Blood viscosity and red cell morphology in subjects suffering from cirrhosis before and after treatment with S-Adenosyl-L-Methionine (SAM)

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Abstract. Alterations of fluidity of the hepatocytic membrane and of the transport related systems are the basis of the cholesteatic syndrome and favour the tissue accumulation of cytotoxic metabolites. S-Adenosyl-L-Methionine (SAM) is a natural molecule which acts as a giver of methylc groups and as an enzymatic activator in several enzymatic actions of transmethylase and of transulphuration and plays a key role in biochemical processes of hepatic cell. The aim of our study was to evaluate the effects of SAM on the restoration of the membrane fluidity and on the hepatic function in general. In studying the fluidity of the cell membrane we evaluated some hemorheological parameters (total blood viscosity and red cell morphology). Fluidity of the red cell membrane is one of the most important elements of red cell rheology. We studied 15 patients (Group A) suffering from micro- and macro-nodular cirrhosis verified through hepatic biopsy, with alcoholic or post-viral causes. We evaluated the values of: blood viscosity (with a cone-plate rheometer by Carri-med), haematocrit, plasma fibrinogen and the erythrocytic morphology at the optical microscope with the Zipursky–Forconi method before and after 7 days of therapy with SAM iv. Data were compared with those of a similar group (Group B) treated with traditional therapy only (hyposodic and hypoprotein diet supplemented with multivitamin preparations, vitamin K in particular, if necessary, and potassium sparing diuretics). We also measured biliary salts, alkaline phosphatase, transaminase and gamma-GT. In the first group we observed a statistically significant reduction of blood viscosity, haematocrit didn’t change significantly; biliary salts reduced in a statistically significant way. Evaluation of red cell morphology showed in all cases a pathological percentage (>15%) of echinocytes and knizocytes which reduced to a mean of 5% after SAM therapy. We observed no further modifications of the other hemorheological parameters. Results demonstrate that SAM has a positive action on the fluidity of the membrane, as indicated by the improvement of haemorheological parameters and by the significant decrease of biliary salts, indicating the presence of cholesteasis.

1. Introduction

S-Adenosyl-L-Methionine (SAM) is a natural molecule, distributed in the tissues according to different concentrations, which acts as a giver of methylc groups and as an enzymatic activator in several enzymatic actions of transmethylase and of transulphuration [1–3]. Regarding the liver, SAM is the starting-point for two important metabolic pathways: transmethylation and transulphuration [4]. Transmethylation implies the transfer of a methyl group (–CH₃) from SAM to a wide number of molecules like phospholipids, neurotransmitters, polynucleotides and proteins [5,6]. The majority of transmethylation reactions take place in the hepatocyte. One of the most important is the byosynthesis of membrane...
phospholipids [7]. The cholesterol/phospholipids ratio and the degree of saturation of the fatty acids bound to glycerol in phospholipids are very important factors which fix and preserve membrane fluidity. Membrane fluidity is essential so that proteins (enzymes, receptors, ion-pumps) function at their best. We can remember the Na/K ATPase pump which in the hepatocyte plays an important role in the genesis of biliary flow [8–10]. SAM also activates transulphuration which leads to the synthesis of endogenous sulphurated products: homocysteine, cysteine, coenzyme A, taurine, sulphates and glutathione: the last one, thanks to its antioxidant action, acts in various detoxication processes [11,12]. In pathological conditions, like cirrhosis, there is an alteration of transulphuration which causes a lack of cysteine, a direct forerunner of the glutathione, and of taurine and sulphates which cause an accumulation of biliary acids in the hepatocyte and contribute to the rise of the cholesteatic syndrome [13–15]. Cholesteatic syndrome, favoured by alterations in membrane fluidity, is characteristic of chronic hepatopathies and hepatic cirrhosis and appears from a morphological point of view with an accumulation of bile in hepatocytes and, from a functional point of view, with a deficit of canalicular biliary flow [16–18]. This syndrome inhibits the physiological processes of regeneration of the hepatocytes, induces hepatic fibrosis and makes hepatocytes more susceptible to the damage induced by autoimmune phenomena [19,20]. Thus, the key role of the SAM in the biochemistry of the cell and in particular of the hepatocyte is evident. The alterations of the plasmatic membranes evident in conditions of hepatic damage with cholesteatic syndrome, are not a peculiarity of the hepatocytes but involve also other groups of cells and, among these, the circulating cells [21,22]. According to some well documented studies, abnormal plasmatic lipoproteins which circulate in hepatopathic patients modify the lipid composition of the erythrocytic membranes through a mechanism of equilibration and exchange of these lipoprotein components. It has been demonstrated that, in cholesteatic syndrome secondary to serious chronic hepatopathies like cirrhosis, erythrocytes are overloaded with cholesterol resulting in distension of their membranes and presence of targetocytes; if the phenomenon persists there is a modification of the cholesterol/phospholipids ratio and some rarer pathological shapes of red cells, like knizocytes and echinocytes may appear. The lipoprotein dysfunction induced in the extra-hepatic cells membranes represents a general characteristic of severe hepatopathy; this alteration can lead to a marked intravascular haemolysis and it is an index of adverse prognosis [23, 24]. Alterations in the circulating cells cause a worsening in blood rheology and can be displayed through the methods of filtration, of whole blood viscosity and above all through the study of red cell morphology [25].

