

MORPHOMETRY OF CONNECTIVE TISSUE IN RAT LIVERS

Otto LÖW, Ulrich GABLER, Andreas MÜLLER, Petra GABLER,
Harald SCHUBERT, Hans-Christoph LÖW and Georg MACHNIK

Friedrich-Schiller-University Jena, Klinikum, Bachstr. 18,
D-07740 Jena, Germany

ABSTRACT

Thioacetamide (TAA) induced fibrosis in the rat liver was used as a model for testing preparations for a prevention of cirrhosis. The homogeneity of collagen distribution between the lobes allows the morphometry of a single lobe as a measure for the total organ, but it is always better to estimate about 5 stage sections with equal distances, and these sections should be measured totally.

A test period of 3 months results in a markable fibrosis, sufficient for testing antifibrotic preparations.

Zinc ions in drinking water decrease the collagen percentages in comparison to untreated animals and reduce the TAA-induced fibrosis during simultaneously application over a 3 month period, since an addition only in the 3rd month diminishes this effect. No differences are seen after an interruption of the TAA-poisoning after 3 months and a feeding with zinc sulphate only in the 4th month, compared to clear drinking water.

Key words: connective tissue, methodology, rat liver, thioacetamide, zinc sulphate.

INTRODUCTION

Morphometry of the connective tissue in the liver is not an unknown method both for diagnostic purposes in human pathology (Nakamura et al., 1965; Ludwig and Elveback, 1972; Volmer and Lüders, 1981; Zhou, 1990; Hall et al., 1991; Sarosi et al., 1991; Moragas et al., 1992; Navasa et al., 1992; Nohlgard et al., 1993; Zhao et al., 1993) as well as for experiments (Ryoo and Buschmann, 1983; Gabler, 1984; Löw and Gabler, 1988; Gabler and Gabler, 1989; James et al., 1990; Jiang et al., 1990; Jiang et al., 1992; Löw, 1992; Machnik et al., 1992; Szende et al., 1992). Although biochemical analysis of blood and liver is necessary for testing drugs to prevent or diminish liver cirrhosis, the morphometry of the structural changes offers beside its own value additionally the possibility for correlations with other data.

MATERIAL AND METHODS

Female rats (strain Uje-Wist) received TAA (about 25 mg/kg bw/d) and/or zinc sulphate (Zn1 500 mg/l, Zn2 1000 mg/l) in drinking water (daily amount about 17 ml) mostly for 3 months; TAA3 = zinc only in the 3rd month, TAA4 zinc only in the 4th month after interruption of the TAA-poisoning.

Decapitation in ether narcosis, fixation 10 % formol, embedding in paraffin, section thickness about 7 µm, staining HE, GOLDNER, Sirius Red or Direct Red in picric acid, magnification 400x, sometimes 100x, for the Quantimet 500 pixel distance 2.6 µm and fields of 1.24 mm².

Equipments: Eltinor 3 (ROW Rathenow), Statistic (Carl Zeiss Jena), Quantimet 500 (Leica, Bensheim).

npd = net point distance in mm, sd = slide distance in mm, d = day, bw = body weight

RESULTS

1. METHODOLOGY

The collagen percentages of the different lobes do not cross the range : mean value \pm 10 %. These mean values are calculated via the percentage data per section without consideration of the different areas. The divergencies (diff. %) to the mean values based on hits are comparably small (table 1).

Table 1: Collagen distribution in the lobes of a cirrhotic rat liver

lobe	total liver	l.sinister	l.sinister access.	l.dexter	l.dexter access.	l.caudatus	processus papillaris
vol.cm ³	4.35	1.18	1.66	0.25	0.59	0.28	0.39
mean	9.92	10.51	9.13	10.25	9.42	9.23	9.09
+/-	1.00	1.07	0.56	0.42	1.22	0.47	0.76
diff.%	-1.00	1.15	-1.40	-1.16	-3.78	-0.86	-3.10

Even in groups under equal conditions the collagen may be distributed less or more homogeneously, sometimes with higher values in the central regions. In series of stage sections of the lobus sinister with sd = 1 mm the mean values are calculated on the basis of hits for all slides and further for each 2nd slide, starting with number 1 or 2; this procedure was continued for larger distances up to the half of them. In sections with less inhomogeneity there were calculated no case outside the mean value \pm 10 %, and with 3-4 slides (corresponding to a distance of about 9-10 mm) per lobe no mean value cross the \pm 5% borderlines, since there are a few cases with 2 slides only (distances up to about 15 mm). All mean values of a section with more heterogeneity for 3 (and more) slides are inside of a range of \pm 10 %, corresponding to a stage section distance of about 10 mm, but only the mean values for sections with a distance of 4 mm are inside a range of \pm 5 %; if only 2 slides are measured in such a case, half of the mean values are outside the \pm 5 % borderlines and a third are outside the \pm 10 % range (table 2).

