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Rapid Communication

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THE EFFECT OF NAFAMOSTAT MESILATE (FUT-175) AND GABEXATE MESILATE (FOY) ON MULTIORGAN OXIDANT-ANTIOXIDANT BALANCE IN ACUTE EXPERIMENTAL PANCREATITIS

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This study was undertaken to determine whether synthetic proteinase inhibitors — nafamostat mesilate (FUT-175) and gabexate mesilate (FOY) have any influence on multiorgan oxidant-antioxidant balance in acute haemorrhagic pancreatitis induced in Wistar rats using a retrograde intraductal injection of 5% Na-taurocholate. Rats were treated with FUT-175 $25 \cdot 10^{-3}$ $g \cdot kg^{-1} \cdot h^{-1}$) or FOY ($2.5 \cdot 10^{-3}$ $g \cdot kg^{-1} \cdot h^{-1}$) and sacrificed at 3 h. Malondialdehyde and sulfhydryl groups concentration, as an index of oxidative stress, we measured in pancreatic, lung and liver tissue. In rats with acute pancreatitis treated with these proteinase inhibitors, oxidative stress expressed by malondialdehyde elevation and sulfhydryl groups depletion, was markedly diminished. It was observed in the pancreas and lung, and to a lesser extent in the liver. These effects of FUT-175 or FOY treatment, at least in part, may account for recently postulated favorable systemic effects of such a medication.

K e y w o r d s: *malondialdehyde, oxidative stress; pancreatitis acute experimental; sulfhydryl groups; synthetic proteinase inhibitor*

INTRODUCTION

We have recently found serious, multiorgan disturbances of oxidant-antioxidant balance in early hours of Na-taurocholate-induced acute pancreatitis (Na-TC-induced AP) in the rat (1). Malondialdehyde (MDA) concentration, as an index of oxidative injury, and sulfhydryl groups (SH-groups), known as a major nonenzymatic oxidant, were determined in pancreatic, lung and liver tissue along with enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (CAT). Early and profound oxidative

stress in each organ was evidenced by marked increases in MDA concentrations as well as marked reductions in levels of SH-groups and SOD. A paradoxical increase in CAT activity, perhaps compensatory, was noted in pancreas and lung.

Synthetic trypsin inhibitors have been reported to have favourable effects on experimental pancreatitis (2—7). Unfortunately, most of them exhibited only a prophylatic effect what may explain their lack of efficacy in controlled clinical trials (8). Nevertheless, recent Italian multicenter trial on gabexate mesilate (FOY) in acute pancreatitis has shown that FOY treatment started in the early phases of the disease, reduced the incidence of systemic complications (9). Interestingly, many of experimental data also suggest that beneficial effects of acute pancreatitis treatment with synthetic proteinase inhibitors may result from an amelioration of the systemic effects of the disease (3—5, 7).

The aim of the present study was to evaluate whether synthetic proteinase inhibitors — nafamostat mesilate (FUT-175) and gabexate mesilate (FOY) has any influence on multiorgan oxidant-antioxidant balance in the early hours of Na-TC-induced AP in the rat.

MATERIAL AND METHODS

Experimental Protocol

The experiments were carried out on male Wistar rats (250—280 g), kept on a standard diet. With the rats under ketamine anaesthesia, a heparine-rinsed polyethylene catheter was placed in the jugular vein and brought out throught the suboccipital area of the neck where it was held in position by means of two stitches. Rats were allowed to recover for approximetely 24 h, with free access to water. Acute pancreatitis (Na-TC-induced AP) was induced by retrograde injection of 0.2 ml of 5% Na-taurocholate into the common biliopancreatic duct, according to previously described techniques (1, 10).

