MTT 法在中草药成分干预细胞增殖及活性中的应用*

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摘要：MTT 实验具有快速、方便和经济性好的特点，是一种评估中草药活性成分干预细胞增殖和活性的常规方法，用于悬浮细胞和贴壁细胞，步骤略有差异。中草药分子量较大、结构复杂，某些基团与甲臜分子基团相互作用，导致最大吸收峰漂移，故检测应重视检测波长的选择。同时，应根据检测目的综合考量检测效率、成本和准确性，选择不同的对照。在结果分析中，尽量采用 IC_{50} 值评估中草药成分的效应。此外，某些分子可进入细胞，其吸收峰与甲臜相同或接近，可能导致检测结果失真，清洗贴壁细胞等质控方法有时无法解决此问题，故 MTT 方法可分别与 BrdU、^{3}H – TdR 法、直接细胞计数或台盼蓝染色法等其他评估方法联合应用，取长补短，以快速、准确、客观地反映药物干预效应。

关键词：MTT 法；中草药；细胞增殖；细胞活性
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Application of MTT Method in Intervention of Chinese Herbal Medicine Ingredients in Cell Proliferation and Activity

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Abstract: MTT experiment has the characteristics of rapidity, convenience and good economy. It is a conventional method to evaluate the intervention of Chinese herbal medicine active ingredients in cell proliferation and activity. The steps are slightly different for suspension cells and adherent cells. Chinese herbal medicine has a large molecular weight and a complex structure. Some functional groups interact with formazine molecular groups, leading to the drift of the maximum absorption peak. Therefore, attention should be paid to the selection of detection wavelength in detection. At the same time, the efficiency, cost and accuracy of the test should be comprehensively considered according to the purpose of the test, and different controls should be selected. In the analysis of results, try to use the IC50 value to evaluate the effects of Chinese herbal ingredients. In addition, some molecules can enter the cells, and their absorption peaks are the same or close to that of methazan, which may lead to distortion of the test results. Sometimes quality control methods such as cleaning adherent cells cannot solve this problem. Therefore, MTT method can be used together with other evaluation methods such as BrdU, 3H TdR, direct cell counting or Trypan blue staining, to learn from each other to quickly, accurately and objectively reflect the effect of drug intervention.

Key words: MTT method; Chinese herbal; cell proliferation; cell activity

MTT[3 – (4,5 – dimethylthiazol – 2 – yl) – 2, 5 – diphenyltetrazolium bromide]法是一种检测细胞增殖和活性的方法，在中草药活性成分的体外筛选中应用广泛。MTT 法的检测原理为：在细胞培养环境中加入 MTT，活细胞线粒体中的琥珀酸脱氢酶

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(succinate dehydrogenase, SDH) 还原 MTT, 形成水不溶性的蓝紫色结晶甲瓒, 而死细胞无此功能; 加入二甲基亚砜溶解甲瓒, 检测吸光度值, 以该值反映样品活细胞数量[1-2]。灵敏度高、操作简单和经济性好的优点是这种方法得以广泛应用的原因, 但这种方法也有一定的局限, 如不能用于某些活性药物分子的检测[3-8]。本文就 MTT 法在中草药活性成分的应用和局限进行概括和总结。

1　常用的 MTT 方法

体外培养的细胞, 按照是否能贴附于支持物上生长的性质, 分为悬浮型和贴壁型两大类。这两类细胞的 MTT 操作步骤存在差异。

贴壁细胞: 向细胞培养液中加入 MTT 溶液, 继续培养一段时间。然后终止培养, 加入二甲基亚砜溶液, 震荡溶解甲瓒, 最后检测液体的吸光度。需要注意的是, 某些测试的中药化合物分子, 本身带有颜色, 需要清除这种干扰。可以在加入 MTT 前, 吸弃含药培养液, 用 PBS 或单纯培养液冲洗细胞 2 ~ 3 遍, 再加入 MTT 液。悬浮细胞: 在培养液中加入 MTT 溶液, 培养一定时间后, 离心, 除去上清, 加入二甲基亚砜, 最后检测吸光度。WST - 8[2 - (2 - Methoxy - 4 - nitrophenyl) - 3 - (4 - nitrophenyl) - 5 - (2,4 - disulfophenyl) - 2H - tetrazolium Sodium Salt] 是一种水溶性四唑盐, 为 MTT 的优化产品。它的主要特点是在线粒体内的脱氢酶反应下, 还原生成水溶性的甲瓒, 因此, 可以直接检测液体的吸光度。尽管 WST - 8 操作更为简单, 但存在不能排除药物分子颜色干扰的不足。

