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An Evaluation of Methods for Grading Histologic Injury Following Ischemia/Reperfusion of the Small Bowel

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INTESTINAL injury following ischemia and reperfusion (I/R) of the gut is a common clinical complication that is most often assessed by histologic evaluation of standard hematoxylin and eosin (H&E) stained tissue sections. For this purpose, many different grading systems for bowel injury have been described in the literature. Unfortunately, there is no consensus on how this injury should be graded. Instead, most studies have used their own systems, which often amount to qualitative or semiquantitative descriptions. Thus, it is difficult to compare the results of studies that were evaluated using different grading systems. However, quantitative comparison of results is essential for the development of an understanding of the mechanisms of I/R injury and the development of treatment strategies.

The overall goal of this preliminary study was to determine whether it is possible to recommend a standard histologic grading system that would be broadly suitable for use in typical experimental studies of I/R of the gut. The specific aims of this study were first to identify the histologic grading systems available for assessment of intestinal I/R injury; and second, to examine the validity, reliability, and ease of use of the most commonly used or representative grading systems using tissue sections from a study of I/R of the rat small bowel.

MATERIALS AND METHODS

Literature Study

Using MEDLINE (Ovid Technologies, NY) from 1966 to the present, we searched the literature for articles reporting on histologic intestinal I/R injury. A large number and variety of grading systems were identified. In many of these studies, however, histologic damage was either just described, with or without semiquantitative grades attached to this description, or the authors developed entirely new grading systems to suit their needs. From this literature we selected three systems that were most representative of the different types of grading systems. These were the systems developed by Parks,[1] Sonnino,[2] Chiu[3] and Park.[4] These systems are described briefly below and in Table 1.

Parks' system scores mucosal injury in intestinal villi and in crypts separately from grade 0 to 4, according to the number of villi and crypts affected by epithelial necrosis.[1] Sonnino's system also grades only mucosal injury using a large number of different, cumulative criteria, each of which are scored 0 for a normal or 1 for an abnormal finding.[2] The system developed by Park encompasses a

system that was earlier developed by Chiu and scores by progression of intestinal injury from the tips of the villi into the deeper layers of the gut wall in 8 grades.[3,4] The present study refers to this system as the Park/Chiu system to avoid any confusion due to the names of the authors.

Experimental Procedures

Using the three selected grading systems, we reevaluated histologic damage in tissue sections from a previous study of I/R of the rat small intestine.[5] This study compared two groups of adult male Lewis rats. The first group of rats ($n = 10$) was subjected to 30 minutes of total warm ischemia of the small bowel by occlusion of the superior and inferior mesenteric arteries, followed by 60 minutes of reperfusion, as previously described.[6] The second group ($n = 11$) received sham occlusion. At the end of the reperfusion period the rats were sacrificed and the terminal ileum was removed and processed for histological analysis. The tissues were processed using standard histologic techniques including formalin fixation, dehydration and paraffin embedding, then cut in $4\text{-}\mu m$ sections and stained with H&E.[6] The sections of both groups were coded, and evaluation was carried out by two independent, experienced observers blinded to the treatment groups. The correlation coefficients between the scores of the independent observers were calculated separately for each of the three grading systems. The mean of the scores for each section obtained by the two observers were used to compare the three systems together.

Table 1. Description of Scoring Systems

Parks	Sonnino	Park/Chiu
Villus epithelial cell inflammation and necrosis	0 for normal, 1 for abnormal findings:	Chiu
0. No damage	Villi	0. Normal mucosa
1. Occasional tips affected	Present/absent	1. Subepithelial space at villus tips
2. Majority of tips affected	Normal/abnormal	2. Extension of subepithelial space
3. Majority of tips and some	long, short, narrow, wide	with moderate lifting
villi affected	Epithelium	3. Massive lifting down sides of villi,
4. Tips, mid- and lower	Normal/abnormal cell loss, complete	some denuded tips
portions of majority of villi	denudation, edema	4. Denuded villi, dilated capillaries
affected	Connective tissue	5. Disintegration of lamina propria
Crypt epithelial cell inflammation	Normal/abnormal edema, intra- or	Park
and necrosis:	extravascular infiltrate	6. Crypt layer injury
0. No damage	Lacteals	7. Transmucosal infarction
1. Occasional crypts affected	Present/absent	8. Transmural infarction
2. Scattered crypts affected	Normal/abnormal dilated, occluded	
3. Many crypts affected	Crypts	
4. Majority of crypts affected	Present/absent	
	Normal/abnormal degeneration	
	disorganization	
	Regeneration: present/absent	
	Lamina propria	
	Present/absent	
	Normal/abnormal edema, MMN or PMN	
	infiltrate, intravascular stasis,	
	hemorrhage	
	Muscularis mucosae	
	Present/absent	
	Normal/abnormal intravascular stasis,	
	hemorrhage	
	Bacteria	
	Present/absent	

