PNH-Like Red Cells: Mechanism of Action of Sulfhydryl Compounds on Normal Red Cells

It is known that concentrated solutions of different sulfhydryl compounds at alkaline pH can alter normal red cells in such a way that they become similar in many respects to the red cells of paroxysmal nocturnal haemoglobinuria (PNH)[1-4]; in particular, they lyse in slightly acidified normal serum and in a medium of low ionic strength if complement is present, and display a low acetylcholinesterase (AChE) activity. STICKNEY et al.[5], using GS^{35}H solutions found that radioactivity was firmly bound to PNH-like cells; on the contrary GSSG did not bind to the cell membrane and did not induce the PNH-like lesion. On the basis of this and other evidence, KANN et al.[4] suggested that the sulfhydryl compounds split the membrane -S-S- bonds and reduce them or form with them mixed disulfides. The data so far available, however, do not indicate whether both reactions take place and are important in determining the PNH-like lesion and whether mixed disulfides are formed through a thiol-disulfide or a disulfide-disulfide exchange. This latter mechanism could occur since it is known that sulfhydryl groups in alkaline solution and in the presence of O_2 partly convert to disulfides[6]. In order to investigate these problems, normal red cells were treated with a solution made up of 9 volumes of a 0.685 *M* solution of GSSG and 1 volume of an equimolar solution of GSH.

The control solutions were: 1. A 0.685 *M* solution of GSH (i.e. that used by MERIWETHER et al.[7] to induce the PNH-like lesion); 2. A mixture of 9 volumes of a 0.685 *M* solution of GSSG and 1 volume of saline; 3. A mixture of 9 volumes of saline and 1 volume of the 0.685 *M* solution of GSH; 4. Saline.

All the solutions were brought to pH 8.0 by a 5*N* NaOH solution and incubated with normal red cells for 15 min at 37°C. After several washings, treated red cells were tested in the acidified-serum test and in the sucrose-haemolysis test, and their AChE activity determined. The experiment was replicated 3 times; results were comparable and those of one of them are reported in the Table.

RBCs incubated with the following solutions (pH 8.0)	Acidified-serum test (% lysis)	Sucrose-haemolysis test (% lysis)	Acetylcholinesterase (mU/ml RBCs)	(% inhibition)
Saline	0	0	13,500	0
GSH	66	18	9,490	30
GSH + GSSG (1:9 v/v)	40	0	11,400	16
GSH + Saline (1:9 v/v)	0	0	13,690	0
GSSG + Saline (9:1 v/v)	13	0	13,580	0

Results of one of the experiments performed. The composition of the solutions of the indicated compounds is reported in the text.

It appears from the Table that the mixture of a solution of GSH at a concentration per se not capable of inducing a PNH-like lesion with a solution of GSSG (per se also inactive on the red cells) induces in normal red cells a PNH-like alteration. This, however, is different from that induced by the higher concentration of GSH, since the cells lyse only in the acidified-serum test, and here lysis is less marked than that observed with the usual PNH-like cells. Moreover, the inhibition of AChE activity was less marked in red cells incubated with the GSSG + GSH solution than in those incubated with the GSH solution. Apropos, it may be noted that the enzyme activity-decrease induced by this latter solution is not so marked as that observed in AET-treated cells[2], which thus resemble PNH cells more closely.

The above findings suggest that the formation of a mixed disulfide between the altering compound (GSSG) and the cell membrane can in fact determine the PNH-like lesion, although only partial. It seems likely that the mechanism involved is that of an exchange between the disulfides of the cell membrane and those of GSSG, catalyzed by GS^- groups. These could initiate the reaction by splitting the membrane –S–S– bonds with formation of a mixed disulfide and a Membrane-S^- group. The latter, by reacting with the disulfide reagent, could then produce another mixed disulfide and a new molecule of the initiator, according to the following scheme:

1. G–S^- + Membrane-S–S–Membrane → Membrane-S–S–G + Membrane-S^-
2. Membrane-S^- + G–S–S–G → Membrane-S–S–G + G–S^-

When concentrated solutions of thiols are used, RS^- groups are predominant and a more effective split of the membrane –S–S– bonds occurs. Moreover, it is possible that these bonds, split by the altering solutions, recombine in an abnormal way, so causing an arrangement of membrane proteins and/or lipids that could play a role in the cell abnormality. Furthermore, it cannot be excluded that a certain degree of oxidation of the membrane –SH groups occurs. All these possibilities become acceptable if one bears in mind the complexity of the reactions that occur when sulfhydryl compounds are involved, although they cannot be assessed by the present experiment which indicates only that the split of membrane –S–S– bonds is a fundamental process by which the sulfhydryl compounds induce a PNH-like lesion in normal cells.

Riassunto. Si è studiato se l'incubazione di emazie normali con una soluzione di GSSG addizionato di una piccola quantità di GSH fosse in grado di provocare in loro la lesione simil-EPN (emoglobinuria parossistica notturna). Si è osservato che questa in effetti si produce, probabilmente attraverso un meccanismo di scambio disolfurico tra i legami –S–S– della membrana eritrocitaria e quelli del GSSG, catalizzato da gruppi GS^-.

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