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COMMENTARY

ROLE OF HEPATOCELLULAR REGENERATION AND HEPATOLOBULAR HEALING IN THE FINAL OUTCOME OF LIVER INJURY

A TWO-STAGE MODEL OF TOXICITY

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The ability to overcome injury from physical or chemical agents encountered in the environment is a remarkable attribute of all living organisms. In an attempt to enhance survival from any noxious injury, organisms have developed several lines of defense mechanisms. By and large, such mechanisms may be categorized into two classes, each representing one tier. One is represented by biochemical mechanisms which enable the organism to prevent injury after a noxious insult. The second is a biological response intended to overcome injury, by promoting tissue healing after the fact, as it were. While much attention has been focused on the endogenous biochemical defense mechanisms, which participate in preventing the infliction of cellular and tissue injury, the biology of endogenous mechanisms, which might be recruited to overcome tissue injury after it occurs, has received little attention. This commentary is intended to draw the latter mechanism into greater focus for conceptual and investigative interest as well as for experimental scrutiny and verification.

Mechanism of CCl_4 *hepatotoxicity*

The mechanism of CCl_4-induced hepatotoxicity has been studied extensively [1]. Since the mechanism underlying the toxicology of CCl_4 is central to the consideration of how its toxicity might affect the liver tissue and how this might be modified by other chemicals, it is worthwhile to outline the prevailing concepts concerning the hepatotoxicity of CCl_4. Several reviews have appeared on this topic [1–5]. The leading theory for the mechanism of cellular damage caused by CCl_4 is that the compound is bioactivated by cytochrome P450 mediated reactions to $\cdot CCl_3$ free-radical [1–7], which is further converted to a peroxy radical, $CCl_3O_2^{\cdot}$ [2]. There is evidence for covalent binding of CCl_4 upon bioactivation [1–7]. The $CCl_3O_2^{\cdot}$ radical is also thought to decompose to phosgene and electrophilic Cl^-, which can react with other macromolecules [8]. The free radicals $\cdot CCl_3$ and $CCl_3O_2^{\cdot}$ readily react with polyunsaturated fatty acids of the endoplasmic reticulum and other hepatocellular membranes to initiate the formation of organic lipid peroxides. In the presence of cellular O_2, these organic peroxy radicals in turn can react with other polyunsaturated fatty acids to perpetuate a series of self-propagating chain reactions, a process commonly referred to as "propagation of lipid peroxidation" [1]. The bioactivation of CCl_4 and the initiation of the self-propagating lipid peroxidation, working in tandem, destroy the cellular membranes leading to cell death. The principal hepatic lesion is characterized by centrilobular necrosis [9], the extent of injury depending upon the dose. Demonstration of the metabolism of CCl_4 to $CHCl_3$ and to CO_2, and covalent binding of CCl_4 to liver protein and lipid, lend experimental support to the bioactivation theory of CCl_4 injury [1, 2, 10–12].

Mechanism of $BrCCl_3$ *and* $CHCl_3$ *toxicity*

Hepatotoxicity of $BrCCl_3$, a brominated analog of CCl_4, is also by virtue of its metabolism to the same $\cdot CCl_3$ radical [6, 12–14] formed from CCl_4. Much greater toxicity of this compound [13, 14] in comparison to CCl_4 has been attributed to the relative ease with which the C—Br bond can be cleaved [6]. A clear inverse relationship exists between the bond dissociation energy of this series of halomethanes ($BrCCl_3 < CCl_4 < FCCl_3 < HCCl_3$) and their potency to initiate free-radical reactions [1–5], to produce lipid peroxidation, and to produce liver necrosis.

With regard to $CHCl_3$ the results of several investigations suggest that phosgene, a reactive metabolite of $CHCl_3$, is responsible for its hepatotoxic, nephrotoxic [15] and possibly its carcinogenic [16, 17] effects. Hepatotoxic effects are due to phosgene-mediated cellular glutathione (GSH) depletion in tandem with the increased covalent binding to hepatocellular macromolecules [16, 18]. Although like CCl_4, $CHCl_3$ also needs metabolic activation to exert its full necrogenic potential, unlike CCl_4, lipid peroxidation is not involved in hepatocellular necrosis. A second important distinction is that, unlike CCl_4, metabolism of $CHCl_3$