The following experimental groups were designed: sham-operated rats (n = 6) with i.v. infusion of 0.9% NaCl started immediately after laparotomy and continued during 3 h; sham-operated rats ($n = 6$) with i.v. infusion of FUT-175 ($25 \cdot 10^{-3}$ $g \cdot kg^{-1} \cdot h^{-1}$) started immediately after laparotomy and continued during 3 h (FUT-175, Nafamostat mesilate from Torii & Co., Japan); sham-operated rats ($n = 6$) with i.v. infusion of FOY ($2.5 \cdot 10^{-3}$ $g \cdot kg^{-1} \cdot h^{-1}$) started immediately after laparotomy and continued during 3 h (FOY, Gabexate mesilate from ONO Pharmaceutical Co. Ltd, Japan); rats ($n = 8$) with Na-TC-induced AP lasting 3 h, with i.v. infusion of 0.9% NaCl started immediately after pancreatitis induction; rats ($n = 8$) with Na-TC-induced AP lasting 3 h, with i.v. infusion of FUT-175 ($25 \cdot 10^{-3}$ $g \cdot kg^{-1} \cdot h^{-1}$) started immediately after pancreatitis induction; rats ($n = 8$) with Na-TC-induced AP lasting 3 h, with i.v. infusion of FOY ($2.5 \cdot 10^{-3}$ $g \cdot kg^{-1} \cdot h^{-1}$) started immediately after pancreatitis induction.

The animals were sacrificed, after 3 h of the experiment, by exsanguination (from the heart) under ketamine anaesthesia. Each pancreas, liver and lungs were quickly removed, blotted dry, weighed, and immediately used for the assays of MDA and SH-groups.

Biochemical Determinations

Tissue MDA concentration was measured after the reaction with thiobarbituric acid, according to the method of Salaris and Babbs (11).

Tissue total SH-groups concentration was determined according to the method of Ellman (12), as descibed previously (13).

Samples' protein content was estimated by the method of Lowry et al. (14).

Serum α-amylase activity was assayed by Caraway's method (15).

Statistical Analysis

Results are expressed as mean $\pm$ SD and statistical significance ($P < 0.05$) was analysed by Student's t test. In the figures, vertical bars = 1 SD.

RESULTS

Pancreas, liver and lungs were not macroscopically changed in all groups of sham-operated rats. In rats with Na-TC-induced AP pancreata appeared grossly swollen and enlarged. Areas of haemorrhage and 3—5 ml of peritoneal exudate were present in all rats with pancreatitis.

Fig. 1 presents pancreatic edema development, measured as a pancreas weight gain, and serum amylase activity. Edema of the gland is slightly lower in rats treated with FUT-175, while marked amylasemia diminution was observed in both groups of rats with pancreatitis, treated with FUT-175 or FOY.

Only treace amounts of MDA we found in pancreatic tissue of sham-operated rats treated with saline (*Fig. 2*). Pancreatic MDA level was substantially increased in sham-operated rats with FUT-175 or FOY intravenous infusion. In rats with NA-TC-induced AP treated with saline or FUT-175, but not in that which FOY was given, the disease has caused marked pancreatic MDA elevation. In this last group of animals with pancreatitis, MDA concentration in pancreatic tissue was even lower than in corresponding sham-operated group. Na-TC-induced AP has produced depletion of pancreatic SH-groups (*Fig. 2*). Pancreatic tissue SH-groups concentration was not changed only in rats with pancreatitis treated with FOY.

In lung tissue of rats with Na-TC-induced AP, we have observed the pattern of MDA and SH-groups concentration similar to pancreatic tissue (*Fig. 3*). The exception was enormously high MDA level in lungs of sham-operated rats treated with FOY. Interestingly, otherwise other groups with pancreatitis, in FOY-treated group with Na-TC-induced AP, lung tissue MDA concentration was significantly lower than in corresponding sham-operated rats.

Treatment with FUT-175 or FOY has caused marked MDA rise in liver tissue of sham-operated rats (*Fig. 4*). It is noteworthy that pancreatitis has not