2　波长的选择

MTT 方法通过检测甲瓒的吸光度值间接反映细胞的活性或活细胞数量, 检测波长的确定是关键环节, 文献报道的检测波长存在一定波动。在中国知网的高级检索中, 选择: 摘要检索, 输入 MTT; 文献来源, 输入中草药。查询《中草药》杂志 1999—2022 年发表的应用 MTT 法评估中药活性分子影响细胞增殖或活性的研究论文。找到 509 篇详细描述 MTT 方法的论文, 选择 490 nm 检测波长的有 209 篇, 选择 570 nm 检测波长的有 197 篇, 选择 492 nm 检测波长的有 33 篇, 选择 540 nm 检测波长的有 6 篇, 选择 550 nm 或 590 nm 检测波长的各有 4 篇, 选择 595 nm 或 450 nm 的各有 3 篇, 选择 630 nm 的有 2 篇, 选择 510 nm、500 nm 或 493 nm 的各有 1 篇, 合计 464 篇选择单波长检测; 26 篇选择了检测波长为 570 nm/参比波长 630 nm, 5 篇选择了检测波长为 570 nm/参比波长 620 nm, 4 篇选择了检测波长为 570 nm/参比波长 450 nm, 检测波长为 570 nm/参比波长 490 nm、检测波长为 490 nm/参比波长 650 nm、检测波长为 595 nm/参比波长 630 nm、检测波长为 570 nm/参比波长 655 nm 各有 1 篇, 6 篇未明确检测波长和参比波长, 合计 45 篇选择了双波长检测。上述报告中, 检测波长范围为 450 nm 到 630 nm; 单波长检测应用较多, 达到 91.2%, 双波长检测为 8.8%; 单波长检测中, 490 nm 和 570 nm 应用最多, 分别达到 45.0% 和 42.5%。

检测波长的差异反映了不同的中草药活性分子或培养液等对 MTT 法吸收光谱的影响。甲瓒的吸收高峰在 550 ~ 600 nm, 这也是较多研究选择 570 nm 作为检测波长的依据。但甲瓒的吸收光谱受到多种因素的影响, 溶液的酸碱度或某些分子(残存的培养基分子、细胞残存的分子以及残留的药物分子等)都可能改变甲瓒的吸收光谱。应预先采用全波长扫描, 在 550 nm 的一定波长范围内, 确定最大吸收峰波长, 作为检测波长[9-13]。如果缺乏全波长扫描设备, 则预先分别采用 490 nm 或 570 nm 检测, 选择吸光度值高的波长作为检测波长。

单波长检测受到样本浊度、干扰色和电路(包括噪音、漂移、电压等)等因素的干扰, 因此, 设定另一个参比波长, 能够减少测定干扰和电路干扰, 从而减小了结果的变异系数 CV 值, 提高结果的精确度。但多数情况下, 参比波长检测的光密度 OD 值较小, 不影响对结果的分析和判断; 另外, 双波长检测酶标仪价格昂贵, 普及率不高, 这些可能是单波长检测选择较多的原因。尽管如此, 双波长检测更为灵敏和准确, 应当优先选择应用。

3　对照选择和结果分析

MTT 法经常采用的对照有两种模式: 一种是两孔设置, 样本孔加入含有药物的培养液, 对照孔加入单纯的培养液; 另一种是三孔设置, 除了上述两孔外, 还有一个空白孔, 该孔没有细胞, 仅加入培养液。

两种模式的结果计算如下: 两孔设置的单波长检测按照细胞存活率 = A 药物/A 对照, 判断细胞活力[14-18]; 三孔设置的单波长检测按照细胞存活率 = (A 药物 - A 空白)/(A 对照 - A 空白), 判断细胞

活力[19-23]。双波长检测按照细胞存活率 =（A 检测波长 - A 参比波长）实验组/（A 检测波长 - A 参比波长）对照组，判断细胞活力[24-28]。上述公式中，A 指检测的 *OD* 值。

除了应用上述公式判断细胞活力外，还可以用增殖率、抑制率或细胞增殖抑制率等分析药物的影响[29-33]。如采用细胞增殖抑制率 = 1 - A 给药/A 对照[34-38]或抑制率 = 1 -（A 给药 - A 空白）/（A 对照 - A 空白）[39-41]。还有一些报告，以半数抑制浓度（inhibitory concentration of 50%，IC_{50}）判断药物对细胞增殖的影响[42-48]。

三孔设计考虑到了培养液对结果的影响，分析结果更为准确，但增加了工作量和培养液的消耗，降低了筛选效率。在实际应用中，大量有效成分筛选可选择两孔设计，而准确性要求高的实验则可选择三孔设计。在结果的计算分析中，IC_{50}作为判断药物对细胞增殖、细胞毒性及细胞活力的指标，结果更为严谨和可靠。