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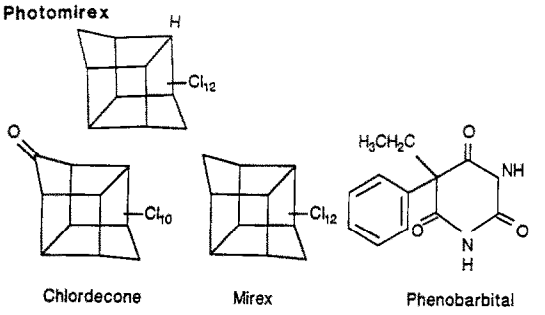

Fig. 1. Structures of chlordecone, mirex, photomirex and phenobarbital.

to a free-radical form has not been associated with its necrogenic action [16]. Recent studies on $CHCl_3$ toxicity have involved mouse hepatocyte primary cultures [19] and the Mongolian gerbil [20–22].

Interactive toxicity of chlordecone and CCl_4

A major toxicological issue from a public health perspective is the possibility of unusual toxicity due to interaction of toxic chemicals upon environmental or occupational exposures to two or more chemicals, at individually harmless levels. While some laboratory models exist for such interactions for the simplest case of only two chemicals, progress in this area has suffered for want of models where the two interactants are individually nontoxic. One such model is available, where prior exposure to nontoxic levels of the pesticide Kepone® (chlordecone, CD) results in a 67-fold amplification of CCl_4 lethality in rats. The mechanism of the remarkable interactive toxicity is of interest in the assessment of risk from exposure to combinations of chemicals.

Prior exposure to a nontoxic level of CD (10 ppm in diet for 15 days) results in a marked amplification of CCl_4 hepatotoxicity [23] and lethality [24, 25]. Neither the close structural analogs of CD, mirex (M) and photomirex (PM), nor phenobarbital (PB) (Fig. 1), exhibit this property [23, 24]. Furthermore, hepatotoxic and lethal effects of $BrCCl_3$ [26, 27] and $CHCl_3$ [28–31] are also potentiated by exposure to 10 ppm dietary CD. This remarkable capacity to potentiate halomethane hepatotoxicity does not appear to be related to CD-induced cytochrome P450 or associated enzymes [23, 32], enhanced bioactivation of CCl_4 [21–23, 33], increased lipid peroxidation [23, 32, 34], or decreased glutathione [34].

Mechanism of the interactive toxicity of chlordecone + CCl_4

Based on several lines of experimental evidence, a hypothesis has been proposed [3–5] for the mechanism of the interactive toxicity of CCl_4 by CD.

First, it became necessary to hypothesize the mechanism for *why an ordinarily nontoxic dose of* CCl_4 is nontoxic [23]. Figure 2 illustrates the mechanism of recovery from the limited liver injury observed after the administration of a low dose of CCl_4 alone. Within 6 hr after the administration of

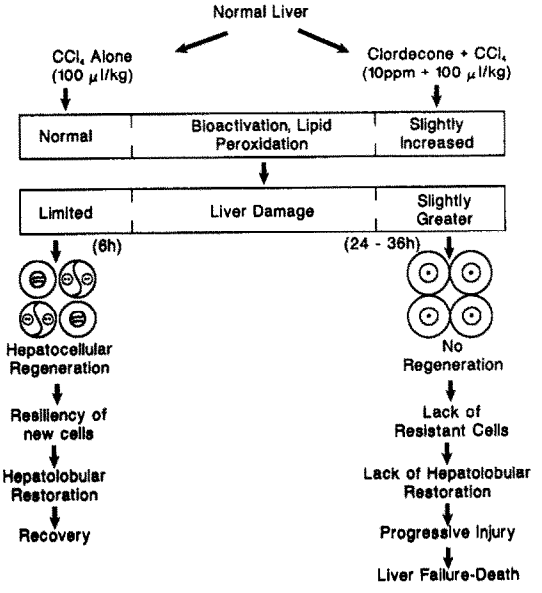

Fig. 2. Proposed mechanism for the highly amplified interactive toxicity of chlordecone + CCl_4. The scheme depicts the concept of suppressed hepatocellular regeneration, simply permitting what is normally limited liver injury caused by a subtoxic dose of CCl_4 to progress in the absence of hepatolobular repair and healing. The limited hepatoxicity from a low dose of CCl_4 is normally controlled and held in check owing to the hepatocellular regeneration and hepatolobular healing. The chlordecone + CCl_4 combination treatment results in uncontrolled permissive progression of injury owing to a lack of tissue repair. These events lead to complete hepatic failure, culminating in animal death. Adapted from Ref. 5.