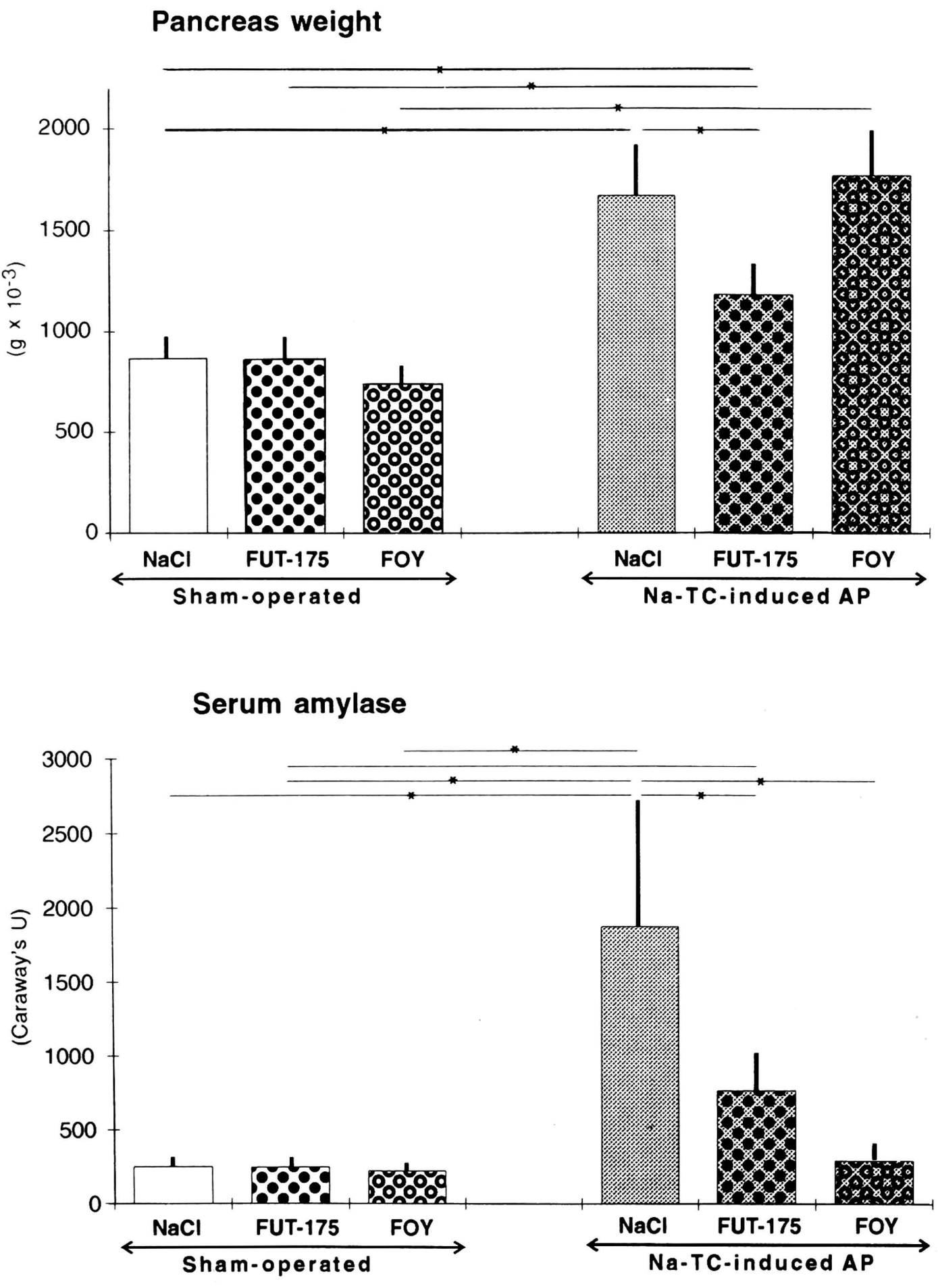
