanas); todos os animais foram submetidos a laparotomia mediana e realizada a injeção da solução E (grupo experimental) ou solução fisiológica (grupo controle). Foram estudadas as alterações bioquímicas e anatomopatológicas (fígado) com 24 horas e quatro semanas de evolução. **Resultados**: Verificou-se que a solução E produz necrose delimitada à área infiltrada apos 24 horas e que ao final de quatro semanas ocorreu regeneração do tecido hepático com formação de discreta área de fibrose. Não foram observadas quaisquer alterações bioquímicas tanto no grupo experimental como controle. **Conclusão**: Frente aos resultados obtidos, é válido considerar-se a possibilidade do emprego da solução proposta, nos casos de metástases hepáticas não ressecáveis.

Descritores: Acido acético. Fenol. Glicerol. Fígado. Cobaias.

Introduction

During surgical procedures, surgeons frequently face limited possibilities of therapeutic action due to the infeasibility of neoplastic tissue total resection, such as when various visceral metastases, and hepatic metastases in particular, exist[1,2]. The major therapeutic modalitites presently used in the treatment of hepatic metastases are: surgical resection, artery ligation, embolization and chemotherapy, ablation methods (laser, radiofrequency, cryotherapy, hyperthermia, microwaves, and necrotizing and cytolytic substances) and genetic therapy[3,4]. To this date, surgical resection, the major therapeutic modality, is the only procedure that may result in cure. Nevertheless the number of patients capable of undergoing surgery (10–15%) is low[2]. Among palliative methods, the ablative approach is distinguished as it uses cytolytic and necrotizing substances such as alcohol or other methods, namely, cryotherapy, radiofrequency, laser and microwaves for localized lesion destruction. These methods are most frequently applied in irresectable cases[5]. It was in this setting (destruction of irresectable metastases) that our line of investigation was developed. The basic idea was to try to destroy tumoral tissue by the use of solutions or substances with

cytolytic or sclerosing action. Through literature review, it was verified that the injection of a solution composed of phenol, glycerin and glacial acetic acid into the prostate of dogs led to the reduction of that organ's volume, and that such reduction would be due to tissue necrosis[6]. 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 tumor[7,8]. In face of such characteristic difficulties posed by hepatic metastasis therapy, new treatments must be developed with the purpose to achieve good efficacy rates, low cost, low frequency of side effects and easy execution. Hence, experimentally investigating the possibility of using such substance (henceforth referred to as solution E) in the destruction of irresectable hepatic metastases is considered to be a worthwhile undertaking.

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' livers and sacrifice occurred after 24 hours (group 1) or 4 weeks (group 2). Saline solution was injected into the animals comprising the control group and their sacrifice took place after 24 hours (group 3) or 4 weeks (group 4). Eight animals were used in each group, and euthanasia was performed by 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 with 3 to 4cm, beginning right under the xiphoid appendix, was performed for hepatic injection. Injection was always accomplished into the upper left lobe using an insulin-type syringe with a 13x4 needle. 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 - Reactoclin kit; bilirubins (Bd, Bi, Bt). MALLOY-EVELYN method - Manual – the laboratory's own kit; glutamic oxalacetic transminase (GOT) - Kinetic UV test - Automation - ABRA - 100 - Reactoclìnic 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 sacrifice, the animal's abdomen was opened in the median line; the liver was macroscopically examined, and slides were prepared and stained by hematoxilin for histopathological examination. Slide reading was performed without previous knowledge of the group to which they belonged.

Results

Chemical dosage

All the dosage amounts for glycemia, gamma-t, alkaline phosphatase, bilirubins, aspartate aminotranspherase and alanine aminotranspherase were within reference values, thus showing no statistically significant difference in any of the dosages at the different studied moments ($p>0.05$). 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 assessment – macroscopic examination

The following phenomena were observed on the occasion of hepatic injection:

- No animals showed alterations in vital signs for and up to 5 minutes after injection of both solution E and saline solution.
- In the case of saline solution, the occurrence of a small pale area was observed around the puncture needle during injection, which was immediately followed by normalization upon completion of the procedure.
- In the case of solution E, a whitish area of approximately 1cm was formed around the infiltration site.