a low dose of CCl_4, limited hepatocellular necrosis inflicted by the same widely accepted mechanisms of CCl_4 bioactivation followed by lipid peroxidation occurs. By mechanisms hitherto unexplored, simultaneously the liver tissue responds by stimulating the early phase of hepatocellular regeneration at 6 hr [35, 36]. The limited hepatocellular necrosis enters the progressive phase between 6 and 12 hr [35–38], while the hepatocellular regeneration and tissue healing processes continue. By 24 hr, no significant liver injury is evident. Any remaining level of tissue injury is overcome by the second phase of hepatocellular division which occurs at 36–48 hr [37–40]. These observations allow one to propose that stimulation of hepatocellular regeneration is a protective response of the liver, occurs very early after the administration of a low dose of CCl_4, and leads to replacement of dead cells, thereby restoring the hepatolobular architecture [3, 4, 23].

Furthermore, this remarkable biological event results in another important protective action. It is known that newly divided liver cells are relatively resistant to toxic chemicals [19, 41]. Therefore, in addition to the restoration of the hepatolobular architecture by cell division, by virtue of the relatively greater resistance of the new cells, the liver tissue is able to overcome the imminence of greater injury

during the progressive phase (6–12 hr), obtunding the spread of injury on the one hand, and speeding up the process of overall recovery through tissue healing on the other (Fig. 2). By 6 hr over 75% of the administered CCl_4 would have been eliminated in the expired air [32] leaving less than 25% for continued injury [23]. Relative resistance of the newly divided cells at this critical time frame, as the animal continues to exhale the remaining CCl_4, would be an added critical defense mechanism. At later time points (12 hr and onwards), most of the CCl_4 would have been eliminated by the animal and hence continued cellular regeneration during this time period and at later time points allows for complete restoration of the hepatolobular architecture during and after the progressive phase of injury [37–40].

Administration of the same low dose of CCl_4 to animals maintained on food contaminated with a low dose of CD results in initial injury by the same mechanisms of bioactivation of CCl_4 and lipid peroxidation (Fig. 2). The liver injury in this case is slightly greater by virtue of the approximately doubled rate of bioactivation of CCl_4 in livers of animals preexposed to CD [4, 23, 32]. The liver injury thus initiated enters the progressive phase between 6 and 12 hr and this phase is accelerated in the absence of tissue repair mechanisms [35–40]. The highly unusual amplification of CCl_4 toxicity relates to the suppression of the initial hepatocellular regeneration, otherwise ordinarily stimulated by CCl_4 within 6 hr (Fig. 2).

The mechanism responsible for the abrogation of this hormetic mechanism of stimulated cell division is of significant interest. At this juncture, experimental observations permit invoking a role for hepatocellular bankruptcy in cellular energy. Under conditions of increased hepatocellular injury, mobilization of hepatic glycogen is initiated in order to stimulate hepatocellular division [36, 42]. Under these conditions of increased demand for cellular energy (augmented need for extrusion of extracellular Ca^{2+} from the cells, protection against free-radical mediated injury, etc.), the hepatocytes are incapacitated due to insufficient availability of cellular energy. Stimulation of cell division, which normally occurs after administration of a low dose of CCl_4, cannot occur. The failure of cell division has two important implications: first, hepatolobular structure cannot be restored; second, unavailability of newly divided, relatively resistant cells predisposes the liver to continuation of liver injury during the progressive phase (6–12 hr and beyond) [3–5, 23, 42, 43]. Permissively progressive injury continues as a consequence of the mitigated tissue repair mechanisms, leading to massive hepatic failure [23–26], followed by death of the animal [3–5].

Increased accumulation of extracellular Ca^{2+} [44] during the progressive phase of liver injury would be consistent with the significant loss of biochemical homeostasis in hepatocytes (see Fig. 4). Earlier histomorphometric [26] as well as biochemical studies [3, 4, 45, 46] have shown that glycogen levels drop very rapidly after CCl_4 administration to CD-treated animals. Increased cytosolic Ca^{2+} [43] would be expected to result in activation of phosphorylase *b* to phosphorylase *a*, the enzyme responsible for glycogenolysis. Phosphorylase *a* activity [42, 43] and precipitous loss of glycogen [35, 36, 43, 44] are experimental observations consistent with the rapid depletion of cellular energy [43] on the one hand, and irreversible increase in cytosolic Ca^{2+} [42] on the other.