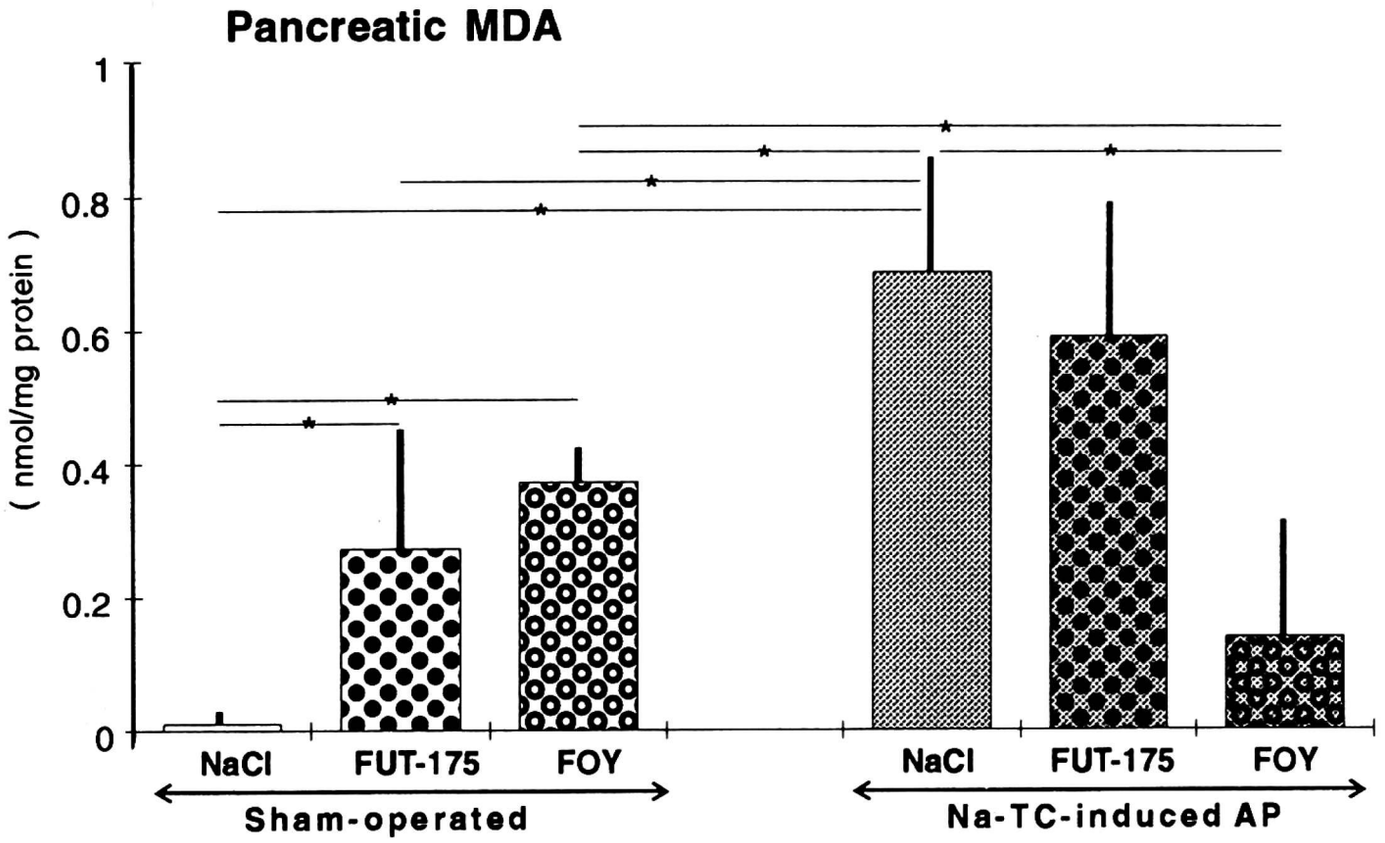


