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Effects of alcoholic injection into the liver in rats as animal model

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ผลของการฉีดแอลกอฮอล์เข้าตับหนู : การทดลอง

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บทคัดย่อ

คณะผู้รายงานได้ทำการศึกษาผลที่เกิดขึ้นจากการฉีดสารต่าง ๆ เข้าในตับหนู จำนวนทั้งสิ้น 86 ตัว โดยแบ่งเป็น 3 กลุ่ม กลุ่มละ 40, 40, และ 6 ตัว ในกลุ่มแรกฉีดเอธิล แอลกอฮอล์ กลุ่มที่สองฉีดน้ำเกลือ และกลุ่มสุดท้ายไม่ได้ฉีดอะไรเลย หนูทุกตัวได้รับการตัดตับเพื่อศึกษากายวิภาคและจุลกายวิภาค นอกจากนั้นยังดูดเลือดจำนวน 5 ซีซี เพื่อศึกษาผลการทำงานของตับ โดยแบ่งเป็น 4 ช่วงเวลา ได้แก่ วันที่ 3,7,14 และ 21 แต่ละช่วงเวลาจะใช้หนู 10 ตัว ผลทางพยาธิของตับพบว่า เกิดบริเวณ necrosis ในเนื้อตับรอบ ๆ ตำแหน่งที่ฉีดแอลกอฮอล์ หลังจากนั้น เกิดกระบวนการ regeneration ไม่พบความเปลี่ยนแปลงในตับหนูของกลุ่มที่เหลือทั้งสองกลุ่ม ผลการทำงานของตับที่มีนัยสำคัญ ได้แก่ SGOT และ ALP ($p<0.05$).

ABSTRACT

Eighty-six rats were divided at random into 3 groups of 40,40 and 6. Intrahepatic injection were performed with 0.5 cc. of ethyl alcohol in group I, saline in group II, and no injection in group III. On the post injection day 3:10 rats each of group I and II and all of group III were sacrificed. The gross and microscopic pathology of livers were recorded. Five cc. of whole blood were sent for biochemical study. The same procedures were then repeated on post injection day 7, 14 and 21 on the first two groups. The results were found on gross pathology were areas of whitish plaque representing parenchymal necrosis on histological examination at the injected site, followed by inflammatory reaction, fibrosis and eventually regeneration. No changes were detected on the last two groups. Significant biochemical changes were noted: SGOT and ALP in saline group ($p<0.05$).

INTRODUCTION

Absolute ethyl alcohol liver tumour injection was first reported by Shinagawa et al.[1] They reported the success of this technique in patients who had hepatocellular carcinoma, less than 2 cm in diameter, the ethanol caused tumour necrosis. Since then, there were several report describing the success of this technique by either percutaneous or laparotomic approaches, with neither biochemical nor systemic complication.[2,3,4,5] At Srinagarind Hospital, this technique has been used in unresectable tumours and in tumours of surgically poor risk patients for more than 7 years. The subjective palliative results so far were satisfactory. However the effects of ethyl alcohol on the tumour and the surrounding healthy liver parenchyma as well as the overall effect on liver function are still not clearly demonstrated. This investigation is designed to study the gross and microscopic morbid anatomy of the liver and its function affected by ethyl alcohol direct injection using rat as an animal model.

MATERIALS AND METHODS

Animals Eighty-six adult rats weighing 240 $\pm$ 50 grams were randomly divided into 3 groups of 40,40 and 6. Intrahepatic injections were performed into left medial or lateral lobe of the liver, 3-5 mm. depth, on day D_0: 0.5 cc. of 95% ethyl alcohol in group I, 0.5 cc. of normal saline in group II. No injection on the controlled group III. On the post injection day D_3:10 rats each of group I and group II and all of group III were sacrificed. The gross pathology of the liver was recorded and all livers were sent for histopathologic study. All procedures were performed under general anaesthesia by using ether as anaesthetic agent. Five cc. of whole blood from each animal were sent bilirubin (TB/DB) and liver enzymes, including SGOT/SGPT and ALP. The same procedures were then repeated on post injection D_7, D_{14} and D_{21} on the remaining first two groups, except one rat in saline group on D_{21} that was missing from its box, so the total number of saline group remained 39.