4　检测方法的质量控制

需要注意的是，某些化合物可能与 MTT 直接发生化学反应产生假阳性结果，如橙皮苷、木犀草素和槲皮素可直接与 MTT 反应，OD 值随着浓度的增加而增加[49-51]。增加药物培养时间或降低药物浓度能够减少假阳性率[52]。另外，在加入 MTT 前，用培养液或 PBS 冲洗贴壁细胞，除去残存药物，是减少这种干扰的有效方法[11,53]。但有文献报道紫草素与甲臜的颜色相近，吸收峰部分重叠，在低浓度紫草素的干预下，MTT 法所测得的 A 值会增加。在高浓度紫草素作用下，几乎没有活细胞，但仍能检测到较高的 OD 值，这种假阳性是进入细胞的紫草素而非甲臜的结果[54]。显然，采用加入 MTT 前清洗细胞的方法无法清除细胞内残存药物的影响，这就需要用其他方法取代 MTT 法进行相关检测。另外，MTT 法应用于增殖检测的基本条件之一，是假设各实验组的单个细胞 SDH 酶活性大致相同。某些药物通过应激、抑制剂非靶效应的细胞适应性代谢及线粒体重编程可明显改变细胞 SDH 活性[55]，影响检验结果，甚至得出与事实相反的结论[5]。这种现象也降低了 MTT 结果的可靠性。

为了防止上述现象导致 MTT 检测结果的失真，需要采取一定的质量控制方法。例如，应用 MTT 之前观察细胞，记录细胞的数量或密度（定性或定量），与 MTT 的结果对照。如果记录显示加药的细胞密度或数量明显减少，MTT 结果为增殖，那么否定 MTT 的结果。另外，可采用直接细胞计数、^{3}H - TdR 法等方法判断细胞增殖，采用^{3}H - TdR 法、台盼蓝染色法或 5 - 溴脱氧尿嘧啶核苷（5 - bromo - 2 - deoxyuridine，Brd U）法等判断细胞活力[49,56-62]。

5　结语

目前，MTT 法已经成为评估中草药活性成分影响细胞增殖、毒性或活性的重要方法。但由于中草药分子量较大、结构复杂，存在某些基团与 MTT 分子基团相互作用，导致最高吸收峰漂移，此外，某些分子可进入细胞，而这些分子的吸收峰与甲臜相似。这些情况可能造成检测结果的失真，甚至产生错误的结论。清洗贴壁细胞等质控方法有时不能解决上述问题，因此，联合应用 BrdU、^{3}H - TdR 法、直接细胞计数或台盼蓝染色法是确保研究结果可靠的方法。但比色方法 BrdU 仍然存在化合物分子引起的吸收峰漂移等问题，^{3}H - TdR 法、直接细胞计数或台盼蓝染色法没有上述问题，但^{3}H - TdR 法有放射性污染的缺点；直接细胞计数不能用于细胞活力的评估，对于贴壁细胞，需要引入消化细胞的步骤，会不可避免地破坏某些细胞，也可能导致结果失真；台盼蓝染色和细胞计数还存在操作烦琐、效率低下、灵敏度低等缺点。因此，应该根据检测的实际情况，选择上述方法与 MTT 法联合应用，综合分析结果（图 1），以快速、准确、客观地反映药物干预效应。

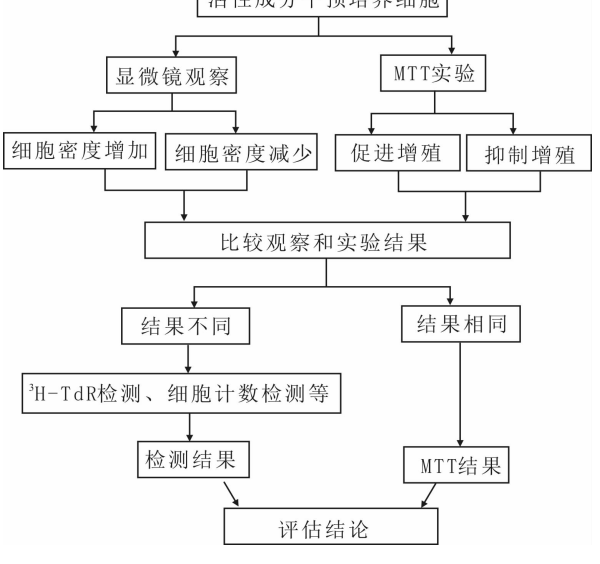

图 1　MTT 法与其他方法联合应用评估活性成分干预细胞的增殖

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