Fig. 1. Pancreatic weight and serum amylase activity in different groups of rats. The data are expressed as mean + SD (* $P < 0.05$).



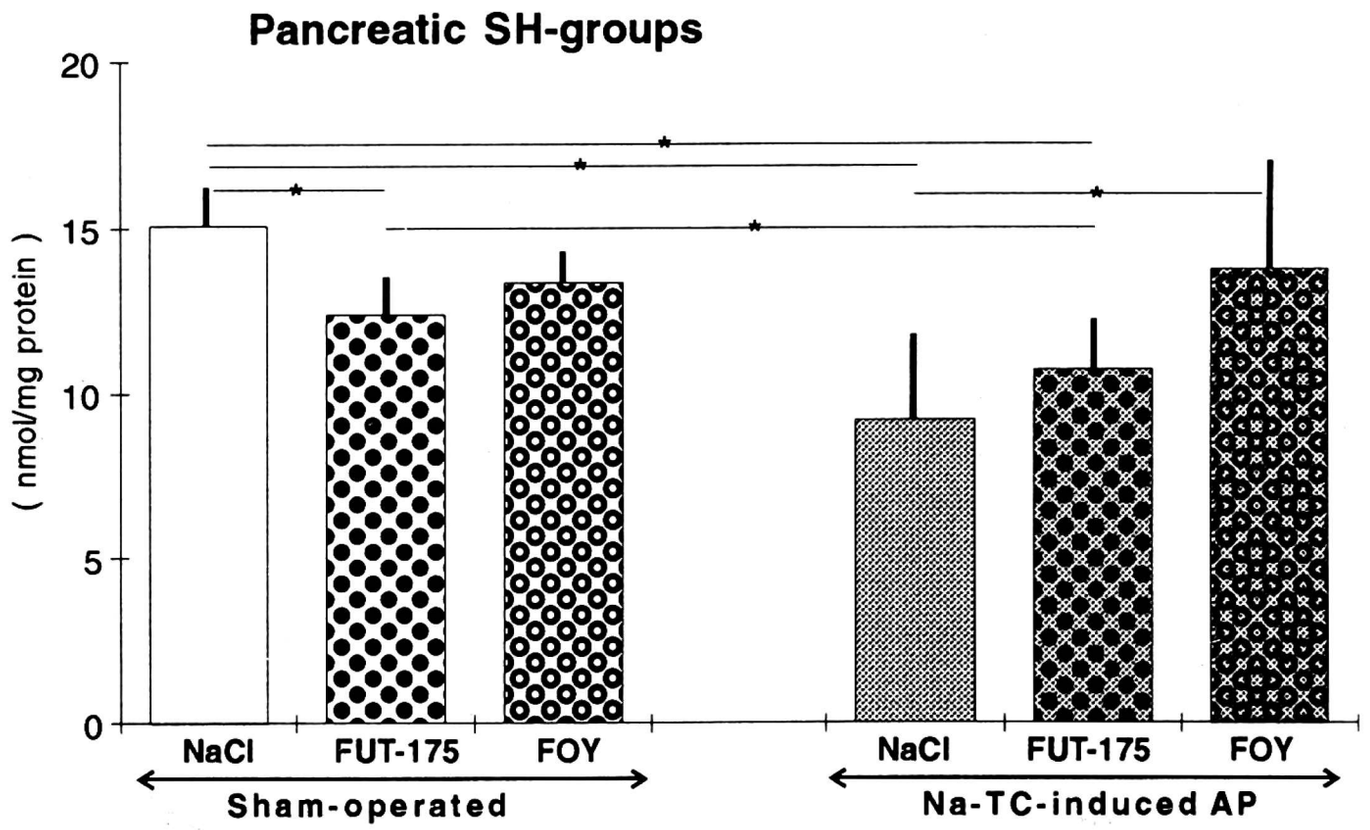


Fig. 2. Pancreatic tissue MDA and SH-groups concentration in different groups of rats. The data are expressed as mean + SD (* $P < 0.05$).