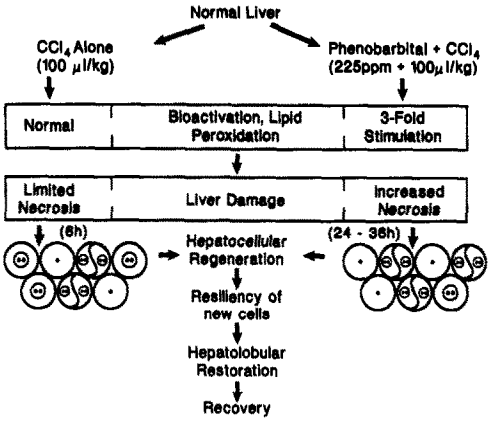

Fig. 3. Proposed mechanism for phenobarbital-induced potentiation of CCl_4-hepatotoxicity in the abence of increased lethality. Normal liver response to a low-dose CCl_4 injury is not abrogated by phenobarbital + CCl_4 interaction. Instead, the early phase of cell division is postponed (from the normal 6 hr to 24 hr). Enhanced putative mechanisms such as increased bioactivation of CCl_4 and resultant increased lipid peroxidation are responsible for increased injury. Because hepatocellular regeneration and tissue repair processes continue albeit a bit later than normal, these healing mechanisms permit tissue restoration resulting in recovery from the enhanced liver injury. This mechanism explains the remarkable recovery from phenobarbital-induced enhancement of CCl_4 liver injury. Despite a remarkably enhanced liver injury by phenobarbital, this is of no real consequence to the survival of the animal. Adapted from Ref. 5.

An intriguing aspect of the experimental framework leading to the proposed mechanism is the observation that PB, even at significantly higher doses (225 ppm in the diet for 15 days), does not potentiate the lethal effect of CCl_4. Although histopathological parameters of liver injury such as hepatocellular necrosis and ballooned cell response are indicative of significantly enhanced hepatotoxicity by PB, if the animals are left alone, this injury does not progress to significantly increased lethality. Hepatic microsomal cytochrome P450 is approximately doubled by prior dietary exposure to 225 ppm PB and the bioactivation of CCl_4 is tripled [23, 32], and these parameters are consistent with the enhanced initiation of liver injury measured by histopathology, elevation of serum transaminases or by hepatic function. Nevertheless, the liver injury neither progresses in an accelerated fashion nor is irreversible, as indicated by the reversal of liver injury accompanied by animal survival [23, 24, 38].

Figure 3 illustrates the proposed mechanism for PB-enhanced liver CCl_4 injury, which is associated with a lack of enhanced lethality. Induction of hepatomicrosomal cytochrome P450 results in

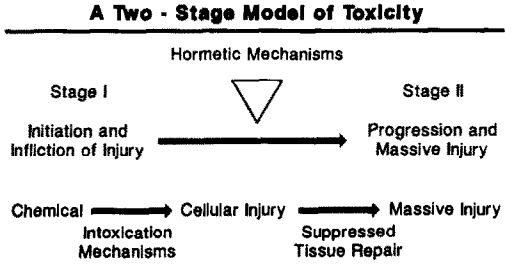

Fig. 4. Scheme illustrating the proposed two-stage model of toxicity. The molecular, cellular and tissue repair mechanisms, which permit healing processes to obtund progression of injury, are collectively referred to as hormetic mechanisms [48] for the purpose of schematic illustration of the two-stage model.

approximate tripling of CCl_4 bioactivation and increased lipid peroxidation [23, 32]. Enhanced liver injury is consistent with these observations (Fig. 3). It should be recalled that the liver is normally able to respond by stimulation of hepatocellular regeneration after a low dose of CCl_4 within 6 hr (Fig. 2). While PB exposure results in greater injury, the ability of the liver to respond by stimulated cell division is not completely compromised, as evidenced by the stimulation of hepatocellular regeneration starting at 24–36 hr and continuing through 72 hr. Therefore, hepatocellular regeneration is stimulated thereby counteracting the enhanced liver injury, which leads to recovery from increase in liver injury. The hypothesis that suppression of hepatocellular regeneration and tissue repair leads to the progression of liver injury was experimentally validated in the partial hepatectomy model [33, 37, 39, 40, 47].