Statistics The BMDP statistical package program version PC90 for PC 80486 DX2-50 was used for screening. The BMDP 2V was used for the Analysis of Variance and Covariance with Repeated Measures in liver enzyme changes (SGPT, SGOT, ALP). And the BMDP 5V was used for the Unbalanced Repeated Measures models with structured covariance matrices in bilirubin changes. Then the SPSS/PC+ was used for paired *t* test and ANOVA (Analysis of Variance) which was significant between times and between groups, respectively. A ρ value of less than 0.05 was considered significant.

RESULTS

Liver of all the ethyl alcohol group showed area of whitish plaque at the injected site with microscopic picture of parenchymal necrosis, vascular thrombosis and acute inflammatory reaction on D_3

examination. Chronic inflammatory reaction, fibrosis and parenchymal regeneration were subsequently seen on D_7,D_{17} and D_{21} examinations. Complete regeneration was detected in 1(10%), 2(20%) and 5(50%) rats on D_7, D_{14} and D_{21}, respectively. Similar examination on the saline injected and the non-injected groups, on changes were detected. (Table 1)

TABLE 1 Microscopic changes related to time in ethyl alcohol group

Day	Maximum diameter (mm.)		Complete Regeneration (n=10,each)
	Necrosis	Inflammation	
3	8.8 ± 4.32	3.2 ± 0.84	1 (10%)
7	6.4 ± 1.19	2.1 ± 0.22	2 (20%)
14	3.3 ± 0.84	1.1 ± 0.55	3 (30%)
21	2.5 ± 2.12	0.88 ± 0.25	4 (40%)

Most of the results of biochemical study showed no significant change, either relating to group or time. Except SGOT : ethyl alcohol group differed significantly from saline group on day 14 and day 21; while ALP : saline group showed significant differences between time from day 3 to day 21. (Figure 1-5)