Table 2: Collagen values in dependence to stage section distance

dist	mm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
less	5%	0	0	0	0	0	0	0	0	22	0	9	0	8	14		
	10	0	0	0	0	0	0	0	0	0	0	0	0	0			
mo	5%	0	0	0	0	20	67	14	25	33	30	45	42	62	50	40	50
	10	0	0	0	0	0	0	0	0	0	0	18	25	15	7	20	38

Probabilities in % for all cases outside the \pm 5 and \pm 10 % ranges

Net point distances up to 0.6 mm have no effect on the collagen data, since the measuring time decreases from 8 hours to about 10 minutes for an area of about 1 cm² (table 3).

The intraobserver differences can be neglected, although it is necessary to measure sections with equal staining procedures. In tests with untrained persons the mean values are slightly higher, if a staining for the nuclei is added to the Sirius Red staining.

Table 3: Net point distance ,collagen values and measuring time

npd (mm)	0.1	0.2	0.3	0.4	0.6
% collagen	20.47	20.25	20.24	22.16	20.11
time (hours)	8	2	0.9	0.5	0.22

Repeated measurements on another slide of such an area show no markable change up to net point distances of 2.56 mm, but the coefficients of variation may increase, and this is valid for collagen percentages from about 3 % up to 40 % (table 4). The number of hits for this area with a npd of 5.16 mm was to small for very accurate data.

Table 4: Net point distance and coefficient of variation

npd (mm)	about 3 %	about 10 %	about 20 %	about 30 %	about 40 %
0.32	9.9	4.9	2.5	3.3	3.1
0.64	26.9	10.7	5.7	5.2	3.5
1.28	73.9	13.1	14.4	19.0	7.7
2.56	67.4	35.1	20.9	28.4	10.4
5.12	(0)	129.1	54.1	119.9	33.8

The heterogeneity in a single section is shown in fig. 1 for squares of 0.64 mm² each with 100 hits (npd 0.08 mm). In the left part most of the values are much lower than in the right part. There was a tendency to slightly increased values during a measurement with a magnification of 100x, compared to 400x, especially for untrained persons (22.41 %, respectively 20.11 %). A subjective index was founded, which includes several histological changes in roughly differentiated 6 stages. Up to stage 4 the curve compared to the morphometric data was a steep straight line, since for the last 2 stages there were observed brider ranges. The total coefficient of correlation was 0.896 (P<0.1 %).

Automatic measurements of 10 squares per 1.24 mm² per section of shame-operated animals with low collagen percentages in slides stained only with Sirius Red let to 1.46 +/- 0.11 % , which is somewhat lower than those after point counting 2.11 +/- 0.43 %.

2. EXPERIMENTS

Although there have been calculated some divergencies for the collagen values in different tests, a time of 3 months always let to acceptable data for a fibrosis (table 5).

Table 5: Test duration of TAA-feeding and collagen values with their standard deviations

months	0.5	1	2	3	6
untreated				1.61 (0.18)	1.80 (0.19)
TAA	1.55 (0.18)	1.77 (0.12)	3.60 (0.56)	10.09 (3.19)	19.68 (1.63)

Compared to untreated animals the application of zinc sulphate causes a small but significant decrease in the collagen data, a distinct dependence to the dosis does not be observed. The marked increase in the collagen percentages after TAA-poisoning was diminished to about a third by simultaneously applicated zinc ions, but less on the half, if the zinc was given just in