2. Aim of the study

Alterations of the cell membrane which has been found in cirrhosis, can be found not only in the hepatocyte but also in other cells like erythrocytes. There is an important rheological alteration in cirrhosis which can be tied to alterations of the erythrocytic membrane and is related to the seriousness of the disease itself. The aim of our study was to evaluate the effect of the S-Adenosyl-L-Methionine (SAM) on some hemorheological parameters and on liver function in general in patients suffering from cirrhosis with cholesteatic syndrome [26,27]. An evaluation of the membrane function, obtained through the determination of the blood viscosity and the observation of red cell morphology, has been made before and after 7 days of therapy with 900 mg of intravenously infused SAM, added to the previous therapy, and evaluating the data described below.
Fig. 1. Erythrocyte morphology: patient suffering from cirrhosis with infrahepatic cholesteasis: before treatment with SAM there is a great number of altered cells (4 echinocytes and 2 knizocytes > 15%).

3. Materials and methods

We studied 15 patients (Group A: 10 males and 5 females between 31 and 83 years of age) suffering from micro- and macro-nodular cirrhosis verified through hepatic biopsy, with alcoholic or post-viral causes and with chronic hepatic insufficiency in ascitic phase and cholesteatic syndrome, without encephalopathy. We treated this group with traditional therapy and with SAM (450 mg bid iv). All the subjects showed altered cholesteatic indexes with an increase of alkaline phosphatase and of biliary salts and alterations of some indexes of hepatic function as well serum albumin, serum bilirubin, PT, GOT, GPT (increased values with GPT > GOT) and gamma-GT. Before and after 7 days of iv therapy with SAM we evaluated the whole blood viscosity with the plate-cone rheometer by Carri-med, haematocrit with Wintrobe method, plasmatic fibrinogen with Clauss method and through a Koagulab MJ. Red cell morphology was evaluated with the Zipursky–Forconi method which is characterised by the observation of the erythrocytes suspended in a viscous medium with an optical microscope, as indicated in Fig. 1 [25]. For total biliary salts the enzymatic method has been used (spectrophotometer); for alkaline phosphatase, GOT, GPT and gamma-GT we used the standard methods of measurement and for the PT, the coagulative method. Statistical analysis was performed with the Student’s t-test for paired data. The values observed in this group of patients have been compared with those of 15 patients (Group B: 9 males and 6 females) matched for age and pathology who were treated with traditional therapy only (hyposodic and hypoprotein diet supplemented with multivitamin preparations, particularly vitamin K in presence of a reduction of prothrombin activity, potassium sparing diuretics like spiranolactone or canreonate): concerning these patients we evaluated the parameters described above after 7 days and we have used these data as controls.

