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CHANGES IN SUCCINATE DEHYDROGENASE ZONATION FOLLOWING CYCLOSPORIN-TREATMENT IN NORMAL AND REGENERATING RAT LIVER

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Abstract: Taking into account the importance acquired by Cyclosporin -A (CsA) in liver transplantation and its hepatocytic regeneration inducing capability, we have started to study other possible effects of this drug over liver functionalism. Succinate dehydrogenase (SD) activity has been quantified by means of a cytophotometric method in liver from normal, CsA-treated and partially hepatectomized (with and without CsA-treatment) rats. The typical periportal localization of the enzyme decreased after partial hepatectomy, and changed to an almost even distribution after CsA-treatment. The decreasing effect of partial hepatectomy and CsA-treatment over SD zonal heterogeneity were added. These findings permit to assume that CsA-induced regeneration is due to a mechanism different from that inducing regeneration after partial hepatectomy.

Key words: Cyclosporin-A, liver, regeneration, histoenzymology, succinate dehydrogenase.

MODIFICATIONS DE L'IMAGE DEFINIE PAR LA REACTION DE LA DESHYDROGENASE SUCCINIQUE APRES LE TRAITEMENT AVEC DE LA CYCLOSPORINE-A DANS LE FOIE NORMAL ET EN REGENERATION

Résumé: En considérant l'importance acquise par la cyclosporine-A (CsA) dans le domaine des transplants hépatiques et sa capacité d'induction de la régénération des hépatocytes, nous avons commencé à étudier d'autres effets possibles de cette drogue sur la fonction hépatique. L'activité de la deshydrogénase succinique (SD) a été mesurée par cytophotométrie de coupes histologiques de rats contrôles, traités avec CsA et après hépatectomie partielle. La plus haute activité SD, correspondant à la zône périportale, a diminué après l'hépatectomie partielle et s'est uniformisée après le traitement avec la CsA. Chez les animaux hépatectomisés et traités simultanément avec la CsA, l'effet observé correspond à la somme des effets obtenus avec les traitements individuels. Ces résultats nous permettent d'affirmer que la régénération induite par le traitement avec CsA est la conséquence d'un mécanisme bien différent de celle induite après hépatectomie partielle.

Mots-clefs: Cyclosporine-A, foie, régénération, histoenzymologie, deshydrogénase succinique.

INTRODUCTION

Cyclosporin A (CsA) is a cyclic polypeptide extracted from the fungi Cylindrocarpon. It was discovered by Dreyfuss et al. (1976), characterized biochemically by Rüegger et al.

(1976), and shown to be immunosuppressive by Borel (1976). It has proved to be a drug with great potential as an immunosuppressive agent in man. The first use of this drug in human liver transplantation was reported by Calne et al. (1979), and it was Starzl et al. (1981) who started using CsA associated with prednisone with encouraging results: the overall 1-year survival rate rose from 33 % to 63 % (Starzl et al., 1984). "The purgatory in which liver transplantation was mired has ended in the cyclosporin era" (Starzl et al., 1984).

Hepatotoxicity of CsA in patients receiving renal allografts was reported by Calne et al. (1978). In a toxicological study of CsA at immunotherapeutic dosage in normal rats, Thomson et al. (1981) demonstrated minor impairment of hepatic function. Similar findings have been reported by other authors (Magreiter et al., 1984; Nogueira and Cutler, 1985; Ota and Bradley, 1983; Williams et al., 1982) and they suggest a transitory and doses-related hepatocyte disfunction being the cause of CsA hepatotoxicity. Reversion of this CsA-induced toxicity may be due to two different mechanisms: recovery of affected hepatocytes or replacement of these hepatocytes by means of hepatocytic regeneration.

Hepatic ability to restore liver mass after surgical ablation of one or more hepatic lobes was established by Higgins and Anderson in 1944, and its mechanism has been largely studied from then to now. Hepatic regeneration also starts after toxic liver injury in order to replace damaged hepatocytes. This is why it may be asserted that cellular lesions and deaths happening during hepatic preservation in liver transplantation are obviated by regenerative mechanisms.

In cases of adult donors, room requirement for the liver graft is a major technical obstacle to liver transplantation in children. To overcome this difficulty orthotopic transplantations with adult livers reduced to the left lobe are being performed (Bismuth and Houssin, 1984). In these cases liver regeneration is a very important event in order to restore hepatic/body weight balance. It may be seen that liver regeneration is not a curiosity of liver capabilities but a very important matter that must be taken into account in human liver transplantation.