RESULTS

The literature search identified 60 papers in which intestinal damage caused by I/R of the gut was assessed using histologic evaluation. A full listing of these references is available on request. In 18 (30%) of these papers intestinal injury was described without grading. Park's system was used in 5 papers (8% of all papers); Sonnino's system in 2 (3%); Chiu's system in 13 (22%), and the system of Park/Chiu was used in 7 (12%). The remaining 15 papers used other grading systems, usually reported only by one research group.

The evaluation of intestinal injury in the two experimental rat groups by the two independent observers using the three selected systems demonstrated that the Park/Chiu system had the best correlation between the two observers ($r^2 = .86$). Parks's system produced a relatively good correlation for the grading of villus damage ($r^2 = .74$); however, the crypt scores showed much greater discrepancies ($r^2 = .26$). Finally, Sonnino's system showed the least overall correlation between the observers ($r^2 = .41$). To better examine the pattern of differences in scoring, we related the differences between scores to the mean scores as shown in Fig 1.[7] The systems by Park/Chiu and Parks showed relatively greater variation between the observers at moderate levels of damage than in the sections with little

damage or those with severe damage; however, the overall differences between the observers were linear. The mean $\pm$ SD differences were 0.64 ± 1.03 for the Park/Chiu and 0.52 ± 1.0 for the Parks system for villi. The Sonnino system showed much greater discrepancies, particularly in the sham control sections, with a mean difference of 4.14 ± 3.1.

When the results of the Park/Chiu system were correlated with the other two systems, it was apparent that Soninno's system, as used by the two experienced observers, suggested that there were significant levels of damage in sham control sections. These were scored as zero with either of the other systems (Fig 2). The correlation between the Park/Chiu and Parks (villi) systems was excellent ($r^2 = .99$), whereas the correlation with the Sonnino system was weaker ($r^2 = .83$).

DISCUSSION

I/R of the small intestine is a common clinical problem, seen for example in neonatal necrotizing enterocolitis and small bowel transplantation, and is thus the subject of considerable ongoing research. The degree of injury caused by intestinal I/R is routinely assessed by histologic evaluation. A commonly used and readily available process for tissue preparation is formalin fixation, dehydration and paraffin embedding, after which sections of 4 μm thickness

Fig 2. The correlation between the Park/Chiu system with both the Parks and Sonnino systems for the mean scores of the two blinded observers. The Parks score for villi correlated very well with the Park/Chiu system ($r^2 = .99$). However, the Sonnino system showed a less good correlation with the Park/Chiu system ($r^2 = .83$), but also a significant baseline shift, with some apparent damage in sham control sections which was not seen with the other two systems. The solid and dotted lines represent the linear correlation and confidence intervals, respectively.
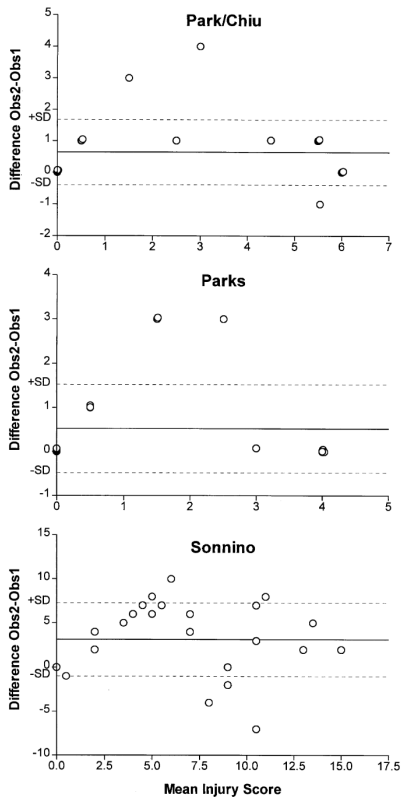
Fig 1. Relationship between the differences between the scores by the two masked observers, and the mean scores for the Park/Chiu, Parks, and Sonnino systems of grading injury, in 21 sections of the small intestine of rats subjected to either sham ischemia or 30 minutes of warm ischemia, followed by 60 minutes of reperfusion. The mean differences are shown by solid lines and the ±1 SD ranges by dotted lines.

are cut and stained with H&E. Intestinal damage may be assessed either by choosing the most affected area for grading or by averaging the scores from sections at standard areas.

