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人淋巴细胞核异常体外测试法

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Nuclear anomaly test in human lymphocytes *in vitro*

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ABSTRACT To assess the usefulness and the sensitivity of the nuclear anomaly test in human lymphocytes we treated *in vitro* human whole blood with various concentrations of mitomycin C, thiotepa, and bimolane. After the blood samples had been stored at 37℃ for 17-18 h, smears of isolated lymphocytes were made. The nuclear anomalies (micronuclei, irregular, karyorrhectic, and pyknotic nuclei) were measured. The concentration-respose relationship and the minimum sensitive concentration of nuclear damage indices to the test mutagens were analyzed. The results showed that all 3 drugs induced a concentration-dependent increase of other nuclear anomalies except pyknotic nucleus in lymphocytes, that the most sensitive index of nuclear damage was the micronucleus assay, that the karyorrhectic assay was as sensitive to MMC and bimolane as the micronucleus assay, and that the irregular nucleus assay and the complex nuclear anomaly assay were less sensitive, but the correlation between concentration and complex nuclear anomalies was the best among various indices of nuclear damage. Therefore, the *in vitro* nuclear anomaly test in lymphocytes of human whole blood could be used to evaluate genotoxic effects of chemicals.

KEY WORDS miutagenicity test; micronucleus test; lymphocytes; mitomycin C; bimolane; thiotepa

提要 乙双吗啉、丝裂霉素 C (MMC)及塞替派体外处理人全血后，观察淋巴细胞核异常的改变(微核、核变形、核裂解和核固缩等)结果表明三种不同类型的化学诱变剂均能诱发大部分核损伤指标，且呈剂量依赖性增加。其中微核细胞率较优。本方法作为一种快速、简便的人体细胞测试系统，可试用于化学品诱变剂活性的评价。

关键词 诱变性试验；微核试验；淋巴细胞；丝裂霉素 C；乙双吗啉；塞替派

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核异常测试法(nuclear anomaly test)是八十年代在实验动物中发展起来的体内评价致癌性的短期测试法[1,2]，其检测诱变剂的敏感性与 SCE 互补，并优于微核检测[3,4]。人的间期淋巴细胞可通过核异常形成微核[5-7]，提示核异常改变有遗传毒理学意义，为探讨在人体细胞中应用核异常的可能性，本实验选用已知诱变剂，进一步评价体外人全血淋巴细胞核异常测试法的实用价值。

MATERIALS AND METHODS

实验用静脉血采自 10 例健康人员，男 5 人、女 5 人，年龄 36 ± *s* 9 a，新采血样根据处理组数立即用培养小瓶分装，每瓶 0.4 ml 全血。待测诱变剂用 RPMI 1640 培养液稀释成各种浓度的工作液，每小瓶中加 0.1 ml，总容量 0.5 ml。乙双吗啉(杭州制药厂)组终浓度分别为 0 (加 0.1 ml 培养液，下同)，5，10，20，40 和 100 $\mu g \cdot ml^{-1}$ 供血员 4 人；MMC (日本协和发酵工业株式会社)组为 0，0.025，0.05，0.1，0.2 和 0.4 $\mu g \cdot ml^{-1}$，供血员 3 人；塞替派(上海海普制药厂)组为 0、0.1、0.2、0.4、0.8 和 1.2 $\mu g \cdot ml^{-1}$ 在 37℃培养箱中放置 17-18 h 按本室常规制片并阅片[10]，每个志愿者计数 5000 个淋巴细胞。

核损伤指标有微核、核变形、核裂解以及核固缩、核空泡(核内出现空泡样变性)等。后两项指标在本实验中无明显改变，未作分析。微核细胞率用平均 1000 个淋巴细胞中具有微核的细胞数表示。核异常率(frequence of nuclear anomaly)为微核细胞率(frequence of micronucleated cells, MNCF)、核变形

率(frequency of irregular nucleus, INF)与核裂解率(frequency of karyorrhectic nucleus, KNF)之和. 上述指标均以‰表示，具体含义见文献[8]. 最低敏感浓度表示诱变剂诱发各种核损伤指标和对照组相比有显著增量($P<0.05$)的最低浓度.