Until now liver regeneration and CsA have been considered important but separate matters in hepatology. However, we have recently established that CsA induces liver regeneration and improves regeneration following partial hepatectomy in rats (García-Alonso et al., 1988). For that reason, considering the importance of liver regeneration and CsA in orthotopic liver transplantation, we have investigated other possible effects exerted by CsA on liver functionalism.

To this end we have studied changes in succinate dehydrogenase (SD) activity following CsA-treatment in normal and regenerating livers. This mitochondrial enzyme is a well-known marker of hepatocyte metabolism (James et al., 1986), and its zonal distribution in liver parenchyma changes characteristically with hepatectomy (Andersen et al., 1984).

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing between 200 and 220 g were used. Four groups of ten animals were considered: control, hepatectomy, CsA and hepatectomy+CsA.

Surgery was performed under nembutal (Sodium pentobarbital) anesthesia (30 mg/kg i.p.) between 4:00 p.m. and 6:00 p.m. to standardize for natural diurnal rhythm. Through a median-line incision reaching 2 cm posteriorly from the xiphoid process of the sternum, the left lateral lobe was easily delivered, securely ligated and then excised. In this way portions of the hepatic parenchyma ranging in extent from 35 to 40 per cent of the total liver were removed.

CsA was dissolved in 95 % ethanol and diluted with Intralipid 10 % (Kavi-Vitrum) to 20 mg/ml and was administered intraperitoneally at a dose of 20 mg/kg for 3 days, starting the day before operation in partially hepatectomized animals.

Animals were sacrificed 40 hrs. after hepatectomy or on the third day of CsA-therapy. Under nembutal anesthesia (35 mgr/kg i.p.) liver was quickly removed and two pieces of 0.5 cm each side were obtained from the median lobe. Samples were washed by gentle shaking in a 0.9 % saline solution, frozen in isopentane/liquid nitrogen, and sliced in cryotome (8 μm).

SD activity was demonstrated using succinate and p-nitrobluetetrazolium as the substrate (Lodja et al., 1976). Twenty air-dried 8 μm cryotome liver sections from each animal were incubated in such medium at 37°C for 45 min. The reaction was stopped by 2-4 hrs. incubation with vaporous formaldehyde.

Six liver sections from each animal were chosen aleatorily and those three showing the best quality were used for quantification. Using a Zeiss-Microspectrocytophotometer under microcomputer control, working with the Mariano-García's method (García and Iorio, 1966), the intensity of SD reaction was measured in six points around every vessel found in the slice. The obtained values were distributed in histograms.

By means of a modification of Bartels method (1979), histograms were resolved into two gaussian curves and were homologated by standardizing the peak of the first one for an arbitrary value in order to superpose those from the same animal. Once the histograms on each animal were accumulated, the intersection point of both gaussian curves on the control animals was calculated. The mean value of this point has been called "frontier" and has been used to establish the limit between the two zones of the liver: dark (functionally more active, called zone B) and light (functionally less active, called zone A). The number of points belonging to each zone and their mean SD-intensity value have been estimated in the four experimental groups.

The accumulated histogram on each group has been represented in a lineal chart (X= SD intensity; Y= relative frequency). Statistical significance of our results has been evaluated by means of the Student's "t" test.

RESULTS

Zonal heterogeneity in liver parenchyma from the control animals was evident with light microscopy. Two kinds of vessels were easily distinguishable: some of them were surrounded by a zone of light parenchyma (zone A), and others were surrounded by a zone of dark parenchyma (zone B, of greater SD-activity). Zone B corresponds with periportal areas, while zone A is identified as centrolobular areas. This zonal heterogeneity as seen with light microscopy decreases after partial hepatectomy, and this diminution is even greater after treatment with CsA (Fig. 1).