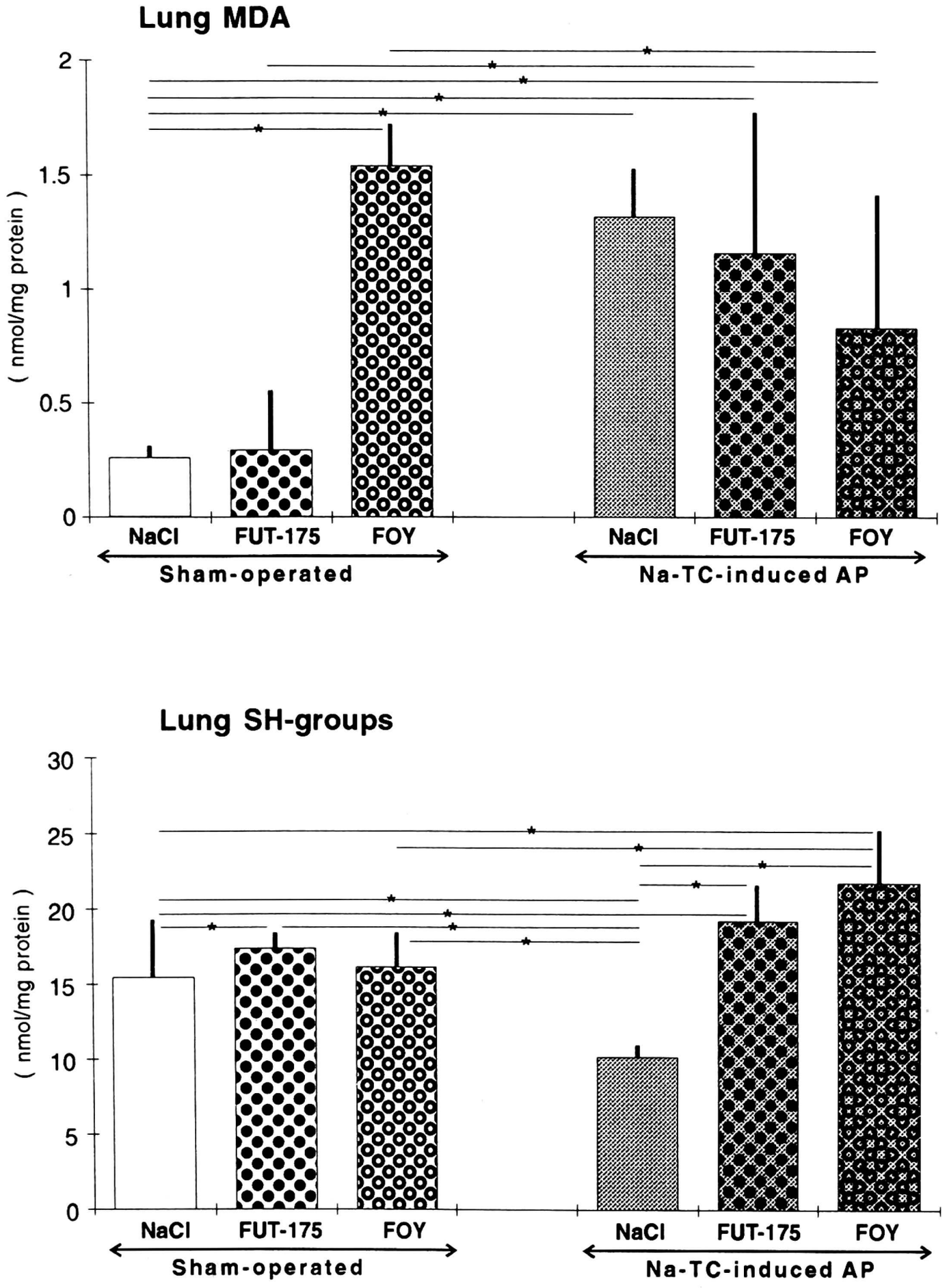


Fig. 3. Lung tissue MDA and SH-groups concentration in different groups of rats. The data are expressed as mean + SD (* $P < 0.05$).