Twenty-four hours later, no abnormal characteristics were observed in the animals receiving saline solution whereas those given solution E showed the presence of a white area of approximately 0.5cm in diameter with necrotic appearance which was well circumscribed and presented no laceration or bleeding. After four weeks, some animals (5) showed a small whitish mark on the site of injection of solution E. No abnormal signs were found in the others.

Histopathology

Small focal areas were found with hepatocyte basophilia on virtually all liver slides from all groups. Although less frequently, points of lymphoid infiltrate were also observed in the portal spaces, which was not observed in the treated animals, however. Additionally, some of the animals presented variable and, in general, low-intensity levels of micro steatosis. The presence of steatosis did not vary whereas the periportal inflammatory infiltrate occurred only after 4 weeks, although with similar frequency in the experimental and control groups,

which showed that such alteration was not related to the experiment, but probably dependent on other factors. Based on the microscopic assessment, only the animals sacrificed after 24 hours and which had been given hepatic infiltration with solution E presented focal-type necrosis (Figure 1). After 4 weeks, only 2 animals were observed to show a fibrosis focal area corresponding to the scar seen under macroscopy. In summary, the solution produced focal liver necrosis which was repaired after 4 weeks and left minimum scars. No other histological alterations that could be attributed to the action of solution E by the sclerosing substance were found.

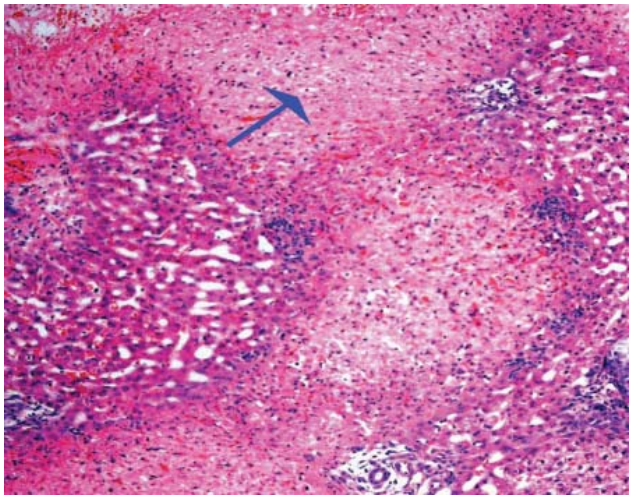

FIGURE1 – Histological section of a guinea pig's liver 24 hours after injection of sclerosing solution (E). Necrosis area (blue arrow). X200.HE staining

TABLE 1 – Biochemical dosages. Absolute values in each animal. Group: Liver infiltration with solution E – 24 hours

MALE	GLYCEMIA mg/dL	ALK. P. U/L	Bd Mg/dL	Bi mg/dL	Bt mg/dL	GOT U/L	GPT U/L	gGt U/L
1	143	56	0.02	0.10	0.12	121	95	17
2	126	73	0.01	0.15	0.16	127	89	19
3	127	97	0.02	0.12	0.14	150	92	15
4	127	69	0.01	0.10	0.11	38	78	14
FEMALE								
5	152	99	0.03	0.10	0.13	106	70	24
6	118	69	0.01	0.10	0.11	102	70	15
7	148	100	0.03	0.15	0.18	90	160	15
8	151	89	0.03	0.13	0.36	76	120	15
Mean	136.5	81.5	0.02	0.12	0.16	101	97	17

TABLE 2 – Biochemical dosages. Absolute values in each animal. Group: liver infiltration with solution E – 4 weeks

MALE	GLYCEMIA mg/dL	ALK. P. U/L	Bd Mg/dL	Bi mg/dL	Bt mg/dL	GOT U/L	GPT U/L	gGt U/L
1	155	69	0.01	0.10	0.11	118	77	15
2	138	73	0.02	0.13	0.15	108	73	19
3	153	59	0.02	0.12	0.14	36	132	15
4	197	92	0.02	0.10	0.12	79	97	11
FEMALE								
5	138	61	0.01	0.10	0.11	113	75	16
6	143	98	0.01	0.10	0.11	68	93	14
7	153	69	0.01	0.09	0.10	45	102	15
8	150	67	0.01	0.09	0.10	45	101	15
Mean	153	73	0.01	0.10	0.12	76	93	15

TABLE 3 - Biochemical dosages. Absolute values in each animal. Group: Liver infiltration with saline solution – 24 hours