4. Results

The results, summarized in Table 1 and Fig. 2, show that there are no statistically significant differences between the basal mean values of the groups as far as hemorrhheological assessment and hepatic function concern. In group A, after 7 days of treatment with traditional therapy and with SAM (450 mg bid iv) we
observed a statistically significant decrease of the mean value of blood viscosity (from 6.19 ± 2.06 to 5.7 ± 2.35 cPs, share rate 10 s⁻¹, \( p < 0.05 \) Student’s “\( t \)” test) while hematocrit and plasmatic fibrinogen do not show any significant difference. The measure of biliary salts, indicating the presence of cholestasis, shows a significant decrease after 7 days (from 59.74 ± 18.37 to 32.81 ± 11.78 μmol/l, \( p < 0.05 \) Student’s “\( t \)” test) while alkaline phosphatase decreases but not significantly. Evaluation of erythrocyte morphology shows in all patients an increase of pathological forms (echinocytes and knizocytes) which reach values above 15% (Fig. 1) and then, after treatment with SAM, decrease in a significant way to 5%. In Group B, treated with traditional therapy only, we observed a modest increase of blood viscosity and alkaline phosphatase, hematocrit and fibrinogen do not show relevant variations while biliary salts further increase in a significant way (from 64.15 ± 32.24 to 88.75 ± 38.5 μmol/l, \( p < 0.05 \) Student’s “\( t \)” test); erythrocyte morphology shows a statistically significant increase of pathological forms (echinocytes and knizocytes) from 13 to 23%. Regarding the evaluation of hepatic function we did not observe significant variations of any parameters in both groups.

5. Discussion and conclusions

Our results demonstrate that by adding 450 mg bid of SAM iv for 7 days to traditional therapy of patients suffering from cirrhosis (Group A) we can obtain a statistically significant decrease of blood viscosity (from 6.19 ± 2.06 to 5.7 ± 2.35 cPs, share rate 10 s⁻¹, \( p < 0.05 \) Student’s “\( t \)” test) while hematocrit and plasmatic fibrinogen do not show any significant difference. The measure of biliary salts, indicating the presence of cholestasis, shows a significant decrease after 7 days (from 59.74 ± 18.37 to 32.81 ± 11.78 μmol/l, \( p < 0.05 \) Student’s “\( t \)” test) while alkaline phosphatase decreases but not significantly. Evaluation of erythrocyte morphology shows in all patients an increase of pathological forms (echinocytes and knizocytes) which reach values above 15% (Fig. 1) and then, after treatment with SAM, decrease in a significant way to 5%. In Group B, treated with traditional therapy only, we observed a modest increase of blood viscosity and alkaline phosphatase, hematocrit and fibrinogen do not show relevant variations while biliary salts further increase in a significant way (from 64.15 ± 32.24 to 88.75 ± 38.5 μmol/l, \( p < 0.05 \) Student’s “\( t \)” test); erythrocyte morphology shows a statistically significant increase of pathological forms (echinocytes and knizocytes) from 13 to 23%. Regarding the evaluation of hepatic function we did not observe significant variations of any parameters in both groups.

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Blood viscosity without changes in hematocrit and fibrinogen values, with a favourable hemorheological reaction directed to red cells [29,30]. This action is confirmed by the other favourable effect on erythrocyte morphology which is the statistically significant decrease of the extreme altered forms [25]. This happens because alterations in the metabolism of membrane phospholipids are corrected, improving fluidity with a normalisation of the cholesterol/phospholipids ratio [22,23]. Several studies have demonstrated that alterations on a erythrocytic level have the same causes of that on a hepatic level and represent a generic characteristic of severe hepatopathy [23,24]. In addition, drugs which can improve erythrocytic membrane fluidity have positive effects on the fluidity of hepatocytic cytoplasmic membrane and improve the course of hepatopathy [31,32]. The favourable action of this drug on the cholestatic syndrome is also demonstrated from the statistically significant decrease of the concentration of biliary salts, which in basal conditions are always above-average and are a direct index of the presence of cholesteasis. In particular we have to consider that in the control group (Group B) this parameter has a completely different trend and so its significance is still greater [15,21]. From a clinical point of view we also observed a reduction of pruritis [31,32]. Regarding the other parameters of hepatic function there is no difference between the groups: this is a consequence of the fact that all patients were subject to traditional therapy and the short period of observation did not allow to evaluate the further positive effects of SAM [26,28,33,34]. In conclusion our study demonstrates that SAM, added to traditional therapy in the cirrhosis, has a positive effect for the cholesteasis and for the hemorheological alterations of the red cell. This effect shows the activity of the drug and leads us to the hypothesis that the hemorheological improvement could be tied to the trend of the hepatic illness itself.
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References