Two-stage model of toxicity: A hypothesis

An intriguing outcome of the work on the mechanism of the interactive toxicity of CD + CCl_4 is the emergence of a concept, which permits the separation of the early events responsible for initiating injury from subsequent events which determine the final outcome of that injury (Fig. 4). Hormetic mechanisms [48] are activated upon exposure to low levels of halomethanes [27, 36, 38–40, 44]. Although the cellular mechanisms responsible for triggering a dramatic mobilization of biochemical events leading to cellular proliferation within 6 hr after exposure to a subtoxic dose of CCl_4 [35, 36, 38–40] are not understood, it is clear that these early events are the critical determinants of the final outcome of injury [3–5]. When this early phase of hepatocellular division is suppressed as has been observed in animals exposed to CD [35, 36, 38–40], a permissive progression of liver injury leading to massive coagulative hepatic necrosis is observed [3–5]. Likewise, experimentally, it can be demonstrated that restoring the tissue hormesis (Fig. 4) results in the obtundation of the progressive phase of injury, permitting tissue healing to proceed.

The pivotal role of cellular proliferation and tissue repair mechanisms in the final outcome of tissue injury becomes self-evident from the following lines of experimental evidence. Prior exposure to 225 ppm PB results in the potentiation of liver injury by the same subtoxic dose of CCl_4 employed in the CD + CCl_4 interaction [23, 24, 38]. The quantitative measures of liver injury at 24 hr after the administration of CCl_4 indicate that the tissue injury is either equivalent to or slightly greater than that seen in CD + CCl_4 interaction [23]. Left alone, the animals undergoing the interactive toxicity of PB + CCl_4 recover, while those experiencing the CD + CCl_4 interaction do not [3–5, 24]. While the enhanced liver injury observed with the interactive toxicity of PB + CCl_4 is consistent with the increased bioactivation of CCl_4 [23, 32], recovery from this injury is consistent with the unsuppressed hepatocellular proliferation and tissue repair [38, 43]. Delayed hepatocellular regeneration and tissue repair from the normal 6 hr to 24–36 hr [5, 38] is the only consequence on stage II of CCl_4 toxicity. Nevertheless, the unsuppressed early phase of tissue repair enables the restoration of hepatolobular structure and function [3–5, 38], and thereby animal survival. These observations suggest the presence of two distinct stages of chemical toxicity (Fig. 4).

Induction of liver regeneration 36–48 hr after the administration of a toxic dose of CCl_4 is well established [4, 5]. The existence of an early phase of cell division (6 hr) was not revealed until experiments with a low, subtoxic dose of CCl_4 were carried out [35, 36, 39, 40]. Experimentally, it is possible to ablate the early phase of hepatocellular regeneration and tissue repair ordinarily stimulated by a low dose of CCl_4, making it in essence a toxic dose. Administration of a large, toxic dose of CCl_4 (2.5 mL/kg) results in ablation of this early phase of cell division [37, 49], indicating that the toxicity associated with a large dose is due to the abolishment of this critical early phase stimulation of tissue repair [3–5]. Administration of the same dose to animals prestimulated by partial hepatectomy, so that they have the ongoing hepatocellular proliferation and tissue repair, results in a remarkable and substantial protection from liver injury and lethality [37]. Such protection is not due to decreased bioactivation of CCl_4 [33].

The hitherto undescribed second stage of acute toxicity independent of stage I of chemical toxicity can be illustrated by another experimental approach. Recent studies with selective colchicine antimitosis of the early phase of hepatocellular division (6 hr) without interfering with the second phase of hepatocellular regeneration (48 hr) have shown a prolongation of liver injury [50]. Liver injury measured through serum enzyme elevations or by morphometric analysis of necrosis was not increased at 6 or 12 hr in colchicine-treated rats, indicating that these findings cannot be explained by colchicine-enhanced bioactivation of CCl_4.

The critical role played by the capacity to respond to CCl_4-hepatotoxicity by stimulation of tissue repair mechanisms at an early time point is illustrated by examining species differences in susceptibility to CCl_4 injury. Mongolian gerbils are extremely sensitive to halomethane hepatotoxicity [20–22]. Gerbils are approximately 35-fold more sensitive to CCl_4 toxicity than Sprague–Dawley rats [21, 22].