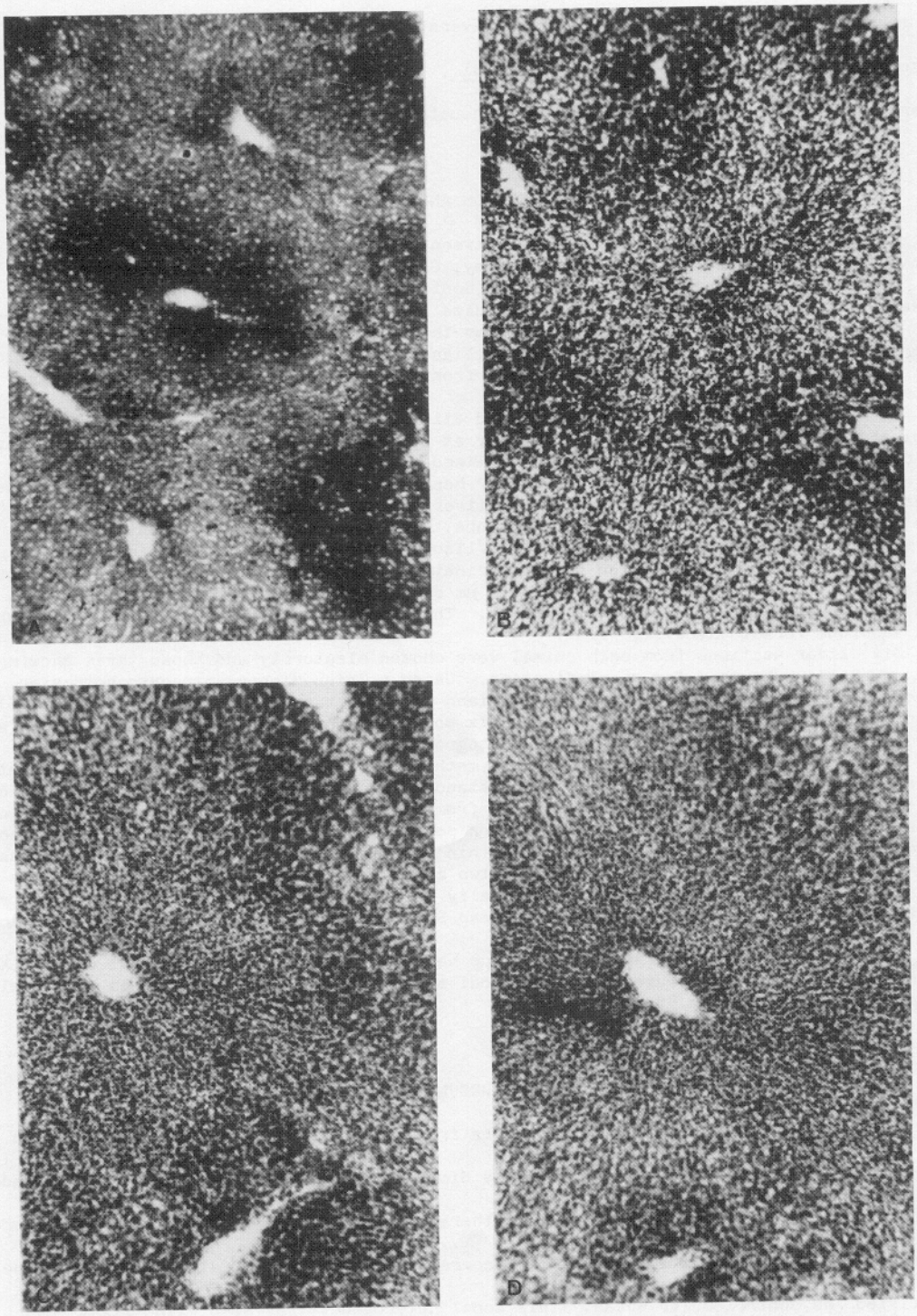

Fig. 1 a–d: SD distribution in liver parenchyma from normal **(a)**, hepatectomized **(b)**, CsA-treated **(c)** and hepatectomized+CsA-treated rats **(d)**. The change from a preferent periportal localization to an almost even distribution may be seen. (x10)

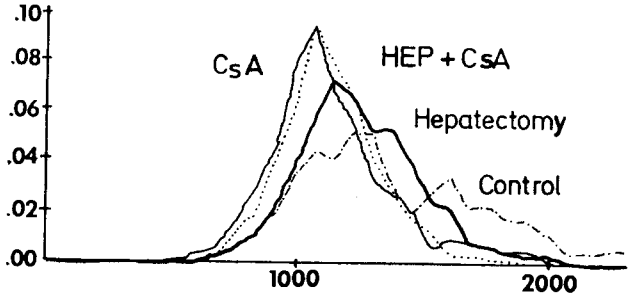

Fig. 2: Lineal charts of SD activity in the four experimental groups. Two events are seen: 1) The curves of the three experimental series are displaced to the left with respect to the control one, and this displacement is greater in CsA-treated animals (both normal and hepatectomized); 2) The second peak of the histogram, decreased in hepatectomized animals, is not evident in CsA-treated animals.