Ideally, to allow comparisons between different studies of I/R of the bowel, the tissue damage would be quantified using a single standard sys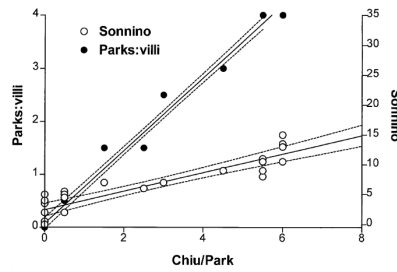tem, applied to sections that are prepared using this standard method. Our literature search has demonstrated that currently there is no consensus on such a standard grading system making comparisons between studies difficult. Yet comparison between studies that is quantitative as well as qualitative is essential for the determination of potential mechanisms underlying injury and for the development of treatment strategies.

An ideal standard grading system should include the following features. The grades should parallel the morphologic appearance of injury with increasing severity of the insult, and be reliable, producing consistent results within and between observers. Biological validity in this context implies that this hypothetical system should assess both the extent of overall damage to the architectural elements of the bowel wall, including the villi and crypts, as well as more detailed evidence of mucosal and submucosal damage, such as epithelial lifting and mucosal cell debris in the lumen. Typically, ischemic injury leads to progression of damage from the villus tips after the briefest or mildest periods of ischemia, extending to the crypts only after much longer periods of ischemia.[3] Of equal importance is that an ideal grading system should be logically presented, easy to learn, and use and have a low inter-observer variability.

From our literature search we concluded that the Park/Chiu system is the closest to meeting these criteria. This combined system grades the progression of morphologic injury from mild to severe. The grades encompass both architectural and more detailed damage and are well defined, which leads to a low inter-observer variability. The Parks system was equally reliable in the present study; however, it does not include injury beyond the mucosa and its structure, which separates villus and crypt damage, made it more cumbersome to use and interpret. In the present

study of I/R-induced intestinal injury in the rat, damage was primarily seen in the villi, and thus there was excellent agreement between the Parks (villi) scores and those of the Park/Chiu system. With a significantly more severe insult, we would have anticipated increasing damage to the crypts which would be linearly reflected in the Park/Chiu system, but would require assessment of two scores with the Parks system.

In many grading systems only one aspect of I/R damage is singled out and graded according to severity. An example of this is the system by Parks, which only assesses the extent of the epithelial cell necrosis. Other systems, like Sonnino's, are very extensive, scoring a wide range of features; however, this system does not directly assess the progression of injury along the villi and crypts. This is demonstrated in Fig 2; although the Sonnino damage scores increased linearly, the most severe damage scored was still only half of the maximum possible, while the Park/Chiu system had reached a very severe score of 6 out of 8. Further, the sham control sections were rated as having significant damage in Sonnino's system, which was not a feature of the other two scales.

All of the grading systems would have benefited from more clear and precise descriptions of the grades. This is likely to have contributed to some of the differences between the observers (see Fig 1). For example, the system used by Parks grades epithelial cell necrosis, but does not describe which characteristics of necrosis should be assessed. It is not clear whether the system purely grades cytologic evidence of necrosis, such as rounding of the normally cuboid epithelial cells, or whether epithelial lifting is also considered to be evidence of epithelial necrosis. Sonnino's system was the most difficult to implement for both observers. A large number of different criteria are used to assess intestinal damage. However, again these criteria are only summarized, and not explained in detail, which leaves the reader to decide what is actually meant by factors such as intravascular stasis, regeneration, and disorganization.

In addition to basic morphologic I/R damage, more detailed features of intestinal I/R injury including leukocyte infiltration, apoptosis, and proliferation are a component of many grading systems such as Sonnino's. However, proper evaluation of such detailed objectives is difficult and time consuming. We propose that it would be more appropriate to assess these objectives separately to the basic morphologic assessment and that these criteria should not be incorporated in a standard grading system. Apoptosis and proliferation are better assessed using specialized techniques, such as immunohistochemical staining and TUNEL.

In conclusion a standard quantitative and qualitative histologic scoring system would make it much easier to compare of studies of I/R. However, to date no one system has been consistently supported in the literature. An ideal system should be comprehensive in scope, assessing the evolution of injury from mild to severe damage. It should also be well described and easy to use to ensure low inter-observer variability. From our assessment of the literature and the present evaluation of representative systems, we propose that the combined grading system of Chiu and Park is the most suitable to be recommended as a standard scoring scale for histological evaluation of intestinal I/R damage. However, better description of the last grades of this system would further strengthen its suitability.

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