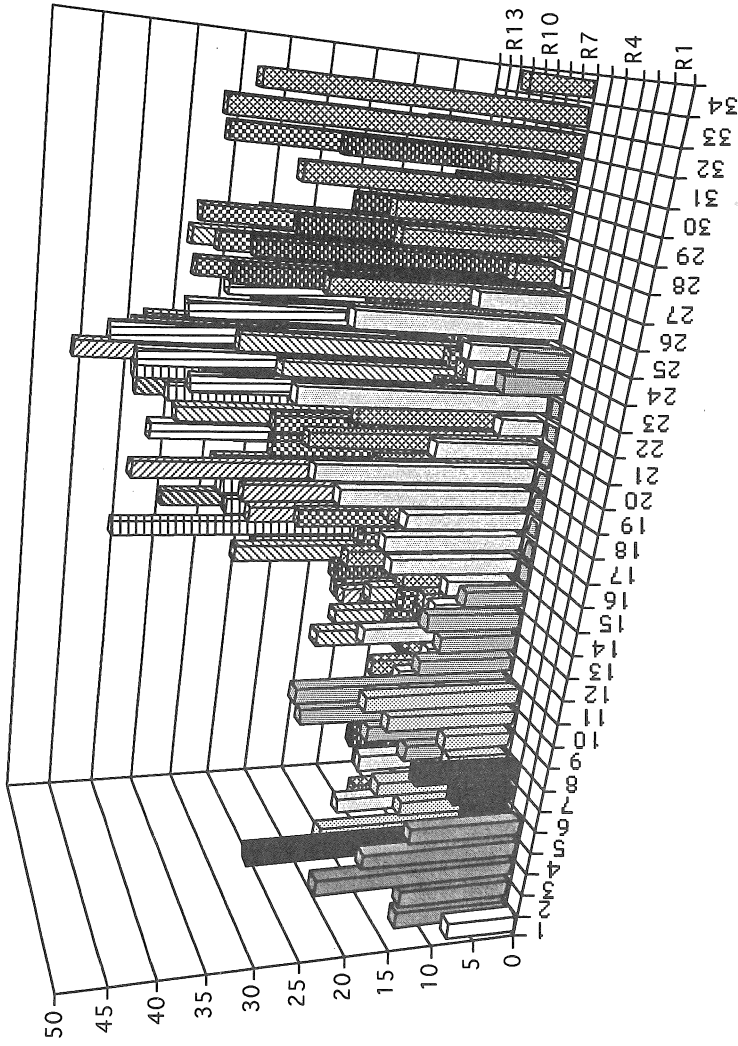


Fig. 1 Heterogeneity of cirrhotic rat liver

the 3rd month.No clear difference was seen after an interruption of the TAA-feeding and a following administration of clear water or zinc sulphate (table 6).

Table 6: Collagen data after application of zinc sulphate and/or TAA (S.I.=Subjective Index)

group	contr.	Zn1	Zn2	TAA	TAA Zn1	TAA Zn2	TAA3 Zn1	TAA3 Zn2	TAA4 H2O	TAA4 Zn1	TAA4 Zn2
mean	2.77	1.76	2.11	30.52	7.16	9.23	14.03	17.79	14.19	13.06	11.88
+/-	0.43	0.17	0.21	5.63	1.98	4.27	7.70	6.81	4.98	7.83	2.61
S.I.	0.0	0.0	0.0	5.86	2.57	2.80	4.25	5.08	4.66	4.33	5.16

The following parameters let to significant correlations with the collagen percentages in the liver ($P < 5\%$): weight of the liver, hydroxy proline in the serum as well as in the liver, collagen peptidase only in the serum. Otherwise the calculations give probability data $> 5\%$ for serum protein, collagen peptidase in the liver and N-acetyl- β -D-glucosaminidase both in liver and serum.

DISCUSSION

The methodological studies let to the following results. The measuring of a single lobe with 3-5 stage sections seems to be sufficient, but all of these slides should be measured totally, because there may be some heterogeneity in the collagen distribution (Gabler, 1984; Löw and Gabler, 1988; Löw, 1992). Such an inhomogeneity may increase the working expense, otherwise the accuracy was decreased. For small percentages not more than 500 hits are necessary for an acceptable accuracy, and for higher ones approximately 300. For a higher accuracy it is not necessary to attach more than 1000 points, therefore a net point distance of 2.5 mm may be sufficient for some stage sections with a sufficient total area. For automatic measuring 10 or more squares should be selected from all over a section area of . Only in a few cases it should be tried to use the total area, but this increases the measuring time. Point counting has the advantage to estimate such an irregular shaped area without complications and to measure more morphological parameters, since automatic estimations demand good contrasts, e.g. a staining only with Sirius Red. This procedure should be preferred, because sometimes it is difficult to attach correctly in sections stained after GOLDNER. It is no question, that morphometric data for single parameters like the connective tissue do not make redundant a common histology.

Zinc sulphate causes a reduction of the collagen percentages compared to untreated animals as well to TAA-poisoned, especially if the zinc ions are applied during the total poisoning period; otherwise the differences become smaller or are blurred. After an interruption of the TAA-feeding the percentages of collagen decrease, therefore it is better to term the changes a cirrhosis-like lesion, because such observations are in contrast to the changes in human cirrhotic livers. A dependence to the concentration of the zinc sulphate could not be demonstrated. The comparisons with biochemical parameters inaugurate a more complex view of the changes (Gabler and Gabler, 1989). Tests with colchicine show no distinct effect (Machnik et al., 1992), which is in contrast to the results by Jiang et al. (1992), who observed better results with colchicine in comparisons with the efficacy of zinc in CCl₄-treated rats.

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