The remarkable and substantial sensitivity does not appear to be due solely to 3.5-fold greater bioactivation of CCl_4 in gerbils, since CCl_4 toxicity is not increased in gerbils by prior exposure to PB in spite of a 5-fold greater bioactivation of CCl_4 [21]. The time–course studies on the ability of gerbils to respond to a subtoxic dose of CCl_4 by stimulation of hepatocellular regeneration and tissue repair reveal an important difference in the biology of the hormetic mechanisms between gerbils and rats [22]. The early phase stimulation of tissue repair in the liver does not manifest itself in gerbils until approximately 40 hr after the administration of CCl_4 [22], in contrast to the 6 hr in the rats [36]. In the absence of the biological mechanism to arrest the progression of liver injury (Fig. 4), the liver injury might be expected to permissively progress much like an unquelched brushfire. Evidence in support of the concept that species differences in chemical toxicity may depend on the differences in the promptness in initiating tissue repair mechanisms among various species comes from another aspect of the interactive toxicity of CD + CCl_4. While gerbils are extremely sensitive to CCl_4, this sensitivity cannot be further increased by prior exposure to CD [20–22]. Since substantial evidence supports the concept that suppression of the early phase of hepatocellular regeneration and tissue repair is the mechanism for the permissive progression of liver injury in the CD + CCl_4 interaction [3–5], lack of this early phase response in the gerbil would be consistent with extremely high sensitivity of gerbils to CCl_4 on the one hand, and a lack of potentiation of CCl_4 toxicity by prior exposure to CD on the other [21, 22].

The interactive toxicity of CD + $CHCl_3$ has been demonstrated in murine species [28–31]. Stimulation of hepatocellular regeneration and tissue repair after a subtoxic dose of $CHCl_3$ allows the mice to overcome the liver injury associated with that dose of $CHCl_3$ [31]. By lowering the dose of $CHCl_3$ used in the CD + $CHCl_3$ studies [30], it is possible to demonstrate potentiation of liver injury, but without the lethality [31]. Such an experimental protocol vividly reveals a decisive role played by the stimulated tissue repair mechanisms in overcoming liver injury [31] and suggests the separation of tissue repair (Stage II) from the inflictive phase (Stage I) as two distinct stages of chemical injury (Fig. 4).

The importance of stimulated tissue repair mechanisms in overcoming liver injury has also been demonstrated through examination of the mechanistic basis for significant strain differences in mice [51, 52]. A SJL/J strain of mice, known to be least susceptible to CCl_4 toxicity, was shown to possess more prompt and efficient tissue repair mechanisms, which permit augmented recovery, whereas the BALB/C strain, known to be more susceptible, was shown to possess less efficient tissue repair mechanisms resulting in retarded recovery [51]. The F_1 cross between these two strains was shown to be intermediate in susceptibility [52]. While the time course of the inflictive phase of injury in the F_1 (SJL/J × BALB/C) was similar to the two parent strains, the tissue repair was at the intermediate level of augmented (SJL/J) and retarded (BALB/C) recovery.

With the advent of the finding that a low dose of CCl_4 is not toxic, not so much because it does not initiate tissue injury but because of the simultaneously stimulated tissue repair mechanisms [35, 36], it became apparent that the stimulation of the early phase of hepatocellular regeneration is in essence an endogenous hormetic mechanism, recruited to overcome tissue injury. One implication of this finding is its possible role in the phenomenon of CCl_4 autoprotection [53–56]. Circumstantial evidence, wherein hepatic microsomal cytochrome P450 decreased by $CoCl_2$ administration to 40% of the normal level did not result in decreased CCl_4 liver injury [47], suggested the possibility that a mechanism(s) other than decreased cytochrome P450 may be involved in CCl_4 autoprotection. Recent studies with the autoprotection model reveal a potential critical role for the hepatocellular regeneration and tissue repair stimulated by the low protective dose [57]. Essentially, the protective dose serves to stimulate tissue repair mechanisms [33, 35, 36, 39, 40] so that even before the large dose known to abolish the early phase stimulation of tissue repair [37] is administered, the tissue repair mechanisms are already in place, resulting in augmentation of tissue repair sufficient to tip the balance between continued injury and recovery in favor of the latter [57]. This experimental model represents another example wherein a selective manipulation of the tissue repair mechanism (Stage II, Fig. 4) without significantly altering the inflictive phase of toxicity (Stage I, Fig. 4) enables one to demonstrate the second stage of acute toxicity independent of Stage I.