RESULTS

1 乙双吗啉体外诱发的核异常改变

乙双吗啉 0, 5, 10, 20, 40 $\mu g \cdot ml^{-1}$ 与 MNCF 和核异常率呈正相关(相关系数 r 分别为 0.95 和 0.99, P 值均 <0.01)，引起 MNCF 与核异常率显著增量的最低敏感浓度均为 10 $\mu g \cdot ml^{-1}$ ($P<0.01$) (Tab 1)，略优于紫露草微核测试法[11]；当用乙双吗啉 5 和 10 $\mu g \cdot ml^{-1}$ 处理时，INF 值上升不明显($P>0.05$)，当剂量达 20 $\mu g \cdot ml^{-1}$ 时才引起显著增加($P<0.05$)，在 0–40 $\mu g \cdot ml^{-1}$，乙双吗啉可引起 KNF 的增加，仅在 10 $\mu g \cdot ml^{-1}$ 时的增量显著($P<0.01$)，其它各剂量引起 KNF 的增加均不显著($P>0.05$)；100 $\mu g \cdot ml^{-1}$ 乙双吗啉诱发的四种核损伤指标改变，与其诱发的各指标最大值相比有下降(Tab 1)，除 MNCF 的 $P>0.05$ 外，其余三个指标均为 $P<0.01$.

2 MMC 体外诱发的核异常改变

用 MMC 0, 0.025, 0.05, 0.1 $\mu g \cdot ml^{-1}$ 处理人全血时，MNCF, INF 和核异常率均与剂量呈正相关(r 分别为 0.91, 0.97 和 0.99, P 值均 <0.05)，它们检测的最低敏感浓度均为 0.025 $\mu g \cdot ml^{-1}$, P 值分别 <0.05; 0.01 和 0.01. 在 0–0.25 $\mu g \cdot ml^{-1}$ 范围内，KNF 与剂量呈依赖性增加($r = 0.98$, $P<0.05$)，检测的最低敏感浓度为 0.025 $\mu g \cdot ml^{-1}$, 0.4 $\mu g \cdot ml^{-1}$ MMC 诱发的四种核损伤指标改变，与其诱发的各指标最大值相比，P 值分别为 MNCF >0.05, INF 和核异常率 <0.01, KNF <0.01.

3 塞替派体外诱发的核异常改变

用塞替派 0, 0.1, 0.2, 0.4, 0.8, 1.2

Tab 1. Influence of mutagens on frequencies of nuclear anomalies in lymphocytes of human peripheral blood. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Drug	concentration / $\mu g \cdot ml^{-1}$	Volunteers	Micronucleated cells / ‰	Irregular nuclei / ‰	Karyorrhectic nuclei / ‰	Nuclear anomalies / ‰
Bimolane	0	4	1.3 ± 0.0	5 ± 5	0.8 ± 0.7	8 ± 5
	5	4	3.0 ± 2.3*	3.1 ± 1.3*	0.9 ± 1.0*	7 ± 6*
	10	4	6 ± 4**	4 ± 4*	4 ± 4**	14 ± 7**
	20	4	6.3 ± 2.4***	11 ± 7**	2.2 ± 1.1*	20 ± 4***
	40	4	10 ± 5***	18 ± 7***	1.8 ± 1.1*	29 ± 3***
	100	4	6.2 ± 0.6***	3 ± 4*	0.4 ± 0.6*	9 ± 4*
Mitomycin C	0	10	0.8 ± 0.8	22 ± 9	0.1 ± 0.3	23 ± 9
	0.03	10	2.3 ± 1.0**	30 ± 14***	0.8 ± 1.0*	33 ± 15***
	0.05	10	3.5 ± 2.1***	30 ± 10***	2.2 ± 2.2**	35 ± 11***
	0.10	10	4.5 ± 1.1***	45 ± 14***	1.6 ± 1.4***	50 ± 12***
	0.20	10	3.8 ± 2.1***	32 ± 19***	1.0 ± 1.4***	36 ± 17**
	0.40	10	3.1 ± 1.0***	30 ± 15***	0.9 ± 1.8**	34 ± 16***
Thiotepa	0	12	0.9 ± 1.1	16 ± 5	0.6 ± 0.8	17 ± 6
	0.10	12	2.6 ± 1.1***	14 ± 5*	0.8 ± 1.2*	17 ± 6*
	0.20	12	3.1 ± 1.2***	13 ± 4*	0.6 ± 1.0*	17 ± 3*
	0.40	12	4.3 ± 2.2***	14 ± 3*	1.0 ± 0.3*	18 ± 4*
	0.80	12	4.8 ± 2.1***	20 ± 11***	0.5 ± 1.2**	26 ± 9***
	1.20	12	5.7 ± 2.6***	20 ± 7***	0.1 ± 0.3*	26 ± 7***

$\mu g \cdot ml^{-1}$ 处理人体全血时，MNCF 呈剂量依赖性增加，($r = 0.92$，$P<0.01$)，MNCF 检测的最低敏感剂量为 0.1 $\mu g \cdot ml^{-1}$；同时也引起 INF 和核异常率的剂量依赖性增加，r 分别为 0.85，0.94，P 值均 <0.05，INF 与核异常率的最低敏感浓度均为 0.8 $\mu g \cdot ml^{-1}$；在本实验剂量范围内，塞替派未引起 KNF 的显著增减.