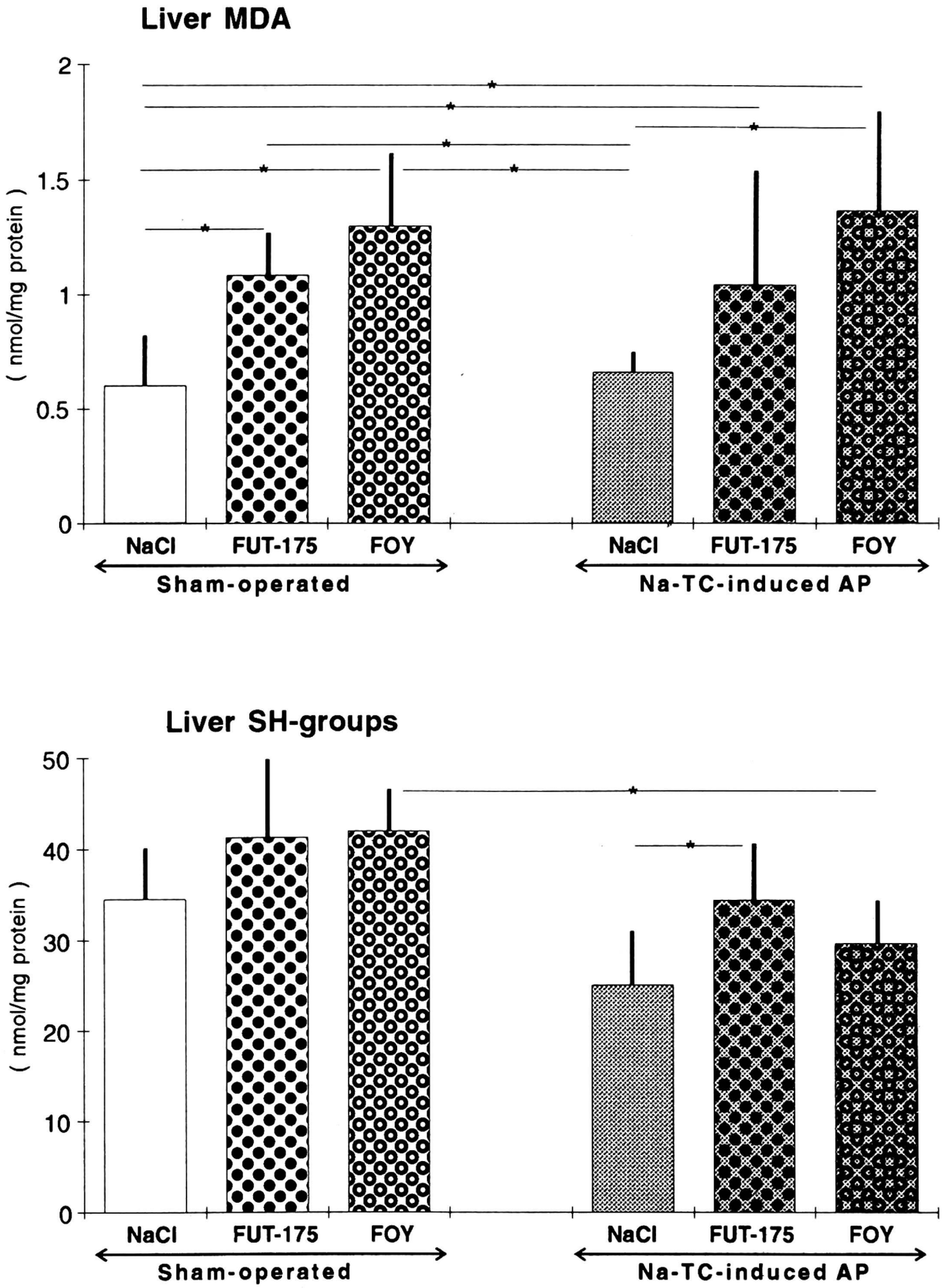


Fig. 4. Liver tissue MDA and SH-groups concentration in different groups of rats. The data are expressed as mean + SD (* $P < 0.05$).

changed these MDA levels. In the same groups of sham-operated rats, liver SH-groups were slightly elevated in comparison to corresponding animls with pancreatitis (not statistically significant). Among rats with Na-TC-induced AP the higher SH-groups concentration we observed in FUT-175 treated group.

DISCUSSION

Oxidative stress, the phenomenon expressed by oxidant-antioxidant imbalance, during acute pancreatitis is especially profound in pancreatic and lung tissue. In recent study, the most pronounced changes we have found in Na-TC-induced AP lasting 3 h. Survival for 24 h was associated with restoration of normality insofar as tissue MDA concentrations were concerned, but pancreas SH-groups remained markedly depleted (1).

In the present study, treatment with FUT-175 or FOY was started immediately after Na-taurocholate injection into the biliopancreatic duct, because it is known that within few minutes, such an injection is followed by all the sign of acute pancreatic (2, 10). Concerning serum amylase levels, we have observed favorable effects of these proteinase inhibitors, what is in agreement with the results obtained by others (2, 6, 7). In the group of rats with pancreatitis treated with FUT-175, also pancreatic oedema formation was notably lower.