MALE	GLYCEMIA mg/dL	ALK. P. U/L	Bd Mg/dL	Bi mg/dL	Bt mg/dL	GOT U/L	GPT U/L	gGt U/L
1	121	17	0.03	0.07	0.10	84	27	17
2	113	11	0.01	0.09	0.11	115	42	16
3	168	22	0.07	0.03	0.12	86	36	8
4	118	18	0.05	0.05	0.10	21	39	22
FEMALE								
5	117	16	0.06	0.04	0.10	119	73	8
6	118	10	0.02	0.08	0.08	105	181	17
7	103	4	0.04	0.06	0.10	115	117	9
8	113	7	0.04	0.06	0.09	94	156	6
Mean	121	11	0.04	0.06	0.10	92	84	13

TABLE 4 - Biochemical dosages. Absolute values in each animal. Group: Liver infiltration with saline solution – 4 weeks

MALE	GLYCEMIA mg/dL	ALK. P. U/L	Bd Mg/dL	Bi mg/dL	Bt mg/dL	GOT U/L	GPT U/L	gGt U/L
1	105	8	0.03	0.07	0.10	127	52	11
2	81	9	0.07	0.13	0.20	152	95	10
3	96	6	0.04	0.06	0.10	195	130	16
4	98	6	0.05	0.06	0.12	250	124	15
FEMALE								
5	109	10	0.03	0.07	0.10	104	18	9
6	100	9	0.06	0.06	0.10	150	120	12
7	130	11	0.06	0.06	0.11	25	23	23
8	115	11	0.08	0.08	0.10	56	25	11
Mean	104	9	0.05	0.07	0.11	132	73	13

Discussion

Guinea pigs were used as experimental animals due to the fact that such animals are very sensitive to drug effects[8]. Also, the type of anesthesia administered must be pointed out. Since venous anesthesia was not possible, 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 intrathoracic 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 was standardized since it enabled the definition of an appropriate area in the liver immediately after injection. 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 histotoxic action results from protein denaturation[9,10] . According to Goodman and Gilman[10], 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[9,10]. Systemic toxicity[10] is shown by the transitory stimulation of the CNS, and the carcinogenic potential of phenol is disputable, according to those authors. Phenol is mainly eliminated by the kidneys, and most of it is excreted in the first 24 hours[11]. Glacial acetic acid also features bactericidal action when the 5% solution in smaller concentrations is bacteriostatic[10]. 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[10,11]. 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[10]. 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. Solution E, containing such components, was empirically used, as previously mentioned, for protatic patients in the early 21st century in India by Roberts[6]. Since then, it has been used by other authors, but always in the prostate[6,12].
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.

Alterations between the animals in the treated and control groups were not observed. The experimental groups were analyzed at 24 hours and at 4 weeks of development, since in a pilot experiment, it was observed that after 24 hours, the infiltrated area presented rather visible necrosis, and that after 4 weeks, it would be possible to verify whether replacement by fibrous or by hepatic tissue had occurred. Our results showed that the injection of solution E into the liver did not lead to biochemical alterations related to the hepatic function. From the histopathological point of view, the injection of solution E caused a necrosis area in the infiltrated region which was perfectly diagnosed (macro and microscopically) after 24 hours. After 4 weeks of development, necrosis was not shown, and the liver presented normal appearance during both the macroscopic and the microscopic examination; the necrosis area was replaced (only in two animals) by a small extension of minor fibrotic tissue, and normal hepatic tissue was recovered.
The injection of saline solution was not followed by any anatomopathological alterations. Based on the obtained results, it is believed that the possibility of infiltrating hepatic metastases with solution E is feasible. Through such procedure, it would be possible to destroy neoplastic cells by necrosis: the occasional destruction of hepatic tissue near the tumoral area, after a certain period of time, will probably show recovery of normal tissue with a small cicatricial area (at times).

Conclusion

In guinea pigs, the used dose (0.5ml) of the sclerosing solution (E), when infiltrated into the liver:

1. produces necrosis.
2. necrosis is focal, limited to the infiltrated area.
3. it is not followed by clinical alterations in the animal and does not cause mortality.
4. it is not followed by biochemical alterations related to the hepatic function.
5. recovery of hepatic tissue with mild fibrosis occurs after necrosis.
6. it is not followed by clinical alterations and no mortality takes place.

Based on these conclusions of experimental nature, the perspective of using solution E in the treatment of irresectable hepatic metastases is recommended.

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