Another line of evidence to illustrate the importance of the tissue repair mechanisms in determining the final outcome of chemical toxicity comes from experiments designed to understand the mechanisms responsible for the failure of the tissue regenerative and repair mechanism in the interactive toxicity of CD + CCl_4. Much evidence is available to implicate insufficient availability of cellular energy at a time when cell division should have taken place [35, 36, 58]. A remarkable and irreversibly precipitous decline in glycogen levels in the liver [36, 42], a rise in hepatocellular Ca^{2+} [44, 59], and a consequent stimulation of phosphorylase *a* activity, leading to an equally precipitous decline in hepatic ATP [42, 43], are events consistent with the failure of hepatocellular regeneration in the CD + CCl_4 interaction. Only marginal and transient declines in ATP levels in the interactive hepatotoxicity of PB + CCl_4 and M + CCl_4 [43] are consistent with only a postponement of hepatocellular regeneration leading to transiently increased liver injury followed by complete recovery [38]. The concept of insufficient hepatocellular energy being linked to failure of hepatocellular regeneration and tissue repair has gained support from experiments in which the administration of an external source of energy resulted in augmented ATP levels and significant protection [45, 46, 49]. Recent studies in which catechin (cyanidanol), known to increase hepatic ATP levels, was employed provided substantial

Table 1. Chemicals reported to cause non-neoplastic hepatocellular proliferation

Chemicals	References
Acetaminophen	60
Allyl alcohol	60, 61
α-Naphthyl isothiocyanate (ANIT)	62, 63
Carbon tetrachloride	36
Chloroform	64
Ethylene dibromide	65
Galactosamine	66, 67
Thioacetamide	68, 69

protection against the lethal effect of CD + CCl_4 [45, 46]. Protection by catechin was accompanied by a restoration of the stimulation of hepatolobular repair and tissue healing [46]. The most interesting aspect of catechin protection against the interactive toxicity of CD + CCl_4 is that protection does not appear to be the result of decreased infliction of hepatic injury [45, 46], as evidenced by a lack of difference in injury up to 24 hr after CCl_4 administration [46]. Cyanidanol protection was clearly due to restored hepatocellular regeneration and tissue repair. These observations provide substantial evidence for the separation of Stage I of toxicity responsible for the infliction of tissue injury from the Stage II events responsible for the final outcome of tissue injury.

Although the proposed two stage model of toxicity needs further experimental verification, abundant opportunities to test the concept are available. Many chemicals have been reported to induce hepatocellular regeneration at relatively modest doses, some of which are listed in Table 1. Opportunities to test the conceptual framework being put forth here are available through additional investigations with these models of tissue injury as well as scores of other models in other tissues and organs.

A framework for novel therapeutic avenues and for assessment of risk to public health

Establishing through additional experimental verification if indeed the initial toxic or injurious events, regardless of how they are caused, can be separated from the subsequent events that determine the ultimate outcome of injury offers promising opportunities for developing therapeutic intervention intended to restore the hormetic tissue repair mechanisms. Such a development will open up avenues for two types of antidotal therapy: (1) to decrease the injury by interfering with Stage I of toxicity, and (2) to enhance tissue repair and healing mechanisms to obtund the progressive phase of injury and simultaneously augment recovery from that injury. The present therapeutic approach deals primarily with interfering with Stage I of toxicity.

In addition to these opportunities opening up avenues to develop novel approaches to deal with acute chemical injury, the two-stage concept of chemical toxicity also embodies implications of significant interest in the assessment of risk from exposure to toxic chemicals. The existence of a threshold for chemical toxicity is evident as indicated by the stimulation of the tissue repair mechanism directed to tissue healing and recovery observed after the administration of subtoxic levels of toxic chemicals. The existence of a two-level threshold can be proposed: one threshold for each stage of the two-stage model. The thresholds may be quantitatively the same or different. From a public health perspective, exposure to singular chemicals is seldom involved. Multiple exposures to chemical combinations and or singular components simultaneously, intermittently, or sequentially are almost always the rule. This is true of medicinal compounds; it is true of environment chemicals. Of significant interest from a public health perspective is the finding that the hormetic mechanisms, which contribute to the threshold for physical or chemical toxicity, can be mitigated in the interactive toxicology of chemical and physical agents, resulting in highly accentuated toxicity.

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