Figure 1 : Total billirubin changes

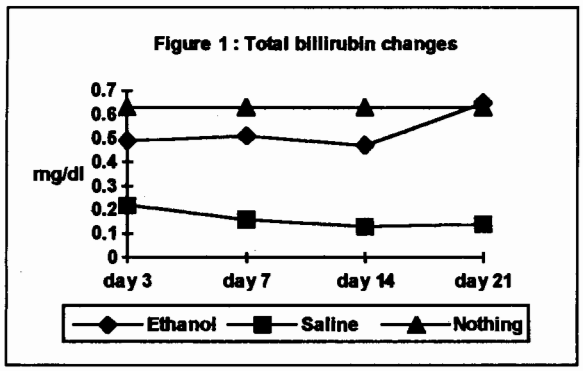


Figure 2 : Direct billirubin changes

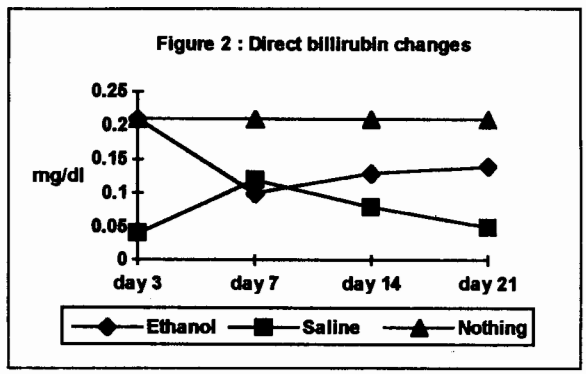


Figure 3 : SGPT change

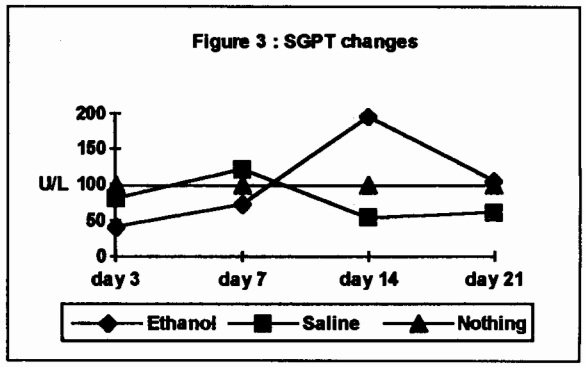


Figure 4 : SGOT change

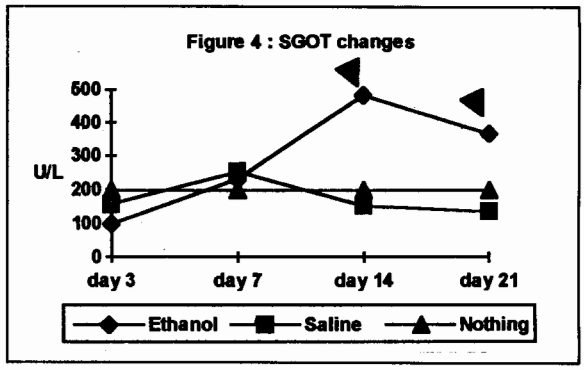
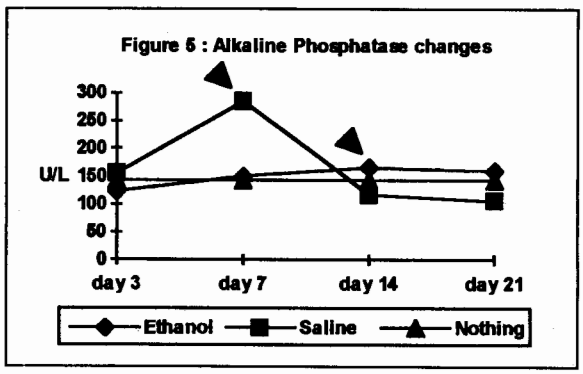


Figure 5 : Alkaline Phosphatase changes



DISCUSSION

When we first began this study, out of 100 rats received, 14 were of inappropriate weight and were excluded (appropriate, 240 $\pm$ 50 grams). The remaining 86 were then divided into 3 groups, 40 for ethyl alcohol, 40 for saline and 6 for no injection. We assumed that no change would occur in no-injected group, so we performed the procedure in this group only once, and used its result as control in all steps of statistical analysis.

There is a definite evidence that ethyl alcohol direct injection causes local cell death of the liver, explaining why SGOT and SGPT increase gradually prior to decline after day 14. Acute inflammatory reaction is seen as a response of the surrounding tissues. This is then followed by chronic inflammatory reaction, fibrosis and eventually parenchymal regeneration. It is probably reasonable then to assume that a similar chain of events occurs if ethyl alcohol direct injection into the tumour mass is employed with local cell death as the main objective. Minimal harm would occur even when a small amount of ethyl alcohol leakage occurs into the surrounding parenchyma provided that normal precaution is taken not to inject into the blood vessels. And further more no significant functional changes if the area of normal parenchymal damage is kept to the minimum.

There is no definite explanation about ALP which increases in saline group while remains unchanged in ethanol group : however the increase in ALP usually has some connection with the obstruction of biliary system.

The biochemical changes were analyzed as followed : firstly with the BMDP 2V for liver enzymes and BMDP 5V for bilirubin because some bilirubin values were missing due to inadequate amount of blood drawn. There were significant changes detected in SGOT (between group), ALP and TB (between time). Secondly, we tested which one was significant in time by paired *t* test : ALP showed significance between day 3 and day 7, day 7 and day 14, day 7 and day 21 ; but no significance was detected in TB. Then ANOVA was used to test group significance : ethyl alcohol differed significantly to saline on day 14 and day 21 (in SGOT). We have no suitable explanation for these contradicted biochemical changes, presumably there were other factors affected these changes as in control group had higher values of liver function test than normal. (Figure 1,2)

In our practice on unresectable liver tumour, we try to limit the amount of absolute ethyl alcohol injection to approximately 1/3 of the tumour volume as a precaution to minimise surrounding liver tissue damage. From this experimental result of the chain of responses of tissue damage and its final sequalae of regeneration, larger volume of absolute ethyl alcohol may be safely injected.

CONCLUSIONS

Ethyl alcohol direct injection causes local cell death of the liver, followed by inflammatory reaction, fibrosis and eventually regeneration of the surrounding parenchyma. Contradiction of biochemical changes, e.g. bilirubin, SGOT, ALP; still needs further explanation.

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