In the last decade, the role of oxygen radicals and other oxidants has been intensively studied in experimental models of acute pancreatitis (16, 17). There is no doubt that these reactive species are generated in excess, within pancreatic tissue during the development of the disease. The effectiveness of antioxidant treatment varied between different models of pancreatitis and was beneficial predominantly when it started before acute pancreatitis induction (17). In our study, pancreatic tissue oxidative stress amelioration was observed in rats with Na-TC-induced AP treated with FOY. It was expressed by profound MDA level reduction what was accompanied by marked elevation of antioxidant represented by SH-groups. Unfortunately, we have no satisfactory explanation of pancreatic tissue MDA elevation in sham-operated rats treated with FUT-175 or FOY. Similar phenomenon was also observed in lung and liver tissue. MDA is a by-product of oxygen radical-mediated peroxidation of unsaturated fatty acids. It seems not likely that these proteinase inhibitors are able to induce directly excessive lipid peroxidation, but may interfere with MDA metabolism or/and tissue clearance. On the other hand, FUT-175 and FOY are as well known as inhibitors of C1r, and C1 esterase and to some extent, of phospholipase A activity (2, 6). Phospholipase A_2 isoenzymes are found in every cell type and play a crucial role in the metabolism and turnover of membrane phospholipids. The polyunsaturated fatty acids of these

phospholipids are highly susceptible to free radical attack and to the propagation reactions that are effectively sustained in unsaturated phospholipid systems. Peroxidized membranes are preferentially hydrolyzed by phospholipase A_2 and oxidized fatty acids are routinely isolated in much greater amounts from oxidized membranes than from unoxidized membranes (18). Finally, such an activity of phospholipase A_2 results in diminution of lipid peroxidation and is considered as a protective or repair function. Therefore, inhibition of this enzyme may theoretically enhance lipid peroxidation followed by MDA accumulation. Taken together, further studies are needed to find satisfactory explanation of pancreatic tissue MDA elevation in sham-opered rats treated with FUT-175 or FOY.

Quite distinct mechanism may be responsible for inhibition of lipid peroxidation and subsequent MDA formation in pancreatic tissue of rats with Na-TC-induced AP treated with FOY. Phagocytic inflammatory cells are known as a potent source of different oxidants (17, 19). Extensive infiltration with these cells is observed in pancreatic and lung tissue in early hours of acute pancreatitis (10, 20). Recently, it was found that synthetic proteinase inhibitors, namely nafamostat mesilate and gabexate mesilate, inhibit the production of various oxidants by human polymorphonuclear leukocytes (21). At the same time, the authors have found that none of these proteinase inhibititors had a direct scavenger-like effect.

Another cause of enhanced MDA production in acute pancreatitis may be the activation of platelets and their eicosanoids metabolism (22). Meanwhile, in the recent study we demonstrated that FUT-175 prevented a decrease in platelet number and inhibited platelet aggregation in bile-induced canine acute pancreatitis (23).

The enzyme xanthine oxidase, which is found in high concentrations throughout the gastrointestinal tract, is known to be a common source of oxygen radicals in ischemic states associated with endothelial cell damage. This enzyme normally exists in tissues in the form of relatively inactive precursor xanthine dehydrogenase. Ischaemia causes an almost immmediate proteolytic cleavage of peptide fragment from the precursor, resulting in irreversible activation to xanthine oxidase (16). Therefore, proteinase inhibitors can certainly inhibit this rout of oxygen radical generation.

Excessive oxidant production may induce oxidative stress with concommitant tissue SH-groups depletion, and subsequent reduction of antioxidant capacity of injured organs. In rats with pancreatitis, the diminution of MDA formation was generally accompanied by elevation of SH-groups level. We believe that preservation of pancreatic and lung thiols, measured as tissue SH-groups, results from lower oxidant generation in acute pancreatitis treated with FUT-175 or FOY. Sustaining of normal thiol level is essential for the organism, since SH-groups determine the function many of enzymes and

other proteins or peptides (22, 24). Interestingly, in lung tissue of rats with Na-TC-induced AP treated with FUT-175 or FOY, SH-groups concentration was even higher than in control, sham-opered rats, what may result from the improvement of tissue SH-groups regeneration.

In conclusion, our study demonstrates that treatment with FUT-175 or FOY has a multiorgan oxidative stress ameliorating effect in Na-TC-induced AP in rats. It is likely that, at least in part, it may account for recently postulated favorable systemic effects of such a medication (3—5,7).

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