DISCUSSION

本实验结果表明，三种化学诱变剂诱发的核损伤改变，剂量效应关系最好的指标是 MNCF，其次是 INF. 核异常率与 PNF 分别对 MMC 和 γ-线在较窄的处理剂量范围内呈线形关系；对三种化学诱变剂采用复合核损伤指标核异常率进行分析，提高了剂量效应间的相关性. 评价化学品的诱变性，最重要的方法学要求之一就是检测的敏感性，对本实验所检测的三种诱变性药物，最优的指标仍是 MNCF，其次是核异常率 KNF 和 INF，但 KNF 对某些诱变剂可不敏感(Tab 1). 然而对 γ-线、INF 和核异常率检测的敏感性优于 MNCF[8]. 可见，各种核损伤指标对不同类型诱变因子的反应各不相同，因此在具体测试过程中，应根据所研究的因子选择应用不同的核损伤指标及其间的组合. 本结果初步提示，对检测化学品的诱变性，最有实用价值的指标是 MNCF，其它指标如核异常率，KNF 和 INF 在反映不同程度的核损伤方面有参考价值.

在用乙双吗啉与 MMC 体外处理淋巴细胞时，在大剂量组观察到四种核损伤指标有不同程度的下降，这可能是由于大剂量组的细胞毒性过大，相反降低了细胞的反应性，类似的情况亦见于大剂量 γ-线处理人淋巴细胞的核异常改变[8].

本实验直接用化学诱变剂体外处理人体外周血，在 37℃ 放置过程中未加含 PHA 的培养液，故此时淋巴细胞属非周期细胞，多处于 G_0 期，但放置 17–18 h 后微核率明显增加 (Tab 1). 显然，此时微核只能通过核损伤后的异常改变形成，而与有丝分裂中染色体畸变无关，证实了我们以往用 γ-线的研究结果[5,6,8]. 然而不论微核通过何种途径形成，其遗传毒理学意义均相同，即引起遗传物质的损伤与丢失，最终也与间期染色体断裂、重排有关. 因此微核及其它核损伤指标均有不同程度的遗传毒理学意义. 近年来还有研究指出，人体外周血红细胞具有一定的氧化作用，可活化间接诱变剂[12]，我们用间接诱变剂环磷酰胺体外处理人全血，亦观察到微核等核损伤指标呈剂量依赖性增加. 体外人全血淋巴细胞核异常测试法具有简便、快速、无需应用 S_9 等代谢激活剂，即可评价各种理化因子对人体细胞的遗传毒理学效应等特点，值得注意.

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用高效液相色谱法研究环氧司坦在兔的药物动力学

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Pharmacokinetics of epostane in rabbits by HPLC method

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ABSTRACT Epostane (Epo), a 3β-hydroxysteroid dehydrogenase inhibitor, interrupted pregnancy in rats, rhesus monkeys, and women. Epo concentrations in serum were determined by high performance liquid chromatography (HPLC) at 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 48 h after intragastric Epo 96 mg · kg^{-1} in rabbits. The concentration-time curve exhibited a 2-compartment open model. The pharmacokinetic parameters were: $T_{\frac{1}{2}ka}$ 0.79 ± 0.08 h, $T_{\frac{1}{2}\alpha}$ 0.96 ± 0.08 h, $T_{\frac{1}{2}\beta}$ 6.6 ± 1.5 h, V_c 14 ± 3 ml · kg^{-1}, AUC 12.0 ± 1.9 μg · h · ml^{-1}, T_{max} 1.8 ± 0.5 h, C_{max} 3.3 ± 0.5 μg · ml^{-1}.

After rat copora luteum were incubated with hCG 10 IU · ml^{-1} and Epo 10 or 100 μg · ml^{-1} for 18 and 48 h, luteal cells showed various degrees of degeneration and progesterone production was significantly inhibited.

KEY WORDS epostane; high pressure liquid chromatography; pharmacokinetics; progesterone; corpus luteum

提要 用高效液相色谱法测定兔 ig Epo 96 mg · kg^{-1} 后 0.25、0.5、1、2、4、8、16、32 及 48 h 的血清中 Epo 浓度，其血药-时间曲线符合 2 室开放模型。主要药物动力学参数：$T_{\frac{1}{2}ka}$ 0.79 ± 0.08 h、$T_{\frac{1}{2}\alpha}$ 0.96 ± 0.08 h、$T_{\frac{1}{2}\beta}$ 6.6 ± 1.5 h、V_c 14 ± 3 ml · kg^{-1}、AUC 12.0 ± 1.9 μg · h · ml^{-1}、T_{max} 1.8 ± 0.5 h、C_{max} 3.3 ± 0.5 μg · ml^{-1}。Epo 可使离体大鼠黄体细胞变性和退化、孕酮生成减少。

关键词 环氧司坦；高压液相色谱法；药物动力学；黄体酮；黄体

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