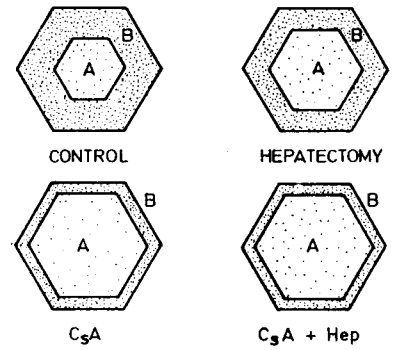

Fig. 3: Graphic representation of the SD activity decrease observed in zone B in our study.

TABLE 1
Mean SD activity value in the four experimental groups

	ZONE A	ZONE B
Control	965 (10)	1546 (196)
Hepatectomy	968 (25)	1328 (19)
CsA	908 (25)	1338 (37)
Hep. + CsA	933 (15)	1297 (34)

* Standard deviation may be seen among parenthesis

TABLE 2
Percentage of points from each zone and A/B proportion

	% A	% B	A/B
Control	56.62 (7.4)	43.38 (7.4)	1.30
Hepatectomy	73.76 (4.6)	26.24 (4.6)	2.81
CsA	86.44 (5.5)	13.56 (5.5)	6.37
Hep. + CsA	88.20 (4.0)	11.80 (4.0)	7.47

* Standard deviation may be seen among parenthesis

The same bipolarity of SD activity shown by light microscopy was observed in the lineal charts representing the four "accumulated histograms" (Fig. 2). The control one presents two clearly separated peaks. The first one corresponds with zone A (centrolobular) and is situated about the standard value of 1,000, with a mean intensity of 965 (Table 1) and accounts for 56.62 % of the total number of points measured (Table 2). The second peak (zone B) is placed about 1,600, with a mean intensity of 1546 and involving 43.38 % of the points.

Regarding the partially hepatectomized animals the first peak is situated around the standard value of 1,000, includes 73.76 % of the points and shows a mean intensity of 968. The second peak is placed about 1,380, involving 26.24 % of the measured points and presenting a mean intensity of 1,328.

The first peak of the histogram on the CsA-treated animals takes in 86.44 % of the points, with a mean intensity of 908. The second peak, including 13.56 % of the points, is located about 1,300, is hardly distinguishable from the first one and shows a mean intensity of 1,338.

The histogram about CsA-treated and partially hepatectomized animals presents a first peak which accounts for 88.2 % of the measured points, with a mean intensity of 933. The second peak, which is undistinguishable from the first one, is situated about 1,250, includes 11.8 % of the points and presents a mean intensity of 1,277.

Superposing the four lineal charts -control, hepatectomy, CsA-treated and hepatectomy+ CsA (Fig. 2)-, two events are seen: 1) The curves of the three experimental series are displaced to the left with respect to the control one, and this displacement is greater on the CsA-treated animals (both normal and hepatectomized); 2) The second peak of the histogram, which decreased in the hepatectomized animals, is not evident on the CsA-treated animals.

The average of points corresponding to the second peak of the histogram (43.38 %) decreased by 39.5 % with hepatectomy (26.24 %). The hepatectomized animals treated with CsA showed a lower dark-zone average (11.8 %), which means a diminution of 72.8 % if compared to the control animals. CsA alone induced a decrease by 68.8 % in zone B (13.56 %) (Fig. 3).

The intensity of the reaction in zone B also decreased by 14-17 % in all three experimental series if compared to the control animals (1,564 versus 1,316 $\pm$ 22). This diminution, statistically significative ($p < 0.05$), was similar in the hepatectomized and CsA-treated animals, but it was significantly greater ($p < 0.05$) when both treatments were associated.

DISCUSSION

Metabolic heterogeneity in rat liver parenchyma is a well established fact which is still being studied. Hepatocytes of the periportal and the perivenous zone are different with respect to enzyme content and ultrastructure (Jungermann and Katz, 1982). Gluconeogenesis is catalyzed preferentially by the periportal cells while glucolysis is mediated mainly by the perivenous hepatocytes (Katz et al., 1977).

Isolated hepatocytes may be fractionated into various subpopulations by using centrifugal elutriation (Summer et al., 1983). Hepatocytes separated by that method differ in enzyme content, and these differences are superposable with those observed between periportal and perivenous hepatocytes (Kiessling et al., 1981).

Therefore, it may be sustained that hepatocytic heterogeneity is a source of liver metabolic zonation (James et al., 1986).

According to these data, other authors suggest that zonal heterogeneity is dynamic and functional rather than static or structural (Andersen et al., 1984; Barberá-Guillem and

Vidal-Banaclocha, 1986). This is why it is not surprising that in determinate circumstances liver zonation may be changed or even be lost.

In our study the zonal heterogeneity, which was present in the normal control rats, was changed after partial hepatectomy because of the diminution SD activity in periportal zone if compared with perivenous area. This agrees with previous studies (Andersen et al., 1984; Brinkmann et al., 1978; Jungermann and Katz, 1982). Biological mechanisms responsible for this change have not been described. We suggest that hepatocytic metabolic changes following partial hepatectomy may be related either to the stimulus inducing regeneration or to environmental changes induced by the regenerative process in liver parenchyma. Since the nature of the initial factor in liver regeneration is still under discussion (Baker, 1986; Caruana and Gage, 1980; Greisler et al., 1979; Ruthenstroth-Bauer et al., 1984) it is not easy to elucidate which of these two suggested mechanisms is responsible of the zonal modifications, or if even both of them must be considered.

Regarding CsA, we can say that it induced a zonal dedifferentiation similar to that happening after partial hepatectomy, but quite more intense. This is a new unexpected finding and we have not found any reference of similar phenomena induced by other pharmacological treatments.

In a previous study we found that CsA induces hepatic regeneration and increases liver regeneration induced by partial hepatectomy (García-Alonso et al., 1988). Because liver regeneration is accompanied by a certain degree of zonal dedifferentiation we initially supposed our results were the logical consequence of liver regeneration induced by CsA. Nevertheless, intensity of CsA-induced liver regeneration is not proportional to the degree of the induced zonal dedifferentiation. Moreover, it has to be considered that CsA-induced and hepatectomy-induced dedifferentiations have been proved to be synergic processes. For this reason, we suggest that CsA, apart from inducing liver regeneration, has a metabolic effect on the hepatocytes, being this effect, which might be direct or indirect responsible for a certain degree of zonal dedifferentiation in liver parenchyma.

Though some reports have described a specific membrane receptor for CsA on the surface of human lymphocytes (Leapman et al., 1983; Ryffel et al., 1982), other authors suggest an unspecific mechanism for the binding of CsA to biological membranes (LeGrue et al., 1983a,b). CsA extremely hydrophobic character can explain its easy insertion into cell membrane, which might increase membrane surface area and fluidity, leading to an uncoupling of the electrochemical membrane hyperpolarization. These modifications in membrane characteristics could

mediate a change in the enzymatic activity of hepatocytes, which would result in a blurring of the zonal heterogeneity in liver parenchyma. This would be in line with our first hypothesis of a direct effect of CsA on hepatocytes.

On the other hand, existing evidence of a relation between immune system, immunomodulatory drugs and liver regeneration (Babaeva et al., 1980,1982; Desser-Wiest and Desser, 1980 a,b; Gieldanowski, 1983; Miyahara et al., 1983; Sobotka et al., 1980; Yokomuro et al., 1983) supports our suggestion about an indirect effect of CsA over hepatocytes, which could be mediated by any of the immune cells.

It has also been said that zonal heterogeneity may be due to ambiental differences inside the liver parenchyma (Vidal-Banaclocha and Barberá-Guillem, 1985). We might, then, assume that CsA in one way or another modifies this differentiating effect: either reducing environmental differences or decreasing hepatocytic sensibility to those ambiental differences. As hepatocytic delimitation is initiated after the first week after birth following endothelial cells differentiation which is already established before birth (James et al., 1986), an inductor role of the endothelial cells in liver zonal heterogeneity might be suggested. This would be in line with our last hypothesis about an indirect effect of CsA, but suggesting another possible mediator different from the immune system: liver endothelial cells.

Anyhow, whether CsA exerts its effects on SD liver parenchyma zonation indirectly through immune or endothelial cells or directly on hepatocytes